A Survey of the Literature of Dental Caries (1952)

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Prepared for the
Food and Nutrition Board
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under the supervision of the
Committee on Dental Health
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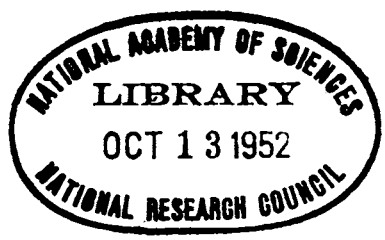

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The preparation of this report was initiated in November 1942, when a special committee was set up under the Food and Nutrition Board with instructions to undertake a critical review of the subject of dental caries, the objective being to provide the Board with a basis for an expression of judgment in the matter. Ten years of persistent labor on the part of Dr. P. C. Jeans and his committee, their numerous consultants, and the authors, superimposed on their full-time professional obligations, have gone into the production of this volume. The ultimate objective remains to be reached. The causes of dental caries are only partially understood at present, although much progress has been made. The Board has encouraged and supported this publication as a public service in the hope that it will help the adjustment of facts and principles into correct relationships and thus serve as a guide both in research and in education.

The Board is grateful to all who have contributed their time and professional talent to the preparation of this volume. The publication has been financially assisted by the voluntary contributions of several industrial firms to whom the Board extends its appreciation.

L. A. MAYNARD, *Chairman*
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CONTENTS

FOREWORD

THE REVIEW of the literature on dental caries sponsored by the National Research Council is a distinct contribution to dentistry. It consists of abstracts of reports in this field with critical summaries by the committee appointed by the National Research Council. The summaries of each section emphasize details of special significance. Bringing together in historical perspective accounts of work that has been done provides, in tangible form, the sources and nature of investigations upon which present knowledge of dental caries rests. It reveals the situation as it is today.

The review is made up of such a wide variety of investigations, involving many phases of biological science, and of evidence often contradictory, that specific consideration of the work of individuals reported is the province of the chemist, the bacteriologist, the pathologist, and the statistician. The ideas and comments following come from a study of the review as a whole and are concerned chiefly with obvious concepts and technics that condition the progress of work in this field as seen in these reports.

As a whole the review reveals limitations resulting from the absence of standardized examination technic in determining caries incidence; the acceptance of deficient statistical data; restricted research interests; the lack of adequate controls in observations and experimental research; and the tendency to overlook the evolutionary status of the dentition in the human organism.

The need for a definite rule of procedure in determining the incidence of dental caries is obvious. Methods of technic reported vary from an examination with mouth mirror and explorer, taking five minutes or less, to one which involves cleaning, drying and X-raying the teeth before recording an estimate of caries incidence. Naturally the comparison of data from such diverse methods is useless. Statistical treatment cannot erase the faults derived from such extreme differences in the methods of examination.

Numerical tabulations of carious areas reported provide quantitative evidence of the extent of its occurrence in a population. Statistics afford a method of treating data of this kind and when taken at their true value reveal trends from which new conceptual schemes can be evolved. The present interest in the relation of fluorine to caries incidence is an example of such procedure. But in this instance as in others it should be remembered that the value of findings depends primarily on the accuracy and unbiased character of the original observations on which they are based.

The tendency to cling to the belief that the tooth is an entity, which characterized the work of many of the earlier investigators, is not in step with the times. The chemico-parasitic theory of dental decay (Miller and Black) has been a dominant influence in the minds of investigators and occupies a conspicuous place in the review. During the 19th century this concept was in harmony with the bacteriological stage in the evolution of medicine resulting from the work of Pasteur. It has served a purpose. From what is known today it has a place in the complex problem of tooth decay. Yet in the face of advances in the general field of biology during the last decade it is clear that the chemico-parasitic theory is not the embodiment of all factors involved. The parallel between the period of life when the incidence of decay is the highest and the time when nutritional requirements are the greatest, childhood and adolescence, together with the change in the character of the tooth with age, is certainly suggestive. Knowledge of the relation of hormones to enzymes involved in calcification processes, the potential origin and appearance of like and mutant characters described in genetics,

and the radioactive tracers of metabolic activity in dental tissues all point to the myopia of interest restricted to the local environment of the teeth.

Accepting the challenge imposed by new knowledge does not mean that the chemico-parasitic theory in its entirety should be discarded. But there are facts that will not fit into it, and from knowledge now available there is reason to believe that these facts may be explained by a more comprehensive scheme of approach. Conant says, "We can put down as one of the principles learned from the history of science that a theory is only overthrown by a better theory, never merely by contradictory facts." The spirit and ability to evolve new concepts and techniques from observations and experiments that have been done is the hall-mark of the true scientist. Those parts of the review, unfortunately few, concerned with the biological aspects of tooth decay and based on a more comprehensive concept than the chemico-parasitic theory are most encouraging. They are consistent with the development of medical science as it has moved from the dramatic period of bacteriology to the experimental medicine envisioned by Claude Bernard and typical of medical science today.

There seems to be a point at issue in the review between the proponents of the chemico-parasitic concept and particularly the nutritionists as to the significance of the metabolism of tooth tissues in the process of decay. Opinions vary from those who believe that dentin and especially enamel of the mature tooth cannot in any degree be influenced by systemic processes, to those who consider the tooth an integral part of the organism, reflecting all its vicissitudes. But sufficient evidence has been presented to support the latter attitude as the more promising approach to an understanding of the nature of tooth decay.

That the importance of adequate controls in research is not generally appreciated is obvious in the review. Theoretically, controlled research in the field of biological phenomena means that two complementary phases, genetic and environmental, are involved in all developmental processes, normal or abnormal. Environment consists of an internal division, the endocrines, central nervous system, and bloodstream; and an external division, the living conditions, diet, infections, etc. Thus in order to determine, for example, the influence of diet on caries incidence, it is necessary to control all other variables, internal and external, and to know the genetic constitution of the subjects observed and experimented upon. Otherwise there is no certainty in the identification and isolation of facts resulting from alteration in the diet. In reality "control" is always a question of degree. The essence of the controlled experiment is the control of relevant variables. Progress depends on the recognition of them in a given experiment or observation. In animal experimentation a higher degree of control is possible than in dealing with human material. The disadvantage here is that it cannot be claimed *a priori* that the same influences operate the same way in man. When working with human beings the relevant variables are so many that the causal significance of one factor is difficult to infer. Genetic constitution is largely unknown, living conditions are seldom identical, strict dietary regulation is difficult both in and out of institutions, within groups of children of the same chronologic age there is usually a wide variation in physiologic age, and emotional states may introduce factors that cannot be anticipated. These are relevant variables difficult to weigh, yet their possible influence on data cannot be ignored. Because of the limitation of controls, the road to facts relative to dental caries by the use of human material is a long one.

It is claimed on the basis of statistical studies that the presence of fluorides in the drinking water and their local application to the teeth reduce the incidence of caries.

INTRODUCTORY REVIEW

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CONTENTS

INTRODUCTORY REVIEW

"It is paradoxical that the hardest of all tissues is so susceptible to lesions in living humans, while it is the most indestructible in the dead. To its remarkable resistance to ages of erosive forces, we owe our knowledge of past fauna and their dietary habits." (Bronner, 1946).

W. D. Miller (1890) writes in his monograph: "The destruction of the hard substances of the teeth commonly known as caries of the teeth, decay of the teeth, tooth-rot, etc., has, more than any other topic in the domain of Dentistry, continued to excite the scientific interest of dentists and physicians for more than two thousand years. The numerous theories which have been held at different times concerning the origin of dental decay prove that the problem is no easy one. Not one of them has as yet been universally accepted." After having read the reviews in this book, one will see that the words of Miller are still true despite an additional 60 years full of active research work. The overall picture gained from the reviews, covering references to more than two thousand publications on the subject, is that dental caries certainly has a complex etiology and that all the different factors involved in the initiation and further development of dental caries are not yet known.

The following statement on "Etiologic Factors" formulated by the "Workshop" on Evaluation of Caries Control Technics (1948), indicates the complicated nature of this disease:

"I. Indirect factors influencing caries
  A. Tooth
    1. Composition
    2. Morphologic characteristics
    3. Position
  B. Saliva
    1. Composition
      a. Organic
      b. Inorganic
    2. pH
    3. Quantity
    4. Viscosity
    5. Antibacterial factors
  C. Diet
    1. Physical factors
      a. Quality of diet
    2. Local factors
      a. Carbohydrate content
      b. Vitamin content
      c. Fluorine intake
II. Direct causes of caries
  A. Acidogenesis
    1. Carbohydrate degradation to acid
  B. Proteolysis."

In the special reviews one will find all the above factors dealt with. The main intention of the present introductory review has been on the one hand to give citation of the most important factors relating to the whole caries problem. As this monograph was meant to be useful also for students of the subject not having the full odontological background, such an overall picture was considered to be of some value. At the same time a discussion of various factors has been undertaken on the basis of the studies and conclusions reported in the various reviews as well as on the basis of other findings.

DENTAL CARIES AS RELATED TO FOOD AND MICROORGANISMS

Ever since Aristotle, 350 years before Christ, asked himself this question: "Why do figs when they are soft and sweet, produce damage to the teeth?," dental caries has been connected with the food eaten. Today no investigator on this subject denies that the food eaten does have something to do in the development of tooth decay. But

the food may act in two distinctly different ways—either as it is taken into the mouth, retained there for a certain length of time and transformed into injurious agencies, or as the metabolized food through nutrition. In the first way the food will act locally, as an environmental factor; and in the second way generally, as a systemic factor. But even after the food has been metabolized, it may act externally on the teeth through the saliva.

From the nature of the enamel—ordinarily the first part of the tooth to be attacked by the caries process—it has been logical to assume that acid is the main destructive agent. Being composed of only around one per cent organic material, chiefly a modified protein, the enamel should be subject to total disintegration by an acid action.

Ever since the formulation of W. D. Miller the source of the acid has been considered to be carbohydrates acted upon by acidogenic bacteria in the mouth. Contrary to the opinion of Miller that every one of the acid-producing bacteria found in the mouth must be recognized as a caries bacterium, the number of types of "caries bacteria" has been reduced during the past years.

As will be seen from the review on Oral Environment and Dental Caries, the discussion of the caries bacteriology has mainly centered around the streptococci and lactobacilli. The literature reported demonstrates that both of these groups are producers of acid strong enough to decalcify the inorganic part of the tooth although one or the other of the strong acid producers may be dominant in the caries picture. From bacteriological studies, Bunting, Jay and coworkers named *Lactobacillus acidophilus* as *the* caries bacterium, and later the *L. acidophilus* count was introduced and widely practiced as a caries-susceptibility test. One of the reasons for characterizing *L. acidophilus* as the caries bacterium was that this bacterium is highly aciduric as well as being acidogenic. As stated in the review of Oral Environment and Dental Caries: "There is no reason why acid producers must be able to live in acids, as the production of the acid is a process separate from that of survival. Many strongly acidogenic bacteria are feebly aciduric . . . rather it is likely that cavity formation is due to the activities of any organisms that form acids and of those that can destroy the organic substance, particularly of dentin . . . Oral streptococci probably account for more destruction of tooth substance in caries than any other forms, as they are rapid acid formers, constantly present, and can penetrate into the deepest part of a lesion in the dentin."

Nevertheless, the literature reported seems to point strongly in favor of *L. acidophilus* as the most typical of the caries bacteria, especially during the earlier stages of the caries process. Further evidence of this will be found in the following studies.

The dental and bacteriologic studies by the Norwegian expedition to Tristan da Cunha 1937–38 (Henricksen and Oeding (1940), Sognnaes (1945)) show a close relationship between the dental status and the presence or absence of *L. acidophilus*. The authors write: "Of the 39 persons who had *L. acidophilus* in their saliva, 21 had one or more soft, apparently active cavities, 12 had signs of an earlier attack of caries (roots or hard cavities), while 6 had sound teeth. Of 136 persons who were negative with respect to *L. acidophilus* only 1 had definitely soft, or slightly soft cavities, 36 evidence of a past carious process while the remaining 92 had sound teeth."

A study strongly pointing to the important role played by *L. acidophilus* is that of Hemmens, Blayney, Bradel and Harrison (1946). By examination of the flora on erupting teeth before they came into contact with their neighbors and until clinical or roentgenological caries could be recognized, it was shown that the increase in number of bacteria during the months prior to making the diagnosis of caries was greatest among *L. acidophilus*. The different

types of streptococci showed a lesser increase, while some other microorganisms had diminished.

Ström (1948) summarizes his studies in Norwegian orphanages as follows:

"Studies are reported on the occurrence of Lactobacilli, yeasts and cocci in the salivas of 116 children in six orphanages in Oslo during the war years 1942–45. The technic of the Michigan group was used. A definite correlation was found between the prevalence of dental caries and the Lactobacillus count, but the correlation was by no means absolute. Between the occurrence of yeasts and cocci in the salivas and the susceptibility to caries, no correlation was found." In a later communication from the same source, Ström (1949) states that 19 of the children who were caries-free did not show *L. acidophilus*, but did show streptococci and staphylococci which produced acid in sugar media. Schourie and coworkers (1949) from their very comprehensive Puerto Rican study conclude: "The correlation coefficient (r) values 0.32 and 0.31 (according to two different groupings) show a relationship between Lactobacillus counts and the DMF* measurements. But such an order of correlation indicates that one could not expect a very accurate prediction of the DMF teeth of a subject from the Lactobacillus counts of the subject." There was also found a real correlation between Lactobacillus and the amount of debris, between the cocci and DMF teeth, but not between cocci and debris nor between yeast and DMF, nor yeast and debris. However, it should be noticed that the saliva specimens for the bacterial study from more than 600 boys 14 years old were taken just once, and thus hardly can be taken as a true picture of the condition.

Different results have been obtained by others, for instance by Boyd and coworkers (1949) in studies of institutional children. They could find no relationship between Lactobacillus counts and the caries picture, and state that *L. acidophilus* counts cannot be used either as a caries diagnostic or as a caries prognostic means. From the studies in 1949 they conclude: "Generally speaking, there was a slight trend towards parallelism of the Lactobacillus counts and rates of progression of caries when massed data were used. However, when the group as a whole was subdivided according to the rate of caries progression, there was little difference between the range of Lactobacillus counts observed among those with least and those with the greatest progression of tooth decay."

The active part played by bacteria is conclusively demonstrated by Blayney and his coworkers (1948) in their studies on germ-free (bred) rats. By feeding a group of germ-free rats and a group of ordinary rats a sterile caries-producing high sugar diet, these workers did not give caries to the germ-free rats, but 95 per cent of the control rats did get caries. Further studies on germ-free rats kept on the caries-producing diet are now in progress, in which different strains of *L. acidophilus* are introduced into the rat's mouth.

W. D. Miller produced artificial caries in human teeth *in vitro* by keeping them in a saliva-bread mixture. Under the microscope he could find no difference between these lesions and the natural caries in pulpless teeth. These experiments have been confirmed by many others. Also, after exposing sterilized human teeth to pure dextrose cultures of *Streptococcus mutans*, Hammond and Tunnicliff (1940) have found caries-like lesions with microorganisms in the dentinal tubules. Bunting (1929) applied a mixture of bread and *L. acidophilus* under a clasp on the teeth in the mouth and produced caries after 1–3 weeks.

Miller's statement that lactic acid was the chief acid, among several other organic acids, in dissolving the inorganic part of enamel and dentin has been corroborated

*DMF— number of teeth decayed, missing or filled per hundred teeth that would be found in corresponding normal mouths.

by many investigators. The source of the acids is the carbohydrates. According to Fosdick and Hansen (1936), the degradation of carbohydrates follows the mechanism of Emden for formation of lactic acid in muscles and of Meyerhof for the action of yeast on carbohydrates. In accordance with this concept, Fosdick and Wessinger (1940) stress the following important steps in the degradation of glucose by yeast from the mouth: a) phosphorylation of glucose; b) production of phosphoglyceric acid; c) degradation to pyruvic acid; d) reduction of pyruvic acid in presence of hexose diphosphate to lactic acid. The strength of the acids is in the order stated here, and hence also their importance in the decalcification process. Neuwirth and Summerson (1942) state: "... all microorganisms can metabolize both lactic acid and pyruvic acid almost as rapidly as they are formed. The metabolism of lactic acid leads to an intermediate production of significant amounts of pyruvic acid, but the further metabolism of pyruvic acid produces no lactic acid."

The microorganisms in the plaque from both caries-free and caries-active persons contain the different enzymes necessary for glycolysis. The hypothesis of Fosdick that *L. acidophilus*, the organism most frequently associated with the acid production, should be deficient in some of the enzymes, especially phosphatase, necessary for high and rapid acid production, and that yeast would complement this, is not substantiated by other investigators in this field. On the contrary, Stephan and Hemmens (1947) found less acid production in high concentration of *L. acidophilus* and yeast cells put together in glucose than by *L. acidophilus* alone. Stephan writes (1948): "It seems probable ... that the chief difference in carbohydrate metabolism between oral microorganisms associated with active caries and those not associated with active caries occurs in the enzymatic steps which occur after the formation of pyruvic acid and lactic acid. Thus, oral microorganisms such as Lactobacilli in plaques permit a more prolonged accumulation of acid intermediates of metabolism (i.e. lactic acid), whereas many other types of oral microorganisms in plaques either relatively quickly oxidize the acid intermediates to carbon dioxide and water or else, like yeasts, reduce them to non-acid substances such as alcohol."

The commonly accepted theory that the microorganisms are producing the enzymes necessary for rapid and strong acid production in the initial enamel decalcification has been opposed by some investigators. Thus Föyn and Hobaek (1947) in a preliminary report showed that the filtrate of saliva having passed Berkefeld's filter failed to yield acid after addition of sucrose, whereas the sediment together with sugar showed an immediate drop in pH and after 15 minutes reached 4.5. This they interpreted in the following way: "The great rapidity with which acids are formed in saliva-sucrose solution mixtures indicates that the breakdown of sucrose is caused by an enzyme system preformed in saliva and not by the microorganisms." This enzyme, a glucosidase, is thus nonfiltrable. The action of the bacterial enzymes, they maintain, is a much slower process. Hill and While (1948) reported similar findings. Even after addition of large amounts of *L. acidophilus* (up to 3000 times as many bacteria as in the saliva) no drop in pH occurred in the filtrate-sugar mixture. While the sediment together with sugar resulted in a drop in pH of two units, the sediment from both cultures together with sugar gave a drop in pH of only one unit. If the saliva-sediment was subjected to a temperature of 60°C for 20 minutes, sufficient to destroy its enzyme content, no acid was formed within the first 90 minutes. The authors conclude: "From this evidence it is difficult to draw a definite conclusion but the suggestions are strong that factors other than the number of Lactobacilli present play an

important factor in acid production." On this point further studies are needed.

The Proteolytic Versus Acid Theory of Enamel Destruction

There is no agreement among the investigators on the acid theory of enamel destruction. Some maintain that the first step in the distingegration of the enamel is not the dissolution of the inorganic salts but a splitting of the organic material. Pincus, one of the best known proponents of the theory of enamel proteolysis, claims to have demonstrated in 1937 enamel lesions on this basis, similar to those well known from acid attack on the enamel. The sterilized sound teeth were placed in cultures of organisms known to attack protein. The pH of the medium when started, and on each subsequent day during the whole experiment, was between 7.2 and 7.4. He writes: ". . . disintegration of enamel may be caused by attack on the organic matrix of enamel . . . The occurrence of lesions of enamel suggests that all enamel erosion is not necessarily caused by acid attack."

However, the results of the carefully conducted *in vitro* studies of Weisberger (1950) do not confirm the findings of Pincus. After incubating extracted impacted teeth for 4 months in a medium consisting primarily of known chemical substances and inoculated with a mixed oral flora, he did not find any change of the enamel surface when no carbohydrate was added. The mixture was changed every week and the pH was about 8. When 1 per cent glucose was added to the medium, the mixture developed a pH of 4 and a pigmented decalcified enamel, resembling caries *in vivo*, in the uncovered area of the tooth crown. On repeating the last experiment but with addition of a buffer keeping the pH at about 8, no change in the enamel was observed. Incubating teeth in the inoculated original medium, without glucose but buffered to pH 4, caused the exposed enamel to become decalcified, but the changes did not resemble natural caries. Weisberger concluded: "In the above experiments glucose not only provides a substrate for acid formation thus producing a H-ion concentration suitable for decalcification of enamel, but also it appears to serve an entirely different role in that only when glucose is present in the medium are we able to produce the pigmented carious-like lesions involving the enamel and dentin." In his studies of the enamel protein, Pincus (1948) has shown that it is not a true keratin as previously maintained. It consists, according to him, of 12.1 per cent nitrogen and 1.2 per cent sulfur. Cystine was present either in small amounts or not at all. Nasmyth's membrane, as well as the "groove" protein, (he used unerupted teeth for the analyses) resist digestion by pepsin and trypsin. The groove protein contains carbohydrate, possibly glucose. In a later publication (1949) Pincus set forth a new hypothesis on the production of dental caries, especially in the grooves. He maintains that Nasmyth's membrane and the other enamel proteins resemble mucoproteins and thus on hydrolysis yield among other substances, sulphuric acid. He has isolated gram-negative bacilli from the mouth capable of producing an enzyme, sulphatase, which releases combined sulphuric acid from the mucoprotein. But the enzyme does not seem to act easily on this unless the protein first is hydrolyzed to release the polysaccharide. The sulphuric acid released will then attack the calcium in the enamel and dissolve it. Calcium sulphate he has found in carious enamel, but not in sound enamel. The gram-negative bacilli needed no other nutrient than the tooth itself.

By using a histochemical technic, Wislocky and Sognnaes (1949) proved that the enamel organic substance contains an acid mucopolysaccharide.

The finding of a carbohydrate fraction in Nasmyth's membrane and in the "groove" protein may be of some importance in the early decalcification of the enamel from

another point of view than that set forth by Pincus. Even before the occlusal surface proper of the 6-year molar is freed of gingiva, the fissure enamel may be heavily decalcified. As soon as a tip of the mesial cusps is uncovered, acid-producing microorganisms may reach the fissure system and act on the carbohydrate material of this organic substance and thus produce acids. In a later stage, carbohydrate together with saliva will diffuse into the spot and give rise to further acid production.

Gottlieb (1947), like Pincus, attributes the first disintegration of the enamel to the action on the protein instead of to the action on the inorganic constituents. He maintains that the pathway into the enamel is the lamellae. As pointed out in the chapter on Pathology of Dental Caries, these organic structures may be irregularly distributed in the enamel. Histological pictures of enamel caries in early stages often show the pathologic invading agencies along these structures before there is any sign of caries between them (Frisbie, Nuckolls and Saunders (1944)). It is, however, too radical a step to draw from these findings a generalization of the process always starting in the lamellae, as maintained by Gottlieb. The correlation of the distribution of the lamellae and the typical localization of caries has not been demonstrated. It is a common observation that the immune middle third of the buccal and lingual surface of the cuspids and bicuspids readily becomes carious when the tooth is rotated 90° either way. There is a question also whether the "lamellae" labelled as such in many of the illustrations really are true lamellae. Frisbie *et al.* are dubious about the lamellae and name them "pseudo lamellae" or "bacterial tracts." It is true, however, that bacteria find a much easier entrance into the enamel through such structures than elsewhere, but histological studies have demonstrated caries to be present frequently without the classical lamellae.

Frisbie, Nuckolls and Saunders (1944) on the basis of their very thorough studies of enamel caries conclude: "The initial carious lesion on the surface of the enamel is probably associated with the formation of the bacterial plaque and the breakdown of the enamel cuticle, thereby uncovering the ends of the enamel rods and interrod matrix. The penetration of this calcified homogenous cuticle may be due to acidogenic bacteria removing the more readily soluble inorganic constituents from this layer.... Once the primary cuticle is destroyed our observations indicate that the essential and primary pathologic lesion is one of proteolysis of the matrix. We envisage the extension of this proteolytic process and liquefaction of the matrix as freeing the less soluble basic tricalcium phosphate from its organic bond, and thus favoring its solution by products of acidogenic bacteria which secondarily penetrate along widening pathways of ingress. Gram-positive organisms spheroidal in form having penetrated the lysed surface matrix are found advancing deep into the matrix well beyond the general area of decomposition. We offer as a working hypothesis that with the establishment of the lesion, caries of the enamel is primarily a proteolysis of the organic matrix resulting from the enzymatic action of microorganisms followed by the subsequent dissociation of the inorganic constituents."

The hypothesis of Pincus on the destruction of the inorganic component of the enamel seems rather speculative. The extensive work of Hurst, Frisbie, Nuckolls and Marshall (1948) and of Hurst, Mullett, Frisbie, Nuckolls and Marshall (1949) exploring the earliest stages of enamel lesions, however, are promising. As long as no technic is available for the simultaneous minute study of both the organic and inorganic parts of the enamel in the first stages of abnormality, it seems very difficult to decide whether the organic or the inorganic part is destroyed first. Cox (1944) suggests two different steps in the caries process of the enamel: the initiation of the first enamel

lesion, followed by the cavity formation. Stress should be laid on studies of the initiation of the enamel lesion.

The Carbohydrate-Bacteria Plaque

Carbohydrates as a source of acid are attached to or within the plaque or debris of the teeth. The connection between debris and caries is again demonstrated by Schourie and coworkers (1949) in their Puerto Rican studies and by Fosdick (1950). The former found in 622 boys 8.3 DMF teeth in the group without debris, 10.4 in the group with moderate debris and 12.4 in the group with heavy debris (here only 16 cases). The character of the plaque differs in physical, chemical and bacteriological properties. The concept of the role of the plaque has changed after Stephan in 1940 introduced the antimony-electrode in the studies of the condition of the mouth. Before that, the plaque was generally considered to store the carbohydrates for a long time, thus giving rise to acid production over a considerable period. Stephan, however, showed by measuring the pH of the plaque *in situ* that the acid production after introducing sugar into the mouth, starts after 1–2 minutes, and has usually stopped within one hour. The pH after using glucose or sucrose may drop to 4.5 or 4.0. The rinse with lactose causes only a minute drop in pH. Fosdick (1948) and Strålfors (1948) later corroborated these findings of Stephan and strongly pointed to the intermittent character of the acid attack on the enamel. Strålfors found a significant relation between the pH minimum of the plaque and the Lactobacillus counts. Individuals with higher pH minima have lower Lactobacillus counts. The same author also measured the buffer capacity of the dental plaque and found this to be much higher than that of saliva. Strålfors suggests that the plaque has the capacity to store acid and to hinder the saliva from neutralizing it.

The prominent role of the plaque in the production of acid was plainly demonstrated by Stephan and Miller (1943). After rinsing the mouth with glucose solution, a pH curve was constructed from both sides of the upper arch, according to Stephan's method. Thereafter, the teeth on the left side were thoroughly brushed and the mouth rinsed again with glucose. The new pH curve showed a much lower drop in pH on the left side compared with that of the right side and compared with the first curve on the left side, that is, before the brushing.

The effectiveness of reducing the amount of debris by thorough use of the tooth brush, especially soon after meals, and thereby reducing the amount of caries has been claimed for many years by the clinicians, but until Fosdick (1950) reported on the reduction of the incidence of caries by brushing the teeth immediately after eating, no valid statistical evidence for such claims had been published. The seemingly carefully conducted studies of Fosdick and coworkers covered 423 university students of an average age of 23.5 years serving as controls, and of 523 students of an average age of 22.7 years requested to brush their teeth within 10 minutes after ingestion of foods or sweets. If this was impossible the mouth was to be rinsed thoroughly with water. The latter group was instructed in a uniform technic of brushing. The control students were supposed to continue with their old habit of mouth cleansing. By thorough clinical examination together with roentgen control, the test group revealed 50–60 per cent less new caries after two years than did the control group. The author writes: "A clinical test on the efficiency of brushing the teeth immediately after the ingestion of foods with a di-calcium phosphate detergent dentifrice indicates that dental caries can be materially reduced by this method. The results are in accord with the generally accepted theory of the carious process and materially strengthen this theory."

As to the relative importance of the different carbohydrates in yielding acid through the action of microorganisms,

Stephan (1948), writes: "It seems clear from these studies that with most acidogenic oral microorganisms and related types, fermentation takes place and acid is produced very frequently from the common monosaccharides, dextrose and fructose, fairly frequently from the disaccharides, sucrose and maltose, less frequently from the disaccharide, lactose, and seldom from the polysaccharides, dextrin and starch." It must be remembered, however, that in the mouth of man ptyalin will convert polysaccharides to maltose and thus become a good source for acid production in the plaque.

In cotton rats starch does not produce the same score in experimental caries as an equivalent amount of sucrose (Schweigert, Potts, Shaw, Zepplin and Phillips (1946)). Shafer (1949) reports from experiments in hamsters that starch gave practically no caries and sucrose gave more than double the amount of caries as did glucose. Few organisms seem to be able to attack starch, and human pytalin does not very readily convert raw starch to maltose. Furthermore, the molecules of starch are so large that they are almost unable to penetrate into the plaque material as do the much smaller sugar molecules. It may therefore be concluded that the monosaccharides and disaccharides, except lactose to a certain degree, are of greater importance in the acid production in the plaque than the polysaccharides.

To the clinician, beginning caries connected with dental plaque is a most common picture. Sometimes it is not possible to observe any debris covering an enamel lesion, but this does not exclude the possibility of a plaque having covered the enamel earlier. Contrary to the statement of Gottlieb that the tenacious plaque is secondary to the enamel lesion, the opposite seems to be true: no plaque, no caries. However, to reverse this and say that a lesion of the enamel is always found when plaque has been present for a certain length of time is erroneous. Among more primitive people without the regular modern daily cleaning of the teeth, including those who have an extremely low incidence of caries, it is common to find heavy, tenacious plaque covering most of the crown. Nevertheless, after scraping off the debris, the enamel may have the most lustrous appearance.

Although it is a common observation that beginning enamel caries is associated with plaque, caries does not always start on habitually unclean teeth or parts of the teeth. This can be attributed to the character of: (1) the plaque itself; (2) the saliva; (3) the state of the tooth at eruption; (4) the reaction of the erupted tooth.

The Plaque

The plaque may differ considerably as to both chemical and physical properties. In this discussion the pigmented plaque described by Bibby (1931), Vallotton (1945) and Pedersen (1947), a brownish or blackish deposit associated with low caries frequency, is excluded.

The chemical characteristics of the ordinary plaque (materia alba) are mostly dependent on the bacterial flora and the content of food remnants. Wild (1941), who made a thorough study of the plaque, characterizes it as the debris which sticks to the habitually unclean parts of the tooth surface. It consists of finely divided food particles (carbohydrates, proteins and fats), saliva (mucin, desquamated epithelial cells) and various microorganisms. The plaque is a product of the oral metabolism, and its caries-causative properties depend on carbohydrates and acidogenic bacteria. The plaque is not very permeable. However, according to Dobbs (1932) and Wilska (1947) it is highly permeable to glucose and the molecules of sucrose also are small enough to penetrate into the plaque easily. The starch molecules, however, as mentioned before, are considered too large to penetrate the ordinary tenacious plaque, but after

the ptyalin has converted the starch to maltose, this may diffuse into the plaque.

As to the role of starch, the findings of Osborn, Noriskin and Staz (1937) suggest that whole wheat meal was less effective than white flour in inducing decalcification of teeth in *in vitro* experiments. This may be accounted for partly by the findings of Kneen and Sandstedt (1946) of an antiamylase factor isolated from wheat, rye and other products. When this factor extracted from raw wheat is added to a mixture of starch and saliva, the degradation of starch is greatly retarded. This inhibitory factor seems to be lost during the industrialization of cereals. The antiamylase factor has been stressed also by Volker (1949). This point needs further study and should be considered in an explanation of the very low caries frequency of people living on a high intake of non-industrialized flour (Roos (1937), Toverud (1938)).

The fact that the degradation of carbohydrates is dependent on the acid phosphatase, and that this enzyme has been found in the plaque, makes it possible that differences in concentration of the enzyme may alter the activity of the plaque (Eggers-Lura 1948). However, the role of this factor in the plaque, as well as in the saliva as such, has not been studied enough to give a clear picture.

Fosdick (1949) emphasizes that the plaque formation is a natural one and that the plaque may also be looked upon as a protection against decalcification of the enamel from acid substances in foods and drinks. The freshly deposited plaque, not infected with acidogenic bacteria, has a very high buffer capacity and may buffer weak acids introduced into the mouth as well as acids produced from weak sugar solutions. The old and infected plaque, however, has a much lower buffer capacity. Fosdick states that both plaques are relatively impermeable to weak sugar solutions but when strong sugar solutions (50 per cent) are deposited the osmotic pressure may be increased from the normal six atmospheres to 60. "Under this terrific pressure sugar and ions are drawn deep into the plaque and perhaps even into faults of the tooth." In the infected plaque, acid is formed in such quantities that the buffer capacity is insufficient to take care of the acid.

Many details of the constituents of the plaque need further study. Differences in the characteristics of the plaque may to a certain extent explain why the enamel is not always attacked beneath the plaque.

The Saliva

The literature on saliva in connection with caries is voluminous. Ericson (1949) in his dissertation on Enamel-Apatite Solubility has given in tabulated form a clear survey of the reported relation between some of the most important factors in the saliva and dental caries. It is of course natural that the saliva, as the fluid constantly bathing the teeth, has caught the interest of the caries researchers. The saliva in this respect may be considered from the standpoint of physical, chemical and antimicrobic properties.

It would be logical to conceive that a large secretion of saliva would be beneficial to the teeth. Very low secretion, as in xerostomia, has been observed to lead to extensive caries in man (Losch and Weisberger (1940); Zaus and Teuscher (1940)). Rapid loss of enamel in rats after removal of salivary glands was observed by Cheyne (1939). Trimble, Etterington and Losch (1938) have found an inverse relationship between rate of secretion and the amount of caries, whereas most of the investigators have found no statistically significant relationship (Karshan (1942)). Ericson (1949) did find a higher rate of flow in caries-free children than in caries-susceptible, but the number of cases is rather small.

The reaction of the saliva: Reports in the literature may show close relationship or no relationship between the pH of resting or stimulated saliva and the caries picture.

The more thoroughly conducted investigations of recent years, however, lead to the conclusion that no direct relationship can be found in children.

It has been shown before, and thoroughly stressed by Schmidt-Nielsen *et al.* (1946), that the most minute precautions have to be taken in measuring the pH of the saliva because it is so liable to be changed both by mechanical and by psychic irritants. The pH of saliva runs normally between 6.4 and 7.2. Since the pH has to drop to 6 or below to attack the enamel, the saliva *per se* cannot be directly connected with the ordinary decalcification of the enamel.

The consistency of saliva has been claimed to play a role in the development of enamel caries (Pickerill). The more viscous saliva was held to give a rather tough and sticky plaque, easily catching the carbohydrate and thus being a factor in a high acid production. This property of the saliva, however, is so intimately connected with the amount of saliva secreted that it is difficult to separate the two factors.

Chemical properties: Numerous chemical analyses of the saliva have been performed in order to clarify the importance of the different substances in regard to caries. Up to this date, however, no full agreement exists. Among the different inorganic components of saliva, the interest has been centered mostly around calcium and phosphorus. Karshan's (1942) reports on significantly higher values for calcium and phosphorus in caries-free persons compared with highly caries-active persons have not been confirmed by investigators of recent years, for example by Becks and Wainwright (1946). As strongly stressed by Schmidt-Nielsen (1946) and later by Ericson (1949) the concentration of the calcium and phosphorus ions is not of such great importance in relation to the dissolution of the enamel as is the concentration of the hydroxyapatite which constitutes the inorganic part of the enamel. In order to calculate the concentration of the apatite the calcium, inorganic phosphorus, pH, and ionic strength of the saliva have to be found. Compared with the generally accepted critical pH point of around 5 for the dissolution of the enamel, Ericson estimates the pH for enamel dissolution in saliva to lie between 5.5 and 6.5.

The buffering capacity of saliva acts chiefly through its phosphates and bicarbonates of sodium and potassium and through the proteins. An increased buffering capacity in caries-resistant individuals has been found by many investigators: Pickerill (1912); Fosdick, Campaigne, and Fancher (1934); Dreizen, Mann, Cline, and Spies (1946); and Ericson (1949). Pickerill found a higher neutralizing power after vigorous chewing and considered this of positive value for the preservation of the teeth. A physiologic basis for this increase has been given by discovery of the carbonic anhydrase in the saliva and salivary glands by Sand (1949). This anhydrase is necessary in the production of bicarbonate. The CO_2 produced in increased amount in the glands by vigorous chewing is readily converted by the anhydrase to carbonic acid. The carbonic acid combines with alkali to form bicarbonate. As a consequence of this, the amount of free CO_2 will be relatively constant, whereas the amount of bicarbonate, the pH, and the neutralizing power of the saliva increase. According to Pickerill, the consumption of foods and drinks containing weak organic acids has a similar effect on the neutralizing power. This finding has not been corroborated by Sand. The low caries frequency in people living on a more primitive diet, usually more hard and tough, may be ascribed partly to an enforced chewing, or to a diet containing weak organic acids (Wallace (1948)).

The higher buffering capacity of saliva in malnourished children and adults suffering from vitamin B complex deficiency than in well nourished children, reported by Dreizen, Mann, Cline and Spies (1946), is interesting but difficult to explain.

Antibacterial and antienzymatic action of saliva: Many investigators have found that pooled saliva has an antibacterial action. Most of this work has dealt with *L. acidophilus*, which has been found to be inhibited in growth in saliva from immune persons more than in saliva from caries-susceptible individuals. Hill (1939) points out that pooled saliva may have an anti-action on the microorganisms through the following ways: a) by the amount of saliva, b) by the presence of bacteriolytic substances, c) by the interaction of different kinds of bacteria and d) by the presence of substances which destroy or inhibit growth or reduce pathogenicity of organisms. One of these factors, (b), probably is identical with the lysozyme also found in tears and blood.

Kesel, O'Donnell, Kirch and Wach (1947) reported that filtrates of 8-day-old cultures of *Aerobacter aerogenes* inhibit growth of *L. acidophilus*. This was confirmed by White and Hill (1949), who also found that filtrates of some cultures of *L. acidophilus* inhibit the growth of *A. aerogenes*. They likewise found that viable *L. acidophilus* had an inhibiting effect on *A. aerogenes*, but not vice versa. The antagonistic reactions between organisms were influenced by the pH of the medium.

Fõyn and Hobaek (1947) found that a mixture of sucrose and saliva from caries-immune persons did not drop in pH as did a mixture of sucrose and saliva from caries-susceptible individuals but, on the contrary, increased in pH to a certain extent. They state: "There exists an inhibitory principle in saliva, governing the enzymatic breakdown of carbohydrates. This inhibitory principle is weakened in periods of reduced health."

Not very much is known of the saliva as a whole and of its various components in regard to the preservation of the teeth. There is, however, strong evidence for an increased acid-neutralizing power by caries-resistant people compared with others. This may be one of the factors determining whether or not caries will develop beneath the plaque. However, the plaque may be of such a consistency that the buffer substances will have difficulty in reaching the place of acid production and the tooth surface. The location of the plaque likewise will play a role. On the proximal surfaces and in deep fissures there is a very slow circulation of the saliva.

A relatively high content of apatite salt in the saliva may counteract the dissolving power of the acid on the enamel apatite. The old observation by Head (1910) of a remineralization of a partly demineralized enamel *in vitro*, thoroughly studied and substantiated by Andresen (1926) is also a function of the optimal composition of the saliva. The remineralization of the enamel will be dealt with later.

The State of the Tooth at Eruption

A statement in the section on Oral Environment and Dental Caries reads: "The only plausible explanation of failure of some teeth or areas of teeth to be attacked in an *in vitro* experiment is a difference in surface substance between different teeth and different parts of the same tooth. However, if there is indeed real structure of dental enamel surfaces that confers resistance to caries, the origin is *in vivo*. The parts played by pre- and posteruptive conditions can only be surmised, but surface conditions of enamel are obviously subject to alteration in either phase of tooth history." This statement may serve as a basis for discussion.

The resistance of the enamel surface may be either of preeruptive, posteruptive or pre-and posteruptive nature. W. D. Miller observed the big difference in the liability of various teeth to be attacked by acids. As a consequence of this he stated a number of predisposing factors. Among these he emphasized the surface of the enamel as well as the inner structure of the tooth. The great resistance of Nasmyth's membrane has often been stressed. Some of the difference of tooth resistance may be attributed

to a possible difference in chemical and physical properties of this cuticle. However, according to the opinion of most investigators, this cuticle is partly worn off soon after the tooth has come into function. (This question will be further discussed later.) So far as is known, no one has been able to describe the difference in resistance of the cuticle *per se*.

The observed difference in resistance of teeth has without doubt also to be attributed to the tooth structure proper, and especially to the surface texture. However, it is a common observation of all students of *in vitro* caries studies to find the decalcification first in spots deprived of the membrane.

Pickerill's findings of more easily penetrable enamel in young teeth than in older teeth, even from the same individual, are substantiated by many subsequent investigators. Teeth of the same age also showed great variation in permeability. The change in permeability with advancing age he attributed to a secondary mineralization of the outer part of the enamel from the saliva. Pickerill also stressed the importance of the roughness of the enamel surface in the liability of the teeth to decay. The smoother the surface of the enamel, the more easily is the enamel kept free from plaque. However, Pickerill did not make any direct comparative caries studies in relation to his physical and chemical studies of the enamel.

Direct studies of the relationship of enamel surface and enamel structure as a whole to caries frequency have been numerous during the last three decades, as will be seen in the section Nutrition and Dental Caries.

Enamel hypoplasia and dental caries: The most extensive studies in this field have been carried out by May Mellanby (1936). In the term "hypoplasia" she included, in addition to macroscopic, all grades of preeruptive defects down to and including the microscopic defects. According to the old terminology the percentage of hypoplastic teeth was found to be around 10 for the permanent and 2 for the temporary teeth. By extension of the term hypoplasia, she found about 80 to 90 per cent of teeth in English children hypoplastic. Mellanby found a very close relationship between hypoplastic teeth and their liability to dental caries. Using Mellanby's method, Bibby *et al.* (1934) obtained essentially the same results.

Even by dividing the teeth as hypoplastic and nonhypoplastic according to the old terminology, a correlation has been found between hypoplasia and dental caries. Calteux (1934) for example reported from Luxembourg that among 1853 children 9–14 years old showing no enamel hypoplasia, 9.18 per cent were caries-free, whereas of 163 children showing hypoplasia only 3.68 per cent were caries-free. The per cent of carious and extracted teeth among the hypoplasia-free children was 19 and among the others 23.7. Even the 6-year molars, showing the highest caries frequency of all teeth, showed the same relationship.

As will be seen from the chapter on Nutrition and Dental Caries, many investigators report the finding of a correlation between good structure of the tooth and its resistance to caries. But it will also be seen that many report no such correlation: Taylor and Day (1939, 1940); Day (1944); Rosenbaum and Mansbach (1944).

Enamel hypoplasia is mainly connected with rickets. The extensive studies of E. and M. Mellanby have chiefly explored this relationship both in experimental studies in animals and by clinical studies in children. The connection between rickets, hypoplasia and caries has chiefly been studied in children many years after the typical clinical picture of rickets has disappeared. The diagnosing of rickets may then be uncertain. Rather few such studies have been undertaken on the basis of case histories or X-ray evidence of the disease. The most reliable studies on this subject are probably

those of Hess and Abramson (1931) and Hess, Abramson and Lewis (1934). These children have been observed from infancy up to 5 or 9 years of age. A slight increase in dental caries in the temporary teeth was observed in the rachitic children compared with the non-rachitic. Another study by Eliot and coworkers (1933, 1934) of 451 children 5–12 years old showed hypoplastic teeth in 34 per cent of the "moderate or severe" rachitic group, compared with 4 per cent in the rickets-free group. Dental caries was observed in 63 per cent of the rachitic children and in 43 per cent of the rickets-free children. The authors do not, however, take it as an absolute causal relationship between hypoplasia and caries, in spite of the fact that the hypoplastic teeth were more often carious than were the normal teeth.

Authors having found a correlation between rickets and dental caries as well as authors having found no such correlation point to the possibility that factors may come in later, that is posteruptively, and change the picture. The question then remains as to whether it is possible that the tooth after eruption can be influenced in such a way as to change its liability to dental caries.

The Reaction of the Erupted Tooth

As has been pointed out in other reviews, the tooth during its development is a very sensitive tissue, recording changes in the metabolism of the organism or in systemic conditions. It will be seen in the section on Nutrition and Pathology that most of the authors, especially those in earlier days, consider the erupted tooth as being of such a nature that no change can take place in the interior of the enamel after the ameloblasts once have disappeared. This view was based chiefly on the earlier studies of the enamel indicating that it contained practically no organic material to convey impulses or materials from within or without the tooth. This view held by the so-called localists was opposed by a small group, the vitalists, who had the idea that the tooth was influenced by the general metabolism so long as it was connected with the bloodstream through its living pulp. A condition for such an effect is the possible permeability of the enamel.

Pickerill was one of the first to demonstrate that aqueous solution of silver nitrate was able to penetrate from the outside into the enamel of extracted human teeth. The teeth of the youngest posteruptive age were more penetrable than older ones, even from the same individual. Such findings have been confirmed by others both on extracted teeth and on teeth *in situ* (Türkheim (1922), Wannenmacher (1937)). However, the validity of this test has been questioned, on the ground that the nitric acid from the solution may have partly decalcified the enamel. The observations on staining of the inner part of enamel *in situ* made by Wannenmacher (red stain) and Strauss and Föcheler (1939) (tobacco stain) prove clearly that the enamel is penetrable *in vivo* from the outside. The last mentioned authors have demonstrated, in ground sections of teeth from chronic smokers, tobacco stain in the enamel as far as the enamel-dentin border, and in some places into the dentin. The enamel surface did not show decalcification or cracks. When such were present, the dentin was discolored to the pulp.

Berggren (1947) studied the possibility that bacterial toxins, placed on the surface of the tooth, could penetrate dog enamel. Tetanus toxin of high titre up to 1:200,000 was used in capsules cemented on the teeth and left there for up to 40 hours. After extraction of the tooth, the pulp suspension was inoculated into mice. When the toxin was applied in ordinary salt solution, no reaction occurred in the mice, but when the toxin was given in a 40–60 per cent glucose solution, the pulp suspension inoculated into mice caused death. Unfortunately, too few cases have been used in these experiments to make them wholly convincing.

However, if this should be true, it is valuable information both as to the question of the permeability of the living enamel and to the role of sugar in the caries picture.

Of greatest interest in the matter of posteruptive changes in the enamel is whether the enamel is permeable from the inside. Fish (1932), Bodecker (1941) and Berggren (1947), by placing dry methylene blue in artificially prepared cavities in human teeth *in situ*, demonstrated that the dye dissolves, penetrates to the pulp, and secondarily passes through other dentinal canals centrifugally into the enamel. Sometimes the dye reaches the surface of the enamel. All agree that the pathway of the dye through the enamel is by way of the less mineralized organic structures such as the prism sheaths, lamellae and tufts. That younger teeth are more easily stained than older teeth is considered to be the result of an increasing keratinization of the organic material. However, the findings of Karlström (1931) of an increased hardness of the enamel with increased tooth age would suggest a deposition of new material instead of only a further keratinization.

The use of radioactive isotopes (Berggren, 1947) in studying the permeability of enamel has thrown still further light on this problem. By injection or by oral administration of radioactive phosphorus (P^{32}) or radioactive sodium (Na^{24}) in animals and in human beings, the radioactive material has been found both in the dentin and in the enamel. Here the passage may be both from the pulp and from the saliva. By capping the teeth, the saliva has been excluded, and even in these cases the isotopes have been demonstrated to be present in the outermost part of the human enamel. By reversing the procedure, cementing onto the tooth a capsule filled with the radioactive material and giving none internally, the isotopes have been recovered from the dentin. The uptake from the outside is larger after a slight etching of the surface (Pedersen and Schmidt-Nielsen, 1942).

After removal of the pulp in dogs' teeth, not more than a quarter of the usual amount of radioactive phosphorus per gram of tooth substance could be found (Gilda, McCauley and Johansson (1943)). The amount in the crown was one-eighth and in the root about one-third of that in teeth with living pulps. In pulpless teeth the P^{32} passes the cementum.

These studies using labeled atoms of the natural salts contained in the teeth give strong evidence of the penetrability of the enamel and dentin by elements normally present in the fluids reaching the teeth from inside as well as from outside.

There is, however, a fundamental difference in the pathway of the organic dyes used and the phosphorus isotopes. As pointed out earlier, the former reach the enamel by way of the organic structures, whereas the P^{32} penetrates through the crystal lattice itself. Whether or not a further calcification takes place, or only an exchange of atoms occurs, has been thoroughly discussed. According to Falkenheim, Neumann and Hodge (1947) it is only a replacement of atoms on the surface of the ultra-microscopic crystals of the hydroxyapatites. As is well known, the apatites, including the apatite of the enamel, are easily changeable by such replacements. The fluorine studies to be mentioned later, however, point to the possibility that a real mineralization may take place. The radioactive sodium is found chiefly in the organic material (dentin). But common to both the dyes and the isotopes is the fact that the young dental material takes up more than does the old and well calcified material.

Positive results have also been obtained by using calcium (Armstrong, 1945) and iodine (Bartelstone, Mandal, Oshry and Seidlin, 1947). Tracer iodine was injected intraperitoneally in cats and rats by the latter authors; radioautographs of the ex-

tracted canine teeth showed clearly the "circulation" of a fluid in the tooth, since the iodine was present in the enamel as well as in the dentin and cementum. Later Bartelstone (1949) reported on the use of radioactive iodine as a tracer in the study of the physiology of human teeth: "In the enamel there was marked uptake at the surface, in the subsurface enamel, on the surface and in the subsurface of abraded enamel, in calcium-poor areas, and in areas of marked organic material, both surface and subsurface. In dentin there was a significant increase over basal uptake in areas of metamorphosed and secondary dentin as well as carious, decalcified, and interglobular dentin." These findings were demonstrated in radioautographs. Iodine was also present in capped teeth.

The topical application of *fluoride* to teeth in animals and man has also widened our concept of the reaction of the enamel after tooth eruption. As will be seen in the section on Fluorine and Dental Caries, this subject has been studied extensively. Gerould (1945) has found by using X-ray and electron diffraction methods that human enamel contained calcium fluoride after being polished and immersed in a 4 per cent solution of NaF for a month. As shown by the electron microscope, such enamel was more acid resistant. Holager and Syrrist (1948) have also demonstrated calcium fluoride in a tooth treated *in situ* with a solution of the same strength. The total length of treatment was 70 minutes. This tooth was cleaned in an ordinary way with pumice and brush. Electron microscope pictures of fluorine-treated enamel (also *in situ*) suggest that the outer layer of the enamel became more acid resistant (dense) (Syrrist, 1949) and as a consequence should be less permeable. Holager and Syrrist (1948) have treated enamel with 2 per cent NaF *in situ* for five minutes once a week during 3 to 4 weeks, extracted the tooth, and then put it into glucose-saliva solution for testing the resistance to acid; when put into carbol fuchsin the dye in some instances penetrated much further into the enamel of the control tooth, not fluorine treated, than into the experimental tooth.

By immersing teeth in sodium acetate of pH 4, Brudevold (1948) found that the unerupted surface enamel was more soluble than the erupted enamel. Enamel from persons 20–40 years old was less soluble than enamel from persons under 20 years. Again it was demonstrated that the enamel of erupted teeth may change its physical and chemical character, becoming less permeable and more resistant to acid.

Hord and Ellis (1949) have found increased microhardness of the enamel surface of dogs' teeth after topical application of fluorides. The same authors (1950) have reported on increased fluorine content of dogs' teeth after similar treatment.

These experiments on the effect of fluoride on the enamel might suggest that not only does an exchange of atoms take place, but an even further mineralization really occurs in the enamel as it does in dentin after tooth eruption, but to a much less degree.

The possibility of remineralizing a slightly decalcified enamel must also be mentioned in this connection. The old findings of Head (1910), of restoring a partly decalcified enamel specimen by keeping it for a long time in fresh saliva, have been extensively studied by Andresen (1926). He and others after him have claimed to be able to reharden the enamel in cases of caries incipience by using a remineralizing salt (Andresen) or paste (Wilson).

The greater penetration of isotopes through the partly etched enamel surface (Pedersen and Schmidt-Nielsen) and the effect on the enamel solubility of applying fluorine in a faintly acid solution (Bibby, 1947, Phillips and Muhler, 1947) seem to form a basis for the concept of the pos-

sibility of remineralization of the enamel. Certainly it is common for the observant practitioner to find that a posteruptive faint chalky spot on the enamel may disappear after some weeks without any other treatment than keeping the enamel so clean that the physiological saliva may come into contact with the partly decalcified enamel.

The findings described by Wolf and Neuwirt (1941, 1948) should also be mentioned. A few days after faint etching of the enamel of vital or pulpless teeth in man, these authors were able to demonstrate in the microscope, by using the relief adhesion method, a layer of a homogeneous calcium salt derived from the saliva covering the treated area. Without proving it, they suggested that such deposition might also take place not only on the surface of the enamel, but also in deeper layers poorly mineralized or partly decalcified.

As has been shown, the enamel after tooth eruption is capable of undergoing various changes. These may be brought about from the inside via the pulp or from the outside via the saliva. These reactions may be considered as being either purely chemical or physico-chemical. Some of the changes take place in both vital and pulpless teeth. The reactions in the enamel in pulpless teeth cannot, of course, be taken as vital, since the connection between the enamel cap and the vital cementum of the root is probably too meager to convey any materials. Bodecker and Lefkowitz (1938) have studied the permeability of pulpless teeth and conclude: "1. Vital teeth are less permeable than teeth immediately after pulp extirpation. 2. The permeability status of pulpless teeth changes. Immediately after pulp extirpation, the permeability of the tooth is highest, reducing slowly thereafter." The immediately increased permeability after pulp removal should be in accordance with general physiological laws.

Can the physiological changes demonstrated in the enamel of vital teeth be regarded as responses to general metabolic processes? Do we find any anatomic-physiological basis for such a conception? In the section Pathology of Dental Caries, the anatomy of the dentin and enamel is thoroughly described. Through the dentin body millions of dentinal tubules run from the pulp outwards to the enamel-dentin border. In each tube is found the so-called Tomes "fiber." The old term "fiber," meaning a solid "cytoplasmic extension of the odontoblast" (Chase, 1948), is probably not correct. The structure is probably a tube containing a fluid, the dental lymph. Between this tube and the walls of the dentinal canal is a space, also carrying a lymph. The concept of the Tomes fiber as a tube seems to be corroborated by electron microscopy (Syrrist, 1949, Scott, Kaplan and Wyckoff, 1949). The existence of a circulating fluid in the dentin has been proved by the fact that when dry stain is sealed into a cavity, not only the dentinal cone between the cavity and the pulp, but also other parts of the tooth not in direct communication with the exposed dentinal canals are stained. The afferent passage, however, might be explained as a diffusion, but the secondary staining, the efferent passage, can be explained on no other basis than a stream of fluid passing from the pulp outwards. This fluid originates from the pulp. As pointed out before, the secondary staining is not confined to the dentin, but may reach almost the surface of the enamel. In the enamel, however, no such channels are present as those in the dentin. In the enamel, therefore, the lamellae, tufts, spindles and prism sheaths have been considered to act as the conveyers of the dyes. Of these organic structures, the prism sheaths and the lamellae may form continuous passages from the dentin outward to the enamel surface, whereas the tufts and spindles are found only in the innermost part of the enamel. All these structures, however, may be in organic connection with the dentin. According to Gustafson (1945): "The tufts are characterized by the fact

that the prism sheaths in particular show a low degree of mineralization; they are also more permeable to fluids than the sheaths in other areas.... The lamellae are abnormally developed tufts with the same structural properties as the tufts." Whether these structures are solid bands or hollow in some sense is not known at present. However, Gustafson writes: "We may assume that the tufts are connected both with the metabolism and with the static build of the tooth. By means of the tufts the enamel is traversed by regular 'tubes' (the prism sheaths).... Their low mineralization opens possibilities of increased metabolism via the tufts, but this metabolism differs only as to degree, not in quality, from that found in the enamel as a whole." Forshufvud (1943) makes this assumption on the basis of experiments on injection of gelatin and different dyes into the blood vessels of a human fetus and animals: "... that closed channels are existing which transport blood plasma, ultra capillary reticular fibrils, and which establish a direct contact between the blood plasma of the blood vessels and the elements of the tissues which are irrigated by nutritive fluid."

The supposition of a fluid in the mature enamel may be strengthened by the finding of labeled iodine in the mature enamel after ingestion of radioactive iodine, as mentioned before.

The phosphorus exchange demonstrated by the use of isotopes need not of course be taken as an expression of real metabolic processes. Such phenomena can also be observed in fully inert materials. They may be of mere physico-chemical character.

One reference in the section, Pathology of Dental Caries, stating that the human enamel is not permeable from the outside may not be tenable. Strong evidence for the permeability from the outside is given by the use of isotopes and by topical application of NaF, as well as by the use of dyes. As has been described before, phosphorus isotopes have been demonstrated in the dentin from teeth covered *in situ* with a cap filled with radioactive phosphorus. The only passage for these atoms into the dentin was through the enamel from the surface. After topical application of sodium fluoride the enamel resistance is increased as shown both clinically and chemically. Such fluoride-treated teeth seem also to show a more dense and homogeneous outer enamel layer by electron-microscopy (Syrrist, 1949). Syrrist has also reported increased fluorine content of the enamel after topical application of 2 per cent NaF on teeth *in situ*. *Thus it cannot be questioned that materials may enter the enamel after tooth eruption. But no evidence of any opposite process taking place from the inside has been brought forth.*

The dental picture in ostitis fibrosa generalisata (hyperfunction of the parathyroid glands) does not confirm the idea of a close connection between the mature tooth and the systemic condition. In such cases with a pronounced decalcification of all the bones Albright, Aub and Bauer (1934) did not find any changes in the patients' teeth by X-ray examinations. This has later been confirmed experimentally on rats by Weinmann and Schour (1945). From these facts the last mentioned authors write: "Osteoclasis in hyperparathyroidism should be regarded as a pathologic exaggeration of the physiologic process of bone resorption which is an integral factor in bone development and bone growth. Resorption processes are entirely lacking in the development and growth of the tooth, with exception of the shedding of deciduous teeth. This might explain the freedom of enamel, dentin and cementum from resorption in hyperparathyroidism."

Vital reactions in the enamel in early enamel caries were supposed to have been demonstrated by Mummery (1926). As to being non-stainable, he writes of the translucent zone: "These facts point to the probability of the translucent zone being an area of denser calcification than the rest

of the enamel, and closely corresponding to the similar evidences of the nature of the translucent zone in the dentin.... Whatever this alteration in the enamel may be, there appears to be a distinct change of structure which is not due to decalcification and is intimately associated with the decayed area, apparently indicating that enamel cannot be a dead inert substance, but is capable of reaction to stimuli, and therefore, like other tissues of the body, endowed with vitality."

These findings of Mummery have been corroborated and interpreted in the same way by many others, for instance by Beust (1931) and Applebaum (1938). Applebaum showed by using Grenz-rays that the translucent zone is not decalcified.

Nishimura (1926), demonstrating the same transparent zone, interpreted this as being a hypermineralization caused by inorganic salts diffusing from the somewhat decalcified area superficial to the transparent zone. According to his interpretation, this zone should have no connection with vital processes. This view was later corroborated by Darling (1943) and by Gottlieb (1943) demonstrating the same microscopic zone by artificial action of acids on the extracted tooth. As far as may be seen from illustrations in Mummery's, Nishimura's, Darling's and Gottlieb's publications, no differences exist in these zones. It is, therefore, doubtful that this zone is caused by vital processes. As far as the present author knows, no one has been able to demonstrate beyond doubt any chemical or histologic evidence of vital reactions in the enamel by caries lesions.

Even if changes occurring in the erupted enamel should be only an increasing keratinization and of non-metabolic nature, as stated by Leicester (1949), this process also tends to make the enamel more resistant. Bibby and van Huysen (1933) *et al.* have demonstrated the great resistance of the enamel keratin to bacterial action. The stressing of the importance of the keratin is also in accordance with the statements in the review of Pathology and Dental Caries: "Well-keratinized impermeable enamel lamellae prevent the most dangerous type of caries, i.e., rapid penetration of the enamel through minute channels, resulting in the deep involvement of the dentin.... Well-keratinized enamel cuticle may retard formation of the initial caries lesion.... Well-keratinized enamel rod sheaths and tufts may reduce rate of carious destruction of enamel after initiation of the lesion."

Concerning the enamel cuticle, it is of interest to remember that this cuticle, Nasmyth's membrane, probably does not exist very long on many places after the tooth has come into function. Thus Chase (1926) from his studies of the membrane states: "Nasmyth's membrane is a vestigial, transitory structure, in the majority of cases destroyed during the early stages of eruption, and probably never found in any considerable time after eruption is completed. Since this is so, it is absurd to attempt to ascribe any function to it." This, however, is not in agreement with the results of newer investigations. Darling (1943) from his extensive studies concludes: "The enamel cuticle is present on the enamel surface of all erupted teeth. It is absent only from those areas subject to natural or artificial attrition or abrasion." Scott, Kaplan and Wyckoff (1949) may be said to have a similar opinion. They write: "The fact that recently erupted teeth are completely covered with cuticle, and the persistence of this cuticle over large areas of the tooth surface of older individuals...." This again is not in agreement with the findings of Syrrist (1949). In his electron microscopy studies of fluoride-treated enamel of the buccal surface of the first upper bicuspids, from 11–13 year old children, he was unable to see the membrane. He suggests the possibility of the membrane having been destroyed by the vigorous brushing of the teeth before the fluoride application. The number of cases studied was very small.

Forshufvud (1948) differs totally from most other investigators in his opinion on the nature and presence of Nasmyth's membrane. He writes: "The so-called enamel cuticle is a typical basement membrane that renews itself continuously with the wear of the teeth."

In spite of the extensive studies of tooth surfaces by Scott *et al.*, there still seems to be uncertainty about the persistence of the enamel cuticle.

As far as the changes in the dentin are concerned, these have to be taken as of true metabolic origin as pointed out in the section Pathology of Dental Caries. Nobody who has studied the subject has denied this. The translucency of the dentin, proved to be caused by a hypermineralization, is a true vital process depending upon the vital pulp. The opaque dentin being sclerosed or not (metamorphosed dentin) is also to be taken as a result of vital processes. The nonsclerosed opaque dentin is probably caused by gas formation. These processes, occurring physiologically, tend to decrease the permeability of the dentin and thus help to protect the pulp against external irritants. No change other than a temporary increase and a later decrease in permeability can be found in the dentin after pulp removal. This decreased permeability is caused by degenerative processes occurring in the Tomes "fiber," or in the dentinal tube.

The reactions in the dentin of a vital tooth to external irritants, as for instance those involved in dental caries (translucency, opacity, secondary dentin formation) are all to be regarded as vital processes. That all these processes retard the caries invasion has been proved without doubt. But whether or not the amount and quality of the alterations are dependent on the state of metabolism has to be further substantiated. As pointed out in the review Nutrition and Dental Caries, M. Mellanby has shown that the amount of secondary dentin in puppies is dependent on the vitamin D in the diet, and that the character of the secondary dentin in children's teeth bears a relationship to the caries susceptibility. It would be natural to conceive that the sclerosis of the dentin as well as the formation of dense secondary dentin is a function of the nutritional state of the individual.

Summary

Dentin: There can be no doubt that vital reactions take place in the dentin of erupted teeth with vital pulps. The amount and quality of secondary dentin have been shown in dogs to be dependent on the quality of diet, especially as far as vitamin D is concerned. Whether or not the same is true in man must be further studied. It is unknown whether sclerotization and opacitization of the dentin are influenced by the systemic condition as a whole. Grave systemic disturbances including those leading to profound decalcification of the bones have not been demonstrated to affect the picture of the tooth via the pulp. But in spite of such negative findings, the possibility still exists. The changes may be of such a nature that we do not have the methods today to detect them, but nevertheless they may make the dentin more easily affected by caries. The dentin is a vital organ whose resistance is continually increasing through the pulp with advancing age under physiological conditions. The clinical observation (assumption) of intermittent break of this resistance during and after reduced bodily resistance must be further elucidated.

Enamel: For the start of the carious process the enamel is, of course, of the greatest interest. But as to the question of vital processes, the pathway must be through the dentin. No chemical or histological changes have been observed which may be considered as evidence of catabolic activity occurring via the pulp of either carious or non-carious teeth. Nevertheless, the amount, nature and arrangement of the different organic structures of the enamel,

together with the organic connections between the vital dentin and enamel, warrant the characterization of the enamel in pulp-living teeth as *vital*. The studies of Sognnaes (1949) on the organic elements of the enamel have thrown still more light on the importance of this structure. The exchange of phosphorus and of calcium and disposition of iodine in experiments with radioactive isotopes given *per os* or injected may also lead in the same direction, although such exchange takes place, but to a lesser degree, when isotopes are deposited only on the surface of the enamel. As stated for the dentin, no changes of negative character have been found to occur in enamel via the pulp in grave systemic disorders. The methods used for clarifying the picture, however, may have been too crude. More work has to be done before the question can be definitely answered.

If the clinical observation (assumption) of increased susceptibility to enamel caries during or after illnesses is true, still another question arises in whether this is caused by the pathway of the pulp or by the saliva. In either case, the origin may be characterized as metabolic.

The enamel spindles are, according to Gustafson, "continuations of the dentinal tubes" into the inner part of the enamel, and "within the spindles we find thick thread formations which under normal conditions probably fill up the whole spindle." When the pulp is living, the dentinal tubes contain the Tomes "fiber" which is a cell projection, that is, a living protoplasm. Thus, at least a part of the enamel contains such living material. Nowhere else in the human body have living cell projections been found to be placed, or left behind, in a "dead" organ.

In order to characterize erupted enamel as a vital tissue, metabolism, which includes anabolism and catabolism, must be demonstrated. The development of the translucent zone in early enamel caries cannot be taken as a vital reaction, as the same zone has been described as a result of acid action on extracted teeth. Any true repair of damaged enamel has not yet been demonstrated. The findings of Forshufvud (1948) must be tested by further experiments before being seriously considered. He states: "Dental enamel is a vital tissue.... The reticular fibers of dentin and enamel originate from the adventitia of the blood capillaries of the pulp.... The reticular fibers are ultra capillaries, which transport blood plasma into the very smallest unit of tissue." On this basis Forshufvud made the following experiment: A piece of a specially treated ox tooth was placed within a cleaned cavity in a living tooth; after 8 days reticular fibers were found in the graft which were connected to the reticular fibers of the tooth substance by means of a basement membrane. Seven weeks after such "treatment," microscopic sections showed that the dead ox-graft placed in a human tooth was occupied by newly formed reticular fibers, and a strong reticular enamel stroma arose. Forshufvud writes: "Clear scientific findings have now definitely proved that the enamel is a nourished tissue capable of developing and healing. It is no longer absurd to imagine biologic treatment of an injury in the enamel."

The presence of a fluid in the enamel has been proved without doubt by the finding of iodine isotopes throughout the enamel after injection of such isotopes. But neither the mere "circulation" of fluid nor the exchange of atoms proves that the tissue is capable of metabolizing. The final proof that any respirations can be detected in the enamel is not yet accomplished. In this connection it is of the greatest interest to our question to know that Leinfelder at the ophthalmological department of the State University of Iowa (1949) has been able to prove that the nucleus of the eye-lens, formerly characterized as an inert substance, is a respiring tissue and thus a living one. After its formation the nucleus has no direct connection

with the cells from which it was formed, as is the case with the enamel.

As a conclusion, the statement of Leicester (1949) seems fairly logical: "... it is clear that the erupted tooth, although less sensitive to external influences than when it was developing, is none the less far from an inert and unreactive structure. It is capable of undergoing physiological changes from within and of reacting with a wide variety of substances which may reach it either from within or without. The modifications which thus can be produced may be of great significance for the subsequent health or injury of the organ."

Thus the enamel of the erupted tooth with living pulp may be considered as a living structure capable of undergoing certain physiological changes from within and from without. The changes so far demonstrated, however, are not of the nature of metabolic processes in the ordinary sense.

LOW CARIES FREQUENCY IN PRIMITIVE PEOPLE AND IN PEOPLE LIVING TOTALLY OR PARTLY ON NON-INDUSTRIALIZED FOODS

In other reviews of this monograph, there are many reports on dental studies of populations experiencing caries frequencies lower than the average and showing reduction of the usual rate under various circumstances. The causes for such differences have been interpreted by the authors in various ways, and it seems difficult to get a clear picture of the situation.

It is evident that primitive peoples have a decidedly lower caries rate than the civilized population. This is true irrespective of geographical differences. But amongst the primitive tribes themselves there may also be variations. In the section on Nutrition and Dental Caries, data are reported together with a discussion of the possible causes of the variations in the occurrence of dental caries in different populations. The data from the African tribe Masai (Orr and Gilks, 1931) and from the primitive Greenlanders (Pedersen 1938, 1947, Baarregaard 1949) showing that only 1.6 to 4.6 per cent of the population have caries, stand as a challenge to the highly civilized population of the U. S. A. and most of the countries of Europe, with 95 to more than 99 per cent of caries-affected persons. But as soon as the primitive life has been touched by civilization in some way, the dental picture changes. This is demonstrated in the most clear-cut way in Greenland. The figures from the trading stations in East Greenland are for the females 51.1 per cent of caries-affected persons compared with the 4.3 per cent from the primitive places.

The most striking difference between primitive and civilized peoples is found in the food, in the kinds of food, and especially in the form in which it is eaten. Common to all primitive people is the fact that they eat food in a more natural or unprocessed state than do the industrialized peoples. Some time after primitive man replaces part of his native foods with those from industry brought to him by civilized man, the caries rate rises. P. O. Pedersen (1946), who has made the most valuable and extensive studies among the Greenlanders, writes: "Natives moving from isolated villages to trading stations will get caries within a short time, while caries susceptibility will decrease in those moving from trading stations to villages, and caries already present will become arrested." This author reports the change in imported foods to substitute for the native foods in West Greenland: The average yearly consumption of imported foods was 19.5 kg. per person in 1855–56, 41.9 in 1901–03, and 145 kg. in 1930–33.

The percentages of the population affected with caries in the main trading stations and outposts of West Greenland for the years 1914, 1924, 1935, 1945, according to Baarregaard (1949) were: 58, 63, 88 and 95 for

the main trading stations and 10, 23, 39 and 40 for the outposts.

By dental examination of 436 persons on the Faeroe Isles, Holst and Kaaber (1940) found a much higher caries frequency in people living in towns than in people living in isolated and more primitive places out in the country.

The difference between the primitive and the civilized diets is chiefly reflected in the modern processing of cereals and the introduction of refined sugar and all the different products made from it. Whether the diet of the primitive man consists mainly of fat and protein as that of the Eskimos, or mainly of cereals and vegetables as that of the Kikuyu tribe, the caries frequency is low as long as he does not mix his food with the white man's food.

Two neighboring African tribes, the Masai with the very low caries frequency of 1.6 in boys and 3.6 in girls, and the Kikuyu with the somewhat higher figures of 13.7 and 13.1, respectively, might show that the high diet of animal origin consumed by the Masai could be preferable to the vegetarian diet of the Kikuyu. However, since the percentage of rickets was as high as 63 in Kikuyu children compared to practically nil in the Masai children, together with a much higher percentage of other diseases and a poorer physique in the former, factors other than the food *per se* acting directly on the teeth could be responsible for this difference.

The Norwegian expedition of 1937–38 to Tristan da Cunha (Henriksen and Oeding (1940), Sognnaes (1945)) found that not more than 44 per cent of the population showed dental caries, and these as a rule in a very moderate way, with few having active caries. From 40 to 65 per cent of the calories were derived from potatoes and the rest from fish, meat, birds, and small amounts of milk and eggs. The accessible amount of refined carbohydrates, including sugar and sugar products, was very limited. *L. acidophilus* as well as very active caries were found chiefly in women. Sognnaes and Armstrong (1941) suggest that the very low caries frequency on this island might partly be ascribed to the somewhat higher fluoride content of the teeth. The superior dental health in Tristan da Cunha earlier has been attributed solely to the very low consumption of refined carbohydrates, especially of sugar. The drinking water was not responsible for the higher fluoride content of the teeth; this was ascribed to the large amount of fish used.

That the fluoride ingested has to be taken into account in evaluating the possible influence of the diet in relation to dental caries is clearly shown by Malherbe and Ockerse's (1944) investigations from South Africa. Thus, several of the older studies of diet in relation to dental caries must be reinterpreted because of possible fluoride content of the drinking water or fluoride otherwise ingested.

The findings of Höye (1938) and Toverud (1938) of the remarkably low caries prevalence in an isolated Norwegian valley, Setesdal, have been under such revaluation because of probable high fluoride content of the drinking water. Toverud ascribed the low caries frequency, 75 per cent caries-affected individuals of the 680 examined or about 75 per cent of the total population in the age range 3 to 85 years, to the high intake of milk, butter, potatoes, whole meal flour and the very low intake of sugar, sugar products and refined flour. The caries figure among school children 7–14 years of age in Oslo was at the same time 99.8 per cent. Analyses of the fluoride content of the drinking water showed very few figures up to 0.3–0.4 p.p.m. (Syrrist, 1950). In this isolated farm area the teeth were much better among those who persisted with the old habit of living, using almost exclusively what was produced on the farm, than among those who brought a considerable amount from the grocery store. A similar picture is described by Roos (1937) from some isolated valleys in Switzerland. The population,

living almost exclusively on home products and using very little of industrialized food (sugar and sweets), was practically free from dental caries. Subsequent analyses have shown in some places in Goms that the ingestion of fluoride was so high that it might have played a role.

In this connection the dental condition on the sugar plantations is of great interest. It has often been stated that people on such plantations have very little caries and this was taken as evidence for the harmless effect of sugar. However, Ottesen (1946) examined 200 children 8–15 years old on the island of Trinidad. Among 100 children examined in the city Port of Spain, 74 showed dental caries with 4 cavities each. On the largest sugar plantation on the island 93 of the 100 children examined had caries. The average number of cavities in these children was 7. The basal diet, which was not supposed to be a very good one, was about the same in both groups, but the consumption of sugar was probably higher in the plantation group than in the other.

It is a common picture from all over the world that people living on what is usually called a "natural" diet, that is, a diet consisting of unrefined and nonindustrialized foods, have very little tooth decay. This is also in the main in accordance with the historic distribution of this disease (Euler (1939), Christophersen and Pedersen (1939), Brinch and Möller-Christensen (1949)).

Whether the nutritive value of the natural food is responsible for the very low caries frequency, and a deficiency results from the substitution of refined and industrialized food for the natural food; whether the former acts as a directly destructive environmental factor; or whether a combination of these two factors leads to the greatly increased caries frequency, has still to be answered. The last supposition is most likely.

LOW CARIES FREQUENCY IN SPECIAL GROUPS OF CHILDREN

May Mellanby with coworkers in 1924 reported on caries reduction in hospitalized children by increasing vitamin D and minerals and reducing the carbohydrates in the diet. From a long series of similar experiments, coupled with animal experiments and other studies, she concluded (1936) "... that a relatively high vitamin D content of the food can do much to diminish the incidence of the caries if the vitamin is given during the development of the teeth; that a beneficial effect may be obtained if the vitamin is given at a fairly late stage of development; and that even when it is given after the eruption of the teeth, the onset and spread of caries is delayed." In some of her experiments on children, however, it is difficult to draw any definite conclusions because the control group cannot be said to constitute a real control. Such is the case in her studies with addition of treacle to the basal diet, where it is not possible to state whether the low vitamin D or the treacle has given the higher caries figure. The basic diet was also different.

Boyd and Drain (1928) observed that diabetic children having a regular acute caries picture when first entering the hospital, after some months showed arrest of the caries process and practically no development of new cavities. This unusual picture they ascribed to the adequacy of the diet used, which was such as to meet fully the requirements of the growing organism and contained plenty of vitamin- and mineral-rich food. As the relation between protein, carbohydrate and fat was as 7:9:21, the amount of carbohydrates was low, and no extra sugar or sugar products were allowed during or between the meals. Later the ratio between carbohydrates and fat was altered to 7:15:11 without any change in the dental picture (Boyd, 1944). Further

studies of the same authors with other collaborators have confirmed the first observations in a large group of diabetic children as well as in other children in hospitals or in children's institutions.

Toverud, Kjösnes and Toverud (1942) described similar results from Norwegian diabetic children studied during 1935 to 1941; 55 children in age range 2–18½ years when the study started (mean age 12 years) and observed for 1–6½ years (mean 4 years), showed an average of 0.9 new carious surface per year. That the children were not more or less immune to caries will be understood by the fact that they had an average of 25.7 carious surfaces at the first inspection. In the age group 12–16 years there was an average yearly increment of 1.2 carious surfaces. What this really means is made clear by comparing this figure with the increment of 7 carious surfaces from the 6th to the 7th grade (12–13 years) reported from the school dental clinics in Oslo during 1939–1940. Fifty per cent of the diabetic children did not show any new carious lesions. The children were given a diet sufficient in protective foods and calories, corresponding to that prescribed for normal children, but with the difference that sugar, sweets and cookies were excluded. The authors conclude: "The daily use of a sufficient diet combined with a very low content of sugar, sweets and cakes is considered to be the cause of the low caries incidence in the diabetic children here examined." Diabetic children living entirely, or almost so, on the so-called free diabetic diet, but with insulin, showed a caries picture about similar to that of the average non-diabetic child. The authors also point to the regularity of meals. These authors, contrary to Boyd and coworkers, stress the low sugar intake in addition to the high intake of protective foods.

Mathis and Frey (1938) state from their studies that the low caries incidence in diabetic children is not caused by the diet *per se*. The unusual finding of no reduced caries rate in diabetic children under medical treatment reported by Ziskin, Siegel and Loughlin (1944) and by Coen (1947) is difficult to discuss from the rather meager data published.

Boyd and Drain's observation on diabetic children led to a long series of studies at the Children's Hospital of the State University of Iowa. These have confirmed the first observed results that a sufficient and well balanced diet reduces the incidence of new caries and usually stops an active caries process. In contrast to Mellanby (1936), McBeath (1932) and McBeath and Verlien (1942) and many others who seem to emphasize particularly the importance of vitamin D, the Iowa group stresses the value of the well balanced and regimented diet. However, they all ascribe the beneficial effect of the diet used solely to its nutritional value.

The contention that addition of vitamin D to a diet otherwise fairly well balanced, even high in refined carbohydrates, will reduce the caries frequency in children is not in accord with the results of a study of 263 children 3–6 years of age reported by Toverud (1948). The percentages of children having taken cod liver oil daily during fall, winter and spring from early infancy were almost evenly distributed among the high, intermediate and low caries groups. However, by far the largest percentage of children with the lowest caries figure had lived on a supposedly good and fairly well balanced diet including cod liver oil and were born of mothers also having had a well fortified diet during pregnancy and lactation. On the other hand, the largest percentage of children with the high caries frequency had lived on a poor diet and were born of mothers also on a poor diet during pregnancy. The same study shows that a good protective diet insofar as the quality and the quantity could be estimated does not compensate entirely for an uncontrolled indulgence of sugar and sweets.

Usually with an increase in protective

foods, a simultaneous decrease in the supplementary foods, such as refined carbohydrates in various forms, will occur (Howe, White and Elliot, 1942). A similar consideration applies to most of the studies reporting a decrease in caries frequency after reducing the amount of sugar and other refined carbohydrates. Here the elimination of some of these very likely will increase the amount of more natural foodstuffs. In both cases several factors may be altered, and it is impossible to tell which factor or factors have been responsible for the improvement of the dental condition (Becks and Jensen, 1948).

The experiments on cotton rats performed by Schweigert, Shaw, Zepplin and Elvehjem (1946), however, show that by isocaloric substitution of some of the high dextrose content with lard, the caries score dropped considerably. Here no recognized change in nutritive value was introduced.

Macy (1942) discussed the relation between protective foods and sugar: "If a dietary is adequate in quantity and quality of all nutriments the healthy child will not have an abnormal craving for sweets, especially sugar. This fact is substantiated by our observations of the free choice of sugar by healthy children over the past ten years. As a diet is carefully adjusted to meet the particular needs of an individual child, the healthy child voluntarily reduces his sugar consumption as his bodily requirements are more perfectly met . . . through a better balanced diet of natural foods. We have permitted freedom in sugar intake among our experimental subjects and have recorded the quantity of sugar voluntarily consumed daily by each individual; we have used this as one of the criteria for adequacy of the food intake. In 519 observations over 2595 days, 29 children voluntarily chose about ten grams (average) of refined sugar daily, which affirms the adequacy of their diets."

Very few studies have been made on an *adequate* number of children from which it is possible to state whether it is the improved nutritional condition or the reduced intake of refined carbohydrates which has been the decisive factor. The thorough study by Koehne, Bunting and Morrell (1934) of 22 normal girls during 18 months is of interest in this respect. Here the "adequate" diet was supposed to be kept constant, and the only variation was the sugar and total calories. The authors state: ". . . we feel justified in stating that diets of uniform quantity containing little artificially sweetened food and partaken of at regular times each day will control the activity of *bacillus acidophilus* in the mouth and the activity of caries in the majority of susceptible persons." They could not find any correlation between caries and the adequacy of the diet. However, the food was probably not adequate, as no extra vitamin D was added and the children were kept indoors during the study. Moreover, the recorded calcium retention was very small.

In contrast to this statement stand the results of the thorough study of children with the celiac disease at the Children's Hospital of the State University of Iowa, (Stearns, Jeans, Catherwood and McKinley (1941)). (This group of children, unable to metabolize starch and fat, received 60 per cent of their calories as glucose (dextrose) which was given in 10 per cent solution flavored with fruit juice.) As judged from metabolism studies, the diet was excellent as far as vitamins, minerals and proteins were concerned. During many years of control the children developed practically no caries. (The sugar was given up to 6 times a day (Stearns, 1948).) This observation, however, was made on a small group of children and on children with a grave metabolic disturbance, and it may not be justifiable to transfer these observations to normal children. The carefully performed study by Mack and Urbach (1949) on 585 institutional children in an age range of 5–15 years also suggests that caries does

not increase on a high sugar diet when the diet otherwise is adequate. The group of children (Institution I) having had the best protective diet, but also the highest sugar intake, had the lowest caries rate. However, there was more than a dietary difference between the groups: "Far more attention was paid in Institution I than in the others to personal dental hygiene because of the location of a dental department within the institution. In the other institutions, however, an effort was made to interest the children in good personal dental care." Two hundred and thirty-four of the boys in Institution I were studied for two more years. The diet was much higher in all nutrients, except for vitamin D, than recommended by the Food and Nutrition Board of the National Research Council. The vitamin D intake during 5 winter months was in accord with the recommendations. During the summer the boys were farming outdoors. The sugar (as such together with sweets) amounted to 103–125 grams per day according to age. The candy was given after a meal, and the children were encouraged to brush their teeth after eating it. The increase in DMF/n value (DMF divided by the number of teeth) during these two years was from 0.195 to 0.272, averaging 0.0032 per month. Mack and Urbach conclude: "Although the children consumed sugar considerably in excess of that of population averages during the two-year period of the study, their increase in dental caries was less than that of averages on a comparable age basis in the Pennsylvania mass studies in human nutrition. The dental care and dental hygiene in the institutions of the study were superior. Undoubtedly larger amounts of sugar and of carbohydrates may be consumed in conjunction with a superior dietary and with excellent dental care and hygiene without impairment of the teeth than pertains with concurrent circumstances less favorable."

CARIES FREQUENCY IN INSTITUTIONAL CHILDREN VERSUS IN "FAMILY" CHILDREN

Most of the observations of reduced caries frequency after diet regulations have been made in children either living in orphanages or other institutions for children or having been hospitalized for long periods. It has been a well known fact for many years (Friel and Shaw (1931)), that the teeth, as a rule, are less attacked by caries in institutional children than in children of similar social standing but brought up in their own homes. Mellanby (1934) reports on two groups, each of 160 children 5–12 years of age. One group (A) consisted of children entering an orphange and the other group of children (B) having lived in an orphanage for at least 3 years. Sixty-seven per cent of the teeth in group A and 80 per cent of the teeth in group B were caries free. Such differences are so generally observed that they have to be regarded as true and taken into account in dealing with such groups (Boyd and Cheyne (1947), Hadjimarkos and Storvick (1949), from U. S. A.; Schiötz (1937) and Collett (1937) from Norway; Dorph (1942) from Denmark). The observations also indicate that the earlier the child enters the institution the better are the teeth.

In spite of the fact that studies of the effect of diet changes in children's institutions have been made on "comparable" groups in the institution, it does not seem that the length of stay in the institution prior to the experiment has been given due attention. The finding of a lower caries frequency in institution children than in "family" children makes it also doubtful whether it is permissible to transfer the results obtained in institutions to the ordinary mode of living in the private

families. It is not possible in practice for private homes to imitate in all respects the diet and mode of living in orphanages or other institutions. In institutions of well-cared-for children the nutritional value of the diet as it is served may, as a rule, be superior to that of the ordinary family. It is likewise rare to see children who have stayed in the institution for some time refusing foods commonly refused at the family table. There is evidence for unusually good teeth in institutions in spite of the diet being low in protective foods. The total amount of ordinary sugar may or may not be lower than usual, but uncontrolled indulgence in candy, soft drinks and all sorts of sweets between the meals is uncommon in such homes. As a consequence of this the total consumption of sugar and sweets is apt to be less than by the average child. The mealtime, like the whole daily routine in children's institutions, is more regular than in the average family.

The emotional life of the child very probably plays a certain role in the caries picture. The well-cared-for children in institutions are as a rule not under the same emotional tension as are children living in private homes. They may also have the feeling of more security compared with many other children. Such a view is expressed by Boyd and Cheyne (1947). The observation by Toverud of a lower caries frequency in feeble-minded children living together with their normal siblings in their own homes may also point to the effect of the emotional life. The same has also to be said of the common clinical observation of increased caries incidence in the puberty period and after certain nervous depressions. During puberty, of course, a purely nutritional factor also may enter the picture. However, that other factors are far more potent than the emotional one is clearly evident from the enormous lowering of the caries frequency in children during the war in the war-torn countries, in spite of the tension on their emotional life from the war condition. Most probably several of the factors mentioned lead to the better dental condition in children living in institutions compared with those living in private families.

Even between children in residential nurseries and day nurseries there may be a significant difference in the caries picture. Knowles (1948) reports on 413 children in residential nurseries and 457 in day nurseries. The diet was the same in both types of nurseries and was considered to be good and well balanced, containing cod liver oil, orange juice, and one pint of milk daily. The day nurseries were open from Monday to Saturday mid-day; these children had their meals in their homes during the week-

TABLE 1

Age group in years	Length of stay in months	Residential nurseries		Day nurseries	
		No. exam.	Average DMF teeth	No. exam.	Average DMF teeth
4	<12	48	2.02	82	5.16
	12–24	53	1.85	64	3.59
	>24	76	0.75	55	4.25

ends. As is seen from Table 1, the children in the day nurseries have much higher caries figures than have the other children.

The protective diet was the same for these two groups of children for more than $\frac{5}{7}$ of every week, and even if hardly any protective foods were served in the private homes during week-ends, the eventual difference in total protective foods between these two groups can hardly be taken as the cause. The explanation must be found elsewhere in the experience of the day nursery children after they left the nursery in the afternoon until they entered it again in the morning, and during week-ends. The following statement from the author may throw some light on this: "Matrons also reported that many children arrived every morning eating cake and at night their

mothers brought them buns or jam tarts to be eaten going home. The children were living under difficult conditions and many were sleeping in shelters as London was subject to air attack." The most probable causes are change in diet balance, increased consumption of refined carbohydrates, and disturbed balance of living as a whole. It is also characteristic that in residential nursery children the DMF rates improve according to the length of stay in the nursery, whereas among day nursery children there is only a small difference with time.

LOW CARIES FREQUENCY IN OUTPATIENT PROPHYLACTIC HEALTH WORK

A clear evidence of the importance of good control of diet and mode of living during early development is demonstrated in two Norwegian Health Stations (K. U. Toverud (1946, 1948) and G. Toverud (1949)). As a result of an out-clinic prophylactic health service in Oslo the children 2½ to 3 years old entering the health station before 1 year of age showed a decrease in number of carious teeth of 53 per cent and children 4 years old a decrease of 29 per cent, compared with those entering the health station after 1 year of age, (see Table 2). In the other station (Skedsmo) the reduction was also by far the greatest in the youngest age group. The health supervision consisted of medical control and advice to pregnant and lactating mothers, infants, and children of preschool age. Furthermore, teaching the mothers the right choice of food for themselves and for the children was an important factor in the health program. The dietary recommended was such that it should meet the requirements during the various growth periods, particularly concerning the calcifying factors: 1 liter of whole milk, cod liver oil, vegetables and fruit, whole wheat bread daily. It was stressed that the consumption of refined carbohydrates, especially of sugar and all kinds of sweets, should be kept as low as possible. The good effect of this outpatient health supervision in the youngest age groups and a less pronounced effect in the older ones may be best explained on the basis of the supervised mother being conscientious about her diet and health during pregnancy and lactation, and more conscientious during the early life of the child than later about what the child should and should not eat. The older the child grows, the more he separates himself from strict regimentation both as to the diet and to the mode of living as a whole.

TABLE 2

Percentage of carious teeth in children admitted to the Sagene Health Station, Oslo, before and after one year of age

Age group (yr.)	Before 1 year (a)		After 1 year (b)		Differences between (a) and (b) divided by standard error of difference*
	Number of children	Per cent carious teeth	Number of children	Per cent carious teeth	
2½–3	563	5.1	114	10.8	4.74
3	554	16.1	200	25.4	5.60
4	332	31.6	191	40.7	4.08
5	123	36.8	171	48.7	3.95
6	21	54.5	129	58.9	0.74

* A quotient larger than 2.5 indicates a *significant* difference.

The lower caries frequency in children entering the health station before one year of age has been attributed partly to the supervision of the mother during pregnancy influencing the development of the teeth and thus the resistance to caries. Some investigators, for instance Massler, Schour and Poncher (1941), maintain that the maternal condition does not influence in any high degree the formation and calcification of prenatal dental tissue. They write: "The almost perfect calcification of the tissue calcified before birth is not sur-

prising when one remembers that the embryo develops in an extremely well protected and favored environment. The embryo or fetus is a parasite, deriving all of its nourishment from the mother and drawing on her calcium reserves in the bones when necessary." This statement, however, is not in agreement with Rushton's findings (1939) from studying the birefringence of deciduous tooth enamel, nor with the findings of Toverud, Häupl and Toverud (1934); Stuart (1945); and K. U. Toverud (1946, 1948). Rushton writes: "These observations lead me to the conclusion that the degree of calcification of antenatal enamel is not greater than that of the tissue formed in the months following birth by the same ameloblasts. It often appears about the same, but more commonly less . . . the observations here recorded must reopen the question of the relation between the quality of antenatal enamel and maternal nutrition. . . ." Toverud, Häupl and Toverud demonstrated great disturbances in the mineralization pattern of teeth and bones in stillborn infants from mothers suffering from vitamin deficiency or having shown preeclamptic symptoms. K. U. Toverud has found a much lower incidence of still births, prematures, neonatal mortality, total infant mortality, and incidence of rickets and osteoporosis in children when the mother had been under regular and close supervision during pregnancy compared with children from unsupervised mothers but of the same social standing (G. Toverud (1950)). In recognition of the importance of even the prematernal period for the offspring, K. U. Toverud (1947) proposed to extend the service of the Health Stations to cover the whole period from early fetal life up to 20 years of age. This plan, which was adopted by a large municipality in Norway, is in accordance with Smith (1949): "Effects on the viability of the fetus, its maturity or prematurity at birth, perhaps even to some extent its freedom from malformations, are more likely to be produced by long term malnutrition than by acute undernutrition in pregnancy. The nutritional state of the mother at conception may well be as important as any changes which can be brought about by dietary work in the prenatal clinic once her pregnancy is well under way."

DENTAL CARIES DURING PREGNANCY

As will be seen from the review on Nutrition and Dental Caries, most of the recent reports on this subject in contrast to some of the earlier reports conclude that there is no increased susceptibility to dental caries during pregnancy (Granados, Glavind and Daru (1949)). However, it does not appear that the studies reported on human beings have been properly conducted. The studies have paid attention to the age and social conditions in both groups but not due attention to the nutrition and health factors. As Toverud (1927) pointed out, such studies must take into account also the diet and health of the pregnant woman. It is of course no answer to the question when the Michigan Workshop (1948) concludes: "Pregnancy and lactation in themselves are not a cause of dental caries." To put it in this way is as if one would blame nature that the most vital function of the female organism should break down the hardest tissue of the body. Pregnancy *per se* is of course not a cause of dental caries; however, pregnancy under unhealthy and unsatisfactory nutritional conditions may be a predisposing cause or a conditioning factor leading to dental caries in the pregnant woman. Research in this field is unusually difficult.

Lactation is more likely to increase the disposition to caries because it is a heavier strain on the mother's mineral metabolism than is the pregnancy (Stearns, 1948).

LOW CARIES FREQUENCY IN MALNOURISHED CHILDREN

In contrast to the groups of children showing reduction in caries frequency after nutritional improvement of the diet is the report by Dreizen, Mann, Spies and Skinner (1947) on the very low caries frequency and the low increment of new caries in malnourished children. The authors point to the deficiency of nicotinic acid and thiamine as a possible cause, as these vitamins are necessary for the breakdown of carbohydrates in the mouth. But they find this unlikely, as Dreizen, Mann, Cline and Spies (1946) had shown that in vitamin B complex deficiency there may be enough vitamins for the growth of *L. acidophilus*. On the other hand, the saliva has a higher buffer capacity. The authors declare themselves unable to give a satisfactory explanation for the better teeth in these malnourished children than in the well nourished ones. There is no information about the weight and height of the children. It would be safe to assume that the malnourished children were small and underweight; if this were the case, it may provide a factor having a role in good dental conditions, since it is a clinical observation that children who are growing very slowly, are slender and have a "dry" appearance usually exhibit a low caries rate. This supposition is strengthened by the observation by Schourie and coworkers (1949) on the caries incidence of 622 fourteen-year old boys from Puerto Rico: the incidence of DMF teeth in the group comprising boys with weight below 69 pounds was 7.5, compared to 9.5 in the weight group above 105 pounds. The three intermediary weight groups showed 8.9, 9.0, and 8.4 respectively.

Mann, Dreizen, Spies and Hunt (1947) reported on another study of malnourished persons. This group of 124 in age range 6–75 years was compared with a well nourished group of 99 in age range 8–54 years. The majority of both groups were natives of north central Alabama. The malnourished group had almost the same vitamin B complex deficiency as those mentioned above; 62 of them showed clinical evidence of multiple deficiency diseases but no history of rickets. The thorough dental examination, including roentgenograms, showed 4.54 DMF surfaces in the malnourished group against 14.94 in the well nourished one. Chemical as well as bacteriological caries susceptibility tests were in accordance with the caries findings; so also was the buffer capacity test of saliva. The staple foods of the malnourished groups were corn bread, syrup and fat pork. The calorie intake was usually 40 per cent below the standard.

These reports demonstrate that low caries frequency and good general health are not always associated and that poor diet in the ordinary sense does not necessarily lead to high caries frequency.

No direct information is given about the dietary condition during the period of tooth development except for the following general statement concerning the studies of the children: The mothers had never been under control during pregnancy (as were the mothers of the control children). The children were usually breast-fed up to 2–3 years. In addition they had corn bread, biscuits, syrup and grits from early age; no orange juice nor cod liver oil was used. (The area in concern, however, had 60 per cent sunshine.) Very little meat, milk, eggs or fruit and vegetables was used. The children had poor appetites, ate irregularly, and preferred high carbohydrate food, much of which was in the form of simple sugars. From this rather meager information it is hard to believe that the teeth would have acquired any high resistance to caries according to the common concept today. However, semi-starvation may have been present in these children and during such conditions the organism is burning its own

tissue and the metabolism is running on a very economical basis. No information, however, is given of the condition of mineralization of the teeth. The total intake of carbohydrates, as well as the intake of refined carbohydrates in proportion to the total calorie intake, was about equal in the malnourished and in the control group.

DENTAL CARIES IN CHILDREN DURING AND AFTER THE SECOND WORLD WAR

Sognnaes (1948) has analyzed available data on the fluctuation in caries incidence in several European countries during and after both World Wars. During these periods a distinct reduction in caries frequency in children has been noticed in many countries. A common change in the dietary has been a reduction in consumption of refined carbohydrates, especially sugar and sweets. As a result of his analysis Sognnaes reaches the conclusion that there is no direct relationship in time between the restriction of sugar and decrease in caries incidence in such degree that the reduction in caries can be ascribed to a change in the oral environment alone.

The percentages of 6- to 7-year old German children with "sound permanent teeth" during and after World War I are cited in Table 3. The percentages denote "children not needing treatment of the permanent teeth," and do not necessarily mean that "sound permanent teeth" are free from caries or fillings in the permanent dentition (Wimmenaur, 1929). In the same table the sugar consumption according to v. Gordon (1936) from 1914–15 through 1920–21 is included. The figures for the subsequent years are computed from other sources and may not be strictly comparable to the others.

A decrease in caries may be noted in 1916–17, two years after the war started, and a reduced sugar consumption appeared that year. From 1917–18 the percentage of children with "sound permanent teeth" increased steadily and reached the maximum in 1923–24. During the same period the figures for the sugar consumption were decidedly lower than during the first two years of war, but no constant lowering is to be seen corresponding to the steady increase in percentage of children with sound teeth. On account of the great fluctuation in the sugar figures, it is rather difficult to get a clear picture of the relation between the dental health and the sugar consumption. However, it seems justifiable to state with Sognnaes that the reduction in caries cannot be ascribed only to the change in the oral environment caused by the sugar reduction.

Sognnaes points to a similar trend in Norway during and after the recent war. He writes: "In summing up this analysis I have failed (1) to establish a uniform increase in any specific food or food factor which can satisfactorily explain the marked reduction in caries and (2) to demonstrate the expected concurrent reduction in caries and sugar during the two wars. I have succeeded, on the other hand, in showing that in several countries there has been a reduction in refined carbohydrates and caries after certain time intervals. . . . The time relationship between the wartime reduction in dental caries and the reduction in the use of refined carbohydrates (assuming there is a causative relationship) can best be explained by an indirect favorable influence

TABLE 3

Year	Percentage of 6- to 7-year-old children with "sound perm. teeth"	Sugar consumption (kg. per person)
1914–15	9.5	25.0
1915–16	8.97	24.8
1916–17	12.87	19.6
1917–18	12.37	21.8
1918–19	20.49	20.0
1919–20	22.9	15.0
1920–21	27.5	18.6
1921–22	32.6	22.1
1922–23	36.8	22.5
1923–24	38.8	15.0
1924–25	24.6	21.2

on the development and maturation of the teeth, not by a change in the oral environment alone. The nature of the favorable influence is not known. It cannot be attributed to a uniform increase in the consumption of any previously demonstrated caries-preventing food or food factor."

The statement of Sognnaes, however, about the sugar consumption in Norway during the recent war refers only to the

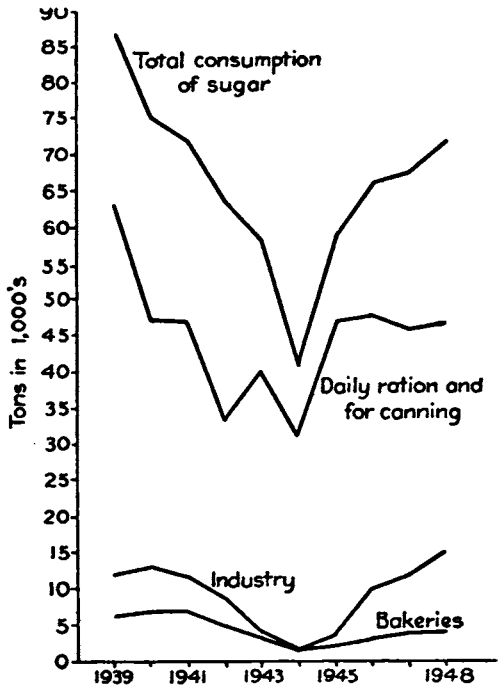

Figure 1.1. Sugar curves from Norway, 1939–1948, according to calculations by H. Skaarer. Reproduced by the courtesy of *J. Am. Diet. Assoc.*

average daily ration of pure sugar. In Figure 1.1 the sugar curves drop heavily year by year from 1939 and reach the bottom in 1944.

Schulerud (1948) in his analysis of the cause of the wartime reduction in caries in Norway reaches the conclusion that the cause is to be found in the drastic decrease in sugar consumption, with the change due solely to the oral environment factor. In another analysis of the same problem in Norway, Alexander (1948) emphasizes a double factor: an increase in tooth resistance and a decrease in environmental factors.

As the fluctuation in caries frequency in Norway, as well as in other countries during and after the last war, may throw some light on the caries problem as a whole, it is worth while to deal with this wartime condition at some length. Toverud (1945, 1946, 1949) has published preliminary reports on a dental survey of 8,000 to 9,000 Norwegian school children 7–14 years of age for each of the years from 1940 to 1949.

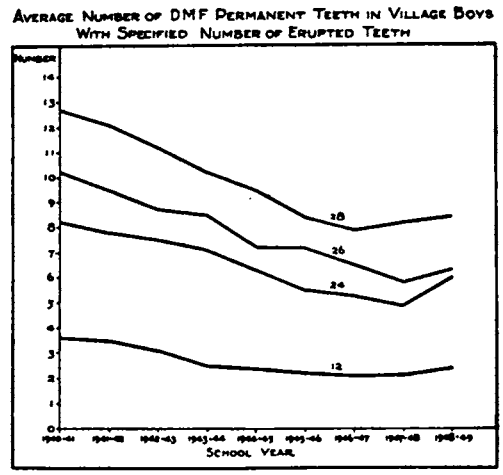

Figure 1.2. Change in average number of DMF permanent teeth in village boys with 12, 24, 26 or 28 erupted permanent teeth. The sugar curve (Fig. 3) started to go up during 1945.

The dental examinations were performed by school dentists. At the same time, 600 to 700 preschool children were studied before, during and after the war. The reduction in number of DMF surfaces per child amounts to 50 to 75 per cent as an average and in some groups to more than 80 per cent. Decrease in caries frequency is clearly seen in the school year 1941–42 (see Figures 1.2 and 1.3).

Nearly all food articles were rationed during the war. Concerning the dietary conditions for the changes shown, it may be stated: the caloric intake was sufficient

up to 6 years, probably suffcient from 6–12 years of age, but deficient above 12 years; the total protein intake was sufficient, but very little meat protein was available; the fat intake was probably sufficient up to 12 years of age, but much too low later; the same is true for the carbohydrate intake; the amount of the different vitamins and minerals, except for iron after 12 years of age, should have been sufficient. This characterization of the diet is generally in accord with the report of household studies by Ström (1948) on 17 Norwegian families during 1942–45.

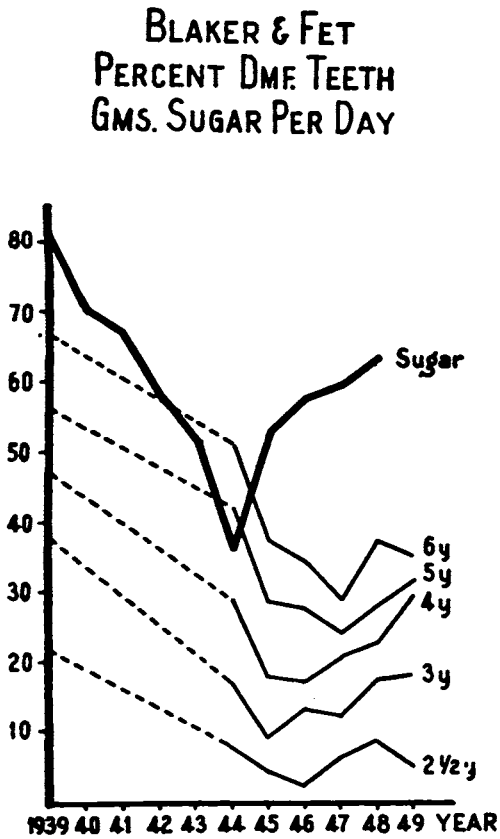

Figure 1.3. Decrease in percent DMF temporary teeth from 1939 to 1949 in two rural municipalities in Norway. The dotted line denotes that no dental examination was performed during that time. The heavy line is the total consumption of sugar in Norway from 1939 to 1948 according to the calculation of H. Skaarer.

The most pronounced change in the diet and eating habits of children from fetal life up to 15 years was the decrease in refined carbohydrates, including sugar and all kinds of sweets, and in meat. As a compensation more flour of high extraction, more potatoes and vegetables, and more fish were used. The daily intake of milk and cod liver oil was probably more regular, as was the whole daily diet. The between-meal eating of cookies and sweets disappeared almost completely.

That the war diet was inferior to that before the war for the physical development of the children is demonstrated in Oslo school children (Stoltenberg, personal communication). From 1940 to 1945 a distinct decrease in the height and weight of 8- to 14-year-old boys and girls was found. In 1947 the growth improved and even passed the values for 1940 (see Figure 1.4). No exact data are at hand for the preschool children, but it seems probable that they were not as physically handicapped as were the school children.

On the basis of the dietary change it would be easy to ascribe the decrease in caries frequency to the reduction in sugar and other refined carbohydrates. The question is whether this affected directly or indirectly the resistance of the tooth, the oral environment, or whether there was a combination of systemic and local factors. The discussion will refer to Tables 4 and 5 demonstrating the fluctuations in the caries incidence of newly erupted permanent teeth and of temporary teeth, as well as to the curves representing the change in sugar consumption. From the table showing caries in the permanent teeth (the 6-year molars and the front teeth) the reduction in carious surfaces is noticeable in 1940–41 and is rather marked in 1941–42. These teeth had their preeruptive mineralization period exclusively before the war. Thus the tooth development and maturation before eruption cannot be referred to the wartime diet. It is unlikely that the resistance should

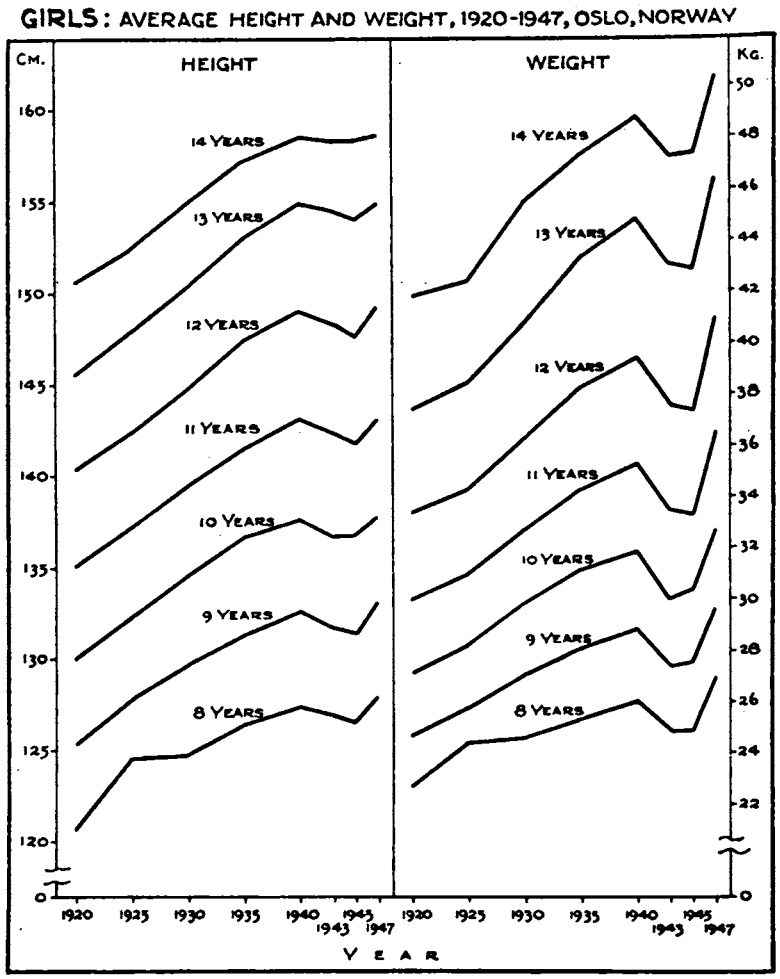

Figure 1.4. Height and weight of 8- to 14-year-old Oslo school girls from 1920 to 1947. The curves for the boys are practically identical with those of the girls. By the courtesy of Dr. Stoltenberg, the school medical officer in chief.

TABLE 4

Carious surfaces in permanent teeth of 7-year-old children from Gjerpen (Alexander 1950)

Year of examination	No. of car. surf. per child	Years of assumed pre-erupt. mineralization
1939–40	3.9	1931–32 to 1937–38
1940–41	3.0	1932–33 to 1938–39
1941–42	2.3	1933–34 to 1939–40
1942–43	1.4	1934–35 to 1940–41
1943–44	0.9	1935–36 to 1941–42
1944–45	0.8	1936–37 to 1942–43
1945–46	0.6	1937–38 to 1943–44
1946–47	0.6	1938–39 to 1944–45
1947–48	1.2	1939–40 to 1945–46

TABLE 5

Percentages of carious surfaces in the temporary teeth of 6-year-old children from the rural municipalities of Blaker and Fet

Year of examination	Per cent car. surf.	Years of assumed pre-erupt. mineralization
1939	38	1931 to 1935
1944	25	1936 to 1940
1945	15	1937 to 1941
1946	11	1938 to 1942
1947	9	1939 to 1943
1948	14	1940 to 1944
1949	15	1941 to 1945

have increased after tooth eruption to such a degree as to lead to a 40 per cent reduction in carious surfaces. However, as has been pointed out before, the enamel may be influenced after its eruption. This is indicated in the preliminary report of the Newburgh-Kingston fluoride studies where Ast (1949) reported 21.6 per cent less DMF teeth in 6-year molars in Newburgh children with 1 part per million fluoride in the drinking water than in the control city of Kingston. The Newburgh children had an average age of 6 years and 2 months when fluoride was added to the water, and the first molars should have been erupted. The posteruptive exposure was 40 months. Even if this condition is not strictly comparable to the wartime condition, it shows that the erupted enamel can be changed.

In regard to the explanation that a change in the oral environment caused the improvement in dental health, it will be seen that the lowest caries figures were recorded during the period 1945 to 1947, and this despite the fact that the sugar consumption increased rapidly from 1944. The delayed action, assuming a change in the oral environment, can hardly be ascribed to anything but an increased resistance incurred during the preeruptive mineralization period, of which the main part occurred during the war. This increased resistance, however, was not able to withstand the steady increase in sugar consumption, as is plainly demonstrated by the figures for 1947–48. Exactly the same conditions are evident in the table showing caries in temporary teeth.

Jay (1947), Kitchen and Permar (1948) and Belding and Belding (1948) have shown that the *L. acidophilus* count drops after sugar restriction and may stay fairly low for some months after the increase in sugar. No bacteriological studies were performed on the Norwegian children, but it may be possible that the decrease in *L. acidophilus* occurred. However, it would be strange to assume that the *L. acidophilus* count would stay low for as long as 3 years after an increase in sugar consumption and still account for the delayed increase in caries.

It is hard to believe that the change in diet after the war could decrease the resistance of the tooth after eruption to such an extent that this alone would account for the increase in caries. After the war ended, the general character of the diet was improved, as demonstrated by the increase in height and weight of Oslo children in 1947 as compared with 1943 and 1945. A logical explanation is that the change in the carbohydrate acted as an environmental factor, together with a nutritional change. Toverud (1949) concludes concerning the decrease in caries: "Based on the rationing of the various food articles our tentative conclusion is that the decrease may be attributed to the lowering in consumption of refined carbohydrates and the increase in consumption of more natural foods, i.e. protective foods. These changes may have resulted in an increased resistance of the teeth and a reduction in the local factors which produce decay." As will be seen, this explanation of the fluctuations in the caries picture in Norway during and after the war differs somewhat from that stated by the reviewer of the section on Nutrition and Dental Caries.

More evidences of the relative role of the postulated general and local factors can not be obtained before more exact studies have been performed on the reaction of the individual teeth in various age groups. Such studies on the Norwegian subjects are being continued.

The hypothesis of the increased resistance of the teeth from lowering of the sugar intake during development, set forth by Sognnaes and supported by Toverud, is strengthened by the experiments of Sognnaes (1948). He found the caries frequency in young hamsters to be as 2:10:20, according to whether they were on a high (67 per cent) sucrose diet after, during or before tooth development.

A decrease in caries frequency in school

children during the recent war has also been reported for other Scandinavian countries. In Finland (Wilska 1946, Ekman 1948), with the worst nutritional conditions among the Scandinavian countries and with the greatest reduction in sugar consumption (reduced to a third of prewar value), the reduction in caries frequency was about the same as in Norway, or even greater. Wilska (1946) writes: "As far as Finland is concerned the only nutritional factor capable of explaining the great reduction of caries incidence is the shortage of sugar."

In Denmark the food restrictions were less severe than in Finland and Norway. Milk and meat were not rationed. The sugar ration was higher than that in Norway, about 50 gm. per day, and pastry and cookies were available. The reduction in caries frequency was not as high as in Finland and Norway (Krohn and Pedersen 1945). Pedersen (1946) writes: "The evidence accumulating from the aforementioned studies seems to bear out that a successful battle can be fought against dental caries by reducing the consumption of refined carbohydrates (refined sugar, white flour, sweets) and by replacing the calories thus removed by protective foods eaten in regular meals. On the other hand we are not entitled to point out any single food item as being responsible for either the presence or absence of dental caries."

Among the Scandinavian countries, neutral Sweden had the best access to protective foods. Some food articles were rationed, but the ration was fairly high. Sandberg (1946) states on the basis of official data from the ministry of health, that, compared with the year 1940, the consumption of milk, fish, flour, bread, potatoes and vegetables had increased during the war. The consumption of cheese, fat, beef, pork, sugar, syrup and all kinds of sweets decreased. This change gave an increased intake of calcium, phosphorus, iron, vitamin B_1 and vitamin C, a small decrease in vitamin A and no change in calories, carbohydrates and protein. The reduction in sugar, syrup and candy from 1940–45 seems to have amounted to about 18 per cent. In 1942–1943 there was a small but distinct reduction in caries frequency but no further reduction during 1944 and 1945 (Maunsbach *et al.* 1947). Sandberg does not draw any definite conclusion as to the cause of the decreased caries incidence, but alludes to a combined effect of more protective foods and less refined carbohydrates.

From England Lady Mellanby and Helen Mellanby (1948) reported a definite decrease in caries frequency in 5-year-old London children during the war. Comparable studies made in 1929, 1943, 1945 and 1947 give the following figures for percentage of "caries-free and almost caries-free" children: 4.7, 24.2, 28.1 and 37.5 respectively. This is certainly a noteworthy improvement in dental health. The structure of the teeth also improved from period to period. On the basis of the special rationing for pregnant and nursing mothers, infants, and young children during and after the war, giving a priority of milk and cod liver oil, the authors conclude: "As in 1943 and 1945 surveys, it is again suggested that improvement is due to the increased calcifying properties of the dietary of this country, and particularly that of pregnant and nursing women and infants and young children. The marked improvement in 1947 is thought to be mainly due to the fact that for the first time in these surveys the diet has been of consistently better calcifying qualities over the whole antenatal and postnatal life of the child concerned." In the description of the diet change and in the evaluation of the dental findings, the authors do not mention the decreased consumption of sugar and other refined carbohydrates, except for the flour. The decrease in sugar consumption, however, has been high. The percentage of the total sugar supplies for the civilians based on the prewar value was 65–69 per cent from 1941 to 1945, 72

per cent in 1946 and 77 per cent in 1947.* Mellanby and Mellanby report that: "There was less arrest or 'spontaneous healing' of decay in individual teeth in 1947 than in 1945, though more than in 1943. The reason for this is not clear." These findings suggest an increased effect of the environmental factor after the end of the war.

H. Mellanby (1949) reports a dental study made early in 1948 on 563 5-year-old children from Wuppertal in Germany. The percentage of caries-free children was 28.4, practically the same as the 28.1 recorded in the London study of 1947. The group "almost caries-free," however, was 22.7 in Germany compared with 9.4 in London. The incidence and extent of caries were "appreciably less for all types in Germany than in London." Based on dietary information obtained during and after the war, the author concludes: ". . . the early diet of these children when they were 1 to 3 years would have appeared to have had better calcifying properties than that of the London children." Unfortunately the author does not give any information about the consumption of refined carbohydrates during these periods.

Knowles (1949) found, on examination in 1945, 51 per cent caries-free 3- to 7-year-old children staying in the Channel Islands during the German occupation, compared with 11 per cent caries-free children evacuated to the British Isles. A reexamination by the author in 1947 in the same localities showed a high increase in dental caries from 1945. The author does not draw any conclusion as to the cause of the change in the caries picture, but from her description of the diet it appears that the amount of protective foods in the Channel Islands was not particularly high during the war. Concerning refined carbohydrates the author writes: "The children had 6 oz. of sugar a week until November 1944, when the ration ceased except for bottle-fed babies. Occasionally small quantities of sweets and biscuits were available for distribution."

Bransby, who has made a careful study of the food situation on the Channel Islands, writes (1949): "The main dietary changes which took place after liberation, compared with occupation, were: an increase in the consumption of meat, bacon, fish, eggs, cheese and vitaminized fats; a slight decrease in consumption of milk; a considerable increase in the consumption of sugar, jam and confectionery; a lowering of the rate of extraction of bread and its fortification with calcium. . . . There was an increase in the intake of nearly all nutrients, including vitamins A and D and calcium." Later Bransby and Knowles (1949) gave a more detailed report of these studies.

From Switzerland Roos (1949) has reported a 37 per cent reduction in numbers of carious teeth in 6-year-old children among a group of several thousand school children. The reduction is based on figures from 1934 and 1945, but he states that the improvement in dental health occurred chiefly during the war. The author writes: "The experience of the War Nutrition Committee has shown with evident clarity that the nutritive value of the prewar diet was insufficient. The chief reason for this was the high proportion of refined foods consumed. For example, sugar consumption before the war was 152,000 tons (149,600 English tons) for a population of 4½ million . . . 60 per cent of the calories were accounted for by refined foodstuffs." During the war, milk, cheese, potatoes, vegetables and fruit were sufficient. The supplies of rice, sugar and flour were particularly low. The flour was of 85 to 90, or above, per cent extraction. "In many respects there were scarcities during the war, but the rationing did not impair the health and general efficiency of the Swiss people; they were on the contrary as a whole better nourished."

From Belgium (Eastman Dental Institute in Brussels) Watry (1949) gives the following percentages of children with caries-

* Personal communication, Dr. E. M. Knowles.

free 6-year molars in the age group 10–12 years: prewar period, 9; 1941, 11; 1942, 16; 1943 and 1944, 20; 1945, 22.5; 1946, 16; 1947, 10. The author writes: "... caries has become more frequent since 1946–47. This seems to confirm the opinion of various observers in this particular field, that the decrease in dental caries is due to the disappearance of certain foods which have reper-

TABLE 6

Total consumption of refined sugar in different countries

(Pounds per capita per year)

Country	1938-39	1948
Hawaii	126.9	127.1
Denmark	121.8	85.9
Sweden	111.9	94.8
United Kingdom	108.1	86.3
Australia	107.1	129.6
Canada	103.9	100.8
U. S. A.	97.1	93.0
Ireland	87.6	61.5
Netherlands	84.8	73.5
Switzerland	81.0	79.3
Norway	72.1	56.6
Belgium	69.1	61.1
Finland	63.4	55.1
Czechoslovakia	62.4	52.9
Germany	56.5	
France	53.5	42.7
Japan	36.3	
Poland	27.8	40.2
Russia	27.0	16.4
Hungary	26.4	21.4
India	23.6	7.0
Italy	19.8	20.0
Spain	16.1	11.1
Roumania	13.7	10.6

(Through the courtesy of Lamborn's Study of World Sugar Prices, New York.)

cussions on the buccal units; sugars, fats, etc...." He points out that the scarcity of food may have affected mastication habits, with some effect on the teeth.

In a group of French children (Paris) Dechaume (1946) reported an increase in caries-free children from 13 per cent in 1942 to 44.8 per cent in 1945. He ascribes the improvement in dental health to the change in diet. He refers especially to the increase in alkali-producing diet, decrease in consumption of sugar and sugar products, and the alcohol prohibition. He does not consider the vitamins an important factor in the preservation of the teeth.

In Holland, Steijling-Lindeboom, Steijling and Tn'T Zandt (1949) carried out an extensive study of the teeth of 1800 school children and 4400 institution children. The caries incidence in age group 8–12 years fell during the war and rose thereafter. The authors suggest that the decrease in caries might be attributed to the findings of a delay in tooth eruption, and the later increase in caries to poor structure of the teeth accounted for by the insufficient food intake. Without a dietary analysis, it is impossible to discuss this suggestion. The caries figures are much higher than those reported by Cady (1946) in 495 Dutch children evacuated to England after the invasion of Holland.

Schour and Massler (1947) report caries frequencies in Italians examined in 1945 as one-half to one-seventh those found in the United States. "The reduced sugar intake in Italy and the high sugar consumption in the U. S. may, in part, explain the difference in the prevalence of caries. The role of vitamin B deficiency in depressing the incidence of caries should also be considered." Unfortunately these authors report no figures from corresponding groups of children in Italy before or early in the war. It is known, however, that the caries frequency in Italians and the sugar consumption before the war were also much lower than those in the United States; Table 6 indicates the sugar consumption in 1938–39 and 1948 in different countries.

Morgan, Wright and van Ravensway (1946) report on the health of 4,618 repatriated American prisoners of war from the Far East. On the basis of the dental survey the authors write: "A detailed study of the dental examination forms has not been completed at this time, but as far as

this study has progressed, no unusual pathologic conditions of the teeth were manifested. The number of carious teeth was less than that of the same age group living under normal conditions, which might be accounted for by the low amount of sugars in their dietary regimen." This good dental health may be contrasted with the reported loss of weight, muscular wasting, high incidence of pulmonary tuberculosis, dermatologic manifestation, and neurologic and psychologic changes.

COMMENTS ON DIETARY STUDIES IN RELATION TO DENTAL CARIES

All of the reports on the wartime decrease in caries frequency, except those of M. and H. Mellanby (1948), Steijling-Lindeboom and coworkers (1948) and to some extent Sognnaes (1948), stress the reduction in consumption of sugar (refined carbohydrates) in a causative relationship, to the improvement in dental health. Explanation on the basis that reduction of sugar consumption influences only the oral environmental factors does not seem to fit all the facts at hand; yet to ignore sugar as an environmental factor seems no more correct. From the data at hand today, the most logical explanation of the reduction in caries frequency during and after the second world war is the assumption of a combination of direct and indirect actions resulting from the decrease in the amount of refined carbohydrates consumed: (1) The reduced amount may have been compensated for by substitution of more natural foods (i.e. protective foods or foods containing necessary trace elements) and in this way improved the resistance of the teeth to caries. (2) The reduction of sugar (refined carbohydrates) *per se* may have improved the mineral metabolism in such a way as to increase the tooth resistance. (3) The change in diet may have improved the quality of the saliva. (4) The reduction in sugar and other refined carbohydrates undoubtedly decreased the effect of oral environmental factors. (5) The caloric intake may have been so low as to introduce a starvation effect, although this does not seem likely (with the possible exception of Holland).

That a combination of two or more of these factors may have been acting together is in agreement with the concept of Jeans (1944): "Production or prevention of caries can be considered as the resultant of the effects of two opposing groups of factors, those tending to produce caries on the one hand and those tending to prevent caries on the other," and of Elvehjem (1948): "There can be no question that sugar plays an important role in dental caries, but several other factors influence the effect of the sugar." Milk has been demonstrated to be one of these factors in cotton rats (Anderson, Smith, Elvehjem and Phillips, 1948). Whole milk added to a cariogenic diet (67 per cent sucrose) gave a 50 per cent reduction in caries indices compared with the controls. Concerning the mode of action Elvehjem writes (1948): "At present it cannot be determined definitely whether milk carries a specific protective factor or whether a large part of the effect of milk is related to its fluidity. Since important changes are noted in the types of diet, it is not at all surprising that important changes take place also in the bacterial flora of the oral cavity for the same reason." A beneficial effect of raw whole milk on the erupted teeth of several groups of English children had been reported by Sprawson (1932). It is hard to believe that milk has been an important factor affecting dental caries in most of the war-torn countries. However, in England the milk consumption was a good deal higher than before the war.

The diet during the war, with more seafood, higher extraction of flour, more potatoes (and unpeeled?) and more vegetables, may have increased the ingestion of fluoride.

Roos (personal communication) mentioned that the Swiss diet during the war did contain more fluoride than before the war. Whether this increase was sufficient to have any preventive effect against dental caries is not known. Increase of fluoride might be expected from increased consumption of salt water fish, as in Norway during the war. However, the farm population showed just as high a decrease in caries frequency as did the city population, and the farmers did not eat proportionately as much fish as did the people in the cities; on the other hand, the farmers used more meat and dairy products. This question is under investigation (Sunde, 1948). Furthermore, according to McClure (1949) the benefit of fluoride comes primarily through the drinking water.

It should be possible to find some common factors in the diet of people or groups of people showing an unusually low caries frequency. In nearly all instances two factors appear: liberal use of so-called natural foods, and restricted use of refined carbohydrates, especially of sugar and sugar products. The first factor, operating chiefly during the developmental and maturation period of the teeth, will most likely produce teeth with a structure having high resistance to decay. But as stated in the section on Nutrition and Dental Caries (Summary), this is not a *conditio sine qua non* for freedom from caries: "If the environment is not suitable for decay, there appears to be no evidence that a tooth must become carious because it is structurally imperfect." The "natural" diets usually contain ample amounts of calcifying factors and may contain one or more trace elements which might be responsible for the resistance of the teeth.

The second factor shows up in all except a few studies (e.g., Boyd, Wessels and Cheyne (1950)). Decreased caries prevalence has been connected with restriction in intake of sugar and sweets in almost all cases. In some instances the total sugar consumption may not have been particularly low, but sweets have not been allowed between meals and the number of meals has usually not exceeded three per day.

FLUORIDE IN DENTAL CARIES PROPHYLAXIS

It was not until 1931 that the condition designated as "mottled enamel" by Black (1916) and McKay (1916), and related to dental caries by McKay (1925), was discovered to be caused by excessive fluorine in the drinking water. Most of the exploratory work in this field was carried out by American scientists, since mottled enamel, or as it is called now, "endemic dental fluorosis," is widespread in the United States. Through the comprehensive epidemiological dental studies in different states, chiefly conducted by the National Institutes of Health, it is clearly shown that there is a close connection between the fluoride content of the drinking water and the dental caries rate in school children. It has been established that dental fluorosis of a disfiguring type will not be expected unless the drinking water contains nearly 2 parts per million of fluoride, and depending, of course, on the amount of water taken in a day and upon other factors. For warmer climates the threshold is lower. The epidemiological studies also revealed that the action of fluoride was not connected exclusively with the clinical picture of dental fluorosis, but that water containing about one p.p.m. of fluoride did not cause disfiguration of the enamel, and had a marked prophylactic action against dental caries. This very important discovery led to the artificial fluoridization of drinking water as an experimental procedure in a number of communities.

Figure 1.5, from Dean (1947), demonstrates clearly the relationship of the caries rate in 7257 children 12–14 years old to the fluoride content of the drinking water.

These field observations and studies led

to intensive investigations both in laboratory animals and in human beings in order to find out if fluoride could be applied in the field of preventive dentistry. Armstrong and Brekhus (1938) found a higher fluoride content in caries-free enamel than in enamel from carious teeth. This could not be confirmed by McClure (1948). Volker, Hodge, Wilson and Voorhis (1940) showed the adsorption of fluorides by enamel. This was confirmed by Sognnaes (1940) on rats showing a reduced caries incidence after topically applied sodium fluoride and by Bibby (1942) and Cheyne (1942) following the same procedure for children.

Topical Application of Fluoride

Through the many clinical reports of the effect of topically applied fluoride on the teeth, especially those of Knutson (1948) and coworkers, it is established that this procedure is capable of reducing the caries increment in children. It has been shown that the maximum effect, about 40 per cent reduction after one year, is obtained by four applications of a 2 per cent sodium fluoride solution at weekly intervals. Before the first application a usual professional cleansing of the teeth has been performed. In the first study of Bibby (1942) the fluoride was applied at 3–4 month intervals. The same was true in the study by Syrrist (1949) where he used 116 children all 12 years old. The teeth were brushed before each application, dried, washed with alcohol and dried again. Before the first application pumice was used, and dental floss on the approximal surfaces. Instead of letting the first application dry for 3 minutes as recorded by Knutson, Syrrist renewed it several times during the 7-minute treatment in order to have a fresh layer of fluid constantly reacting with the tooth surface. (This was considered to be more in accordance with the law of mass action.) Spacing the application permits reaching newly-uncovered surfaces of the teeth during their further eruption. In spite of this more time-consuming procedure, the reduction of caries increment in the 7-times-treated upper quadrant during 2 years, compared with the untreated one, was of about the same order as that published by Bibby and by Knutson and coworkers after one year—40 per cent reduction in DF teeth and 42 per cent reduction in DF surfaces. However, Syrrist, like Krasnow (1949), criticises the practice of expressing the caries reduction as the relative percentage difference instead of the actual difference in caries rate between the treated and untreated material. The actual caries attack after 2 years, in the teeth not carious at the start, was 20.6 per cent in untreated teeth

AMOUNT OF DENTAL CARIES (PERMANENT TEETH) OBSERVED IN 7257 SELECTED 12-14 YEAR OLD WHITE SCHOOL CHILDREN OF 21 CITIES OF 4 STATES CLASSIFIED ACCORDING TO THE FLUORIDE CONCENTRATION OF THE PUBLIC WATER SUPPLY.

NUMBER OF CITIES STUDIED	NUMBER OF CHILDREN EXAMINED	NUMBER OF PERMANENT TEETH SHOWING DENTAL CARIES EXPERIENCE* PER 100 CHILDREN EXAMINED (0 100 200 300 400 500 600 700)	FLUORIDE (F) CONCENTRATION OF PUBLIC WATER SUPPLY IN P.P.M.
11	3867		< 0.5
3	1140		0.5 TO 0.9
4	1403		1.0 TO 1.4
3	847		> 1.4

* DENTAL CARIES EXPERIENCE IS COMPUTED BY TOTALING THE NUMBER OF FILLED TEETH (PAST DENTAL CARIES), THE NUMBER OF TEETH WITH UNTREATED DENTAL CARIES, THE NUMBER OF TEETH INDICATED FOR EXTRACTION, AND THE NUMBER OF TEETH MISSING (PRESUMABLY BECAUSE OF DENTAL CARIES).

Figure 1.5.

and 12.2 in the treated teeth, and 7.3 and 4.1 per cent of the surfaces, respectively.

As the caries frequency differs according to age of the children and geographic distribution, the clearest picture of the condition from a scientific as well as from an economic point of view will be obtained by using and stating both methods. On the basis of his own studies, Syrrist also questions whether fluoride application is really economical in practice as a mass control of caries because of the time used. Syrrist's method, however, is tedious and requires three times as long to treat 4 or 5 teeth as is needed to treat the whole mouth by Knutson's standardized technic.

Sodium fluoride is used most widely but other fluoride salts have been tested. In

in vitro studies lead fluoride and stannous fluoride have shown greater effect than sodium fluoride in decreasing the enamel solubility. However, Klinkenberg and Bibby (1950) did not find so good an effect from 0.06 per cent lead fluoride as from one per cent sodium fluoride in topical application in young adults.

The pH of the solution has likewise been considered. In spite of the fact that an acid solution of fluoride theoretically should be preferable to a neutral solution, and that *in vitro* studies have shown a greater effect in reducing enamel solubility (Bibby, 1947, Phillips and Muhler, 1947), one has to agree with Knutson's statement that a solution of pH 5 or less, that of the solubility point of the enamel, should not be used until further evidence has been obtained.

On the basis of the fact that the youngest enamel is the most permeable, Knutson (1948) has advocated the application of the fluoride at the following ages of the child: 3, 7, 10 and 13 years. However, Klinkenberg and Bibby (1950) have lately reported a 44.5 per cent reduction in DMF surfaces after 4 topical applications of one per cent sodium fluoride to quadrants in young adults, average age 25 years. This is contrary to the earlier findings of Arnold, Dean and Singleton (1944), of no effect after a single application of fluoride to the teeth of young adults.

Mode of Action of Topically Applied Fluoride

It is suggested that the fluoride may act by: (a) changing the enamel to a less soluble substance, (b) making the enamel surface (outer layer) more dense, and (c) reducing (preventing) acid formation. These will be discussed in order.

(a) *In vitro* studies of powdered or whole human enamel treated with sodium fluoride have shown that the enamel became less soluble in weak acids. Zipkin and McClure (1949) demonstrated less erosion of the molar teeth in rats from lactate and citrate solutions when these were given together with fluoride than when they were given alone.

Syrrist (1949) found, in 11 of 22 extracted teeth, less decalcification with a saliva-sugar solution when used *in situ* for 20 minutes with 2 per cent sodium fluoride solution 8 days before extraction than in homologous teeth without treatment. With fluoride treatment of extracted teeth, the difference between treated and untreated teeth increased.

The reduced solubility of the fluoride-treated enamel is attributed to the adsorption of fluoride. However, Armstrong and Knutson (1945) were unable to demonstrate any increased fluoride in the enamel after the usual fluoride application, although the treatment had resulted in a 40 per cent reduction in caries. Schmid (1948) found an appreciable increase in fluoride in enamel after treatment *in vitro* for 30 minutes with a 5 per cent potassium thiocyanate solution followed by treatment with a 10 per cent sodium fluoride solution for 3 hours, but only a small increase after applying the same solutions to teeth *in situ*. Holager and Syrrist (1948), by X-ray diffraction analysis showed a calcium fluoride pattern instead of the normal hydroxyapatite pattern in the enamel after application to the tooth *in situ* of a 4 per cent sodium fluoride solution for 10 minutes during each of 7 sittings; Gerould (1945) made similar observations *in vitro*. In a later study of 20 teeth, Syrrist (1949) applied a 4 per cent sodium fluoride solution for 10 minutes once a week during 4 successive weeks, and a 2 per cent solution in another similar group. One week after the last application the treated teeth, together with the homologous untreated teeth, were extracted. The outer layer of the enamel, or approximately 10 per cent of the whole amount of the enamel, was ground off with a fine stone. The samples from each group were pooled and fluorine analyses made by McClure. The per cent of fluoride of the ash in the "4 per cent group" was 0.0390, compared with 0.020

per cent in the control group, a significant difference. The per cent of fluoride in the "2 per cent group" was 0.0219 per cent and in the control group 0.0149 per cent, an inconclusive difference.

If the fluoride absorbed by the enamel *per se* is responsible for the reduced caries rate after topical application of fluoride, one should have expected to find an increased fluoride content in the enamel after using a 2 per cent sodium fluoride solution as in the last experiment. There is a possibility, however, that the fluorine is to be found in such a superficial layer that it is necessary to reduce the sample for analysis to a still smaller part of the outside enamel than was done by Syrrist.

(b) Gerould (1945), by use of the electron microscope, was able to demonstrate a change in surface structure of treated enamel. A similar electron micrograph by Syrrist (1949) from a tooth treated *in situ* with a 4 per cent sodium fluoride solution for 40 minutes, 10 minutes at a time, showed "dense structure which affords this layer reduced permeability, thereby possibly also acting as a barrier against the invading caries-producing forces" appearing as a 3–4 micron band in the outermost enamel. The same author, however, was unable to demonstrate in polarized light any change in the enamel structure after regular fluoride treatment. The result of some of these studies points to an *ab*sorption of fluorine as well as an *ad*sorption.

(c) Fluoride is a poison to many enzymes, to those involved in glycolysis as well as to proteolytic enzymes. It inactivates the transformation of 2-phospho-D-glyceric acid into enol-phosphopyruvic acid by combining with the magnesium in the enzyme enolate, and thus may prevent or reduce the formation of pyruvic and lactic acids. *In vitro* studies show that acid production is influenced by a concentration of 0.1 p.p.m. fluoride (Hodge and Sognnaes, 1946). This action in the mouth can probably not take place unless some of the fluoride ions first have been liberated by acids. Leicester (1949) points out that the action of the fluoride indirectly may influence the acid-producing organisms. No reduction in *L. acidophilus* counts was found in children's mouths after a full-mouth fluoride treatment (Knutson 1948). This is contrary to the findings in children using natural fluoride-containing waters (Jay and Arnold, 1946), or artificially fluorized waters (Finn and Ast, 1947; Cavies, 1950).

Fluoride in Drinking Water

In 1939 Cox and coworkers reported an increased resistance to dental caries in teeth of rats with a high fluoride diet and thus confirmed the accidental discovery by Miller (1938). Cox wrote: "Our evidence that fluorine aids in the formation of caries-resistant teeth, linked with the findings of Armstrong and Brekhus (1938) and of Dean, Jay, Arnold, McClure and Elvove (1939), shows that a very great reduction of the incidence of human caries can be obtained by supplying in food and water an optimum amount of fluorine during tooth formation."

It was not until 1945 that artificial fluoridization of the drinking water was undertaken experimentally. Grand Rapids, Michigan, and Newburgh, New York, were the first to add 1.0–1.2 p.p.m. fluoride to the drinking water. Dental examinations of the children in these places and of comparable control children were performed. A report by Ast and collaborators (1949) on 3400 children in the Newburgh study after 3 years of fluoridization showed for the first permanent molars the following percentage of reduction in DMF teeth compared with the control group of 2800 children in Kingston: 49, 53, 28, 22, 19, 15, and 4 in children examined at $6\frac{1}{2}$, $7\frac{1}{2}$, $8\frac{1}{2}$, $9\frac{1}{2}$, $10\frac{1}{2}$, $11\frac{1}{2}$, and $12\frac{1}{2}$ years of age respectively. The crowns of the teeth in even the youngest age groups were "fully formed" when the fluoride addition started. The fluoridization of water benefited not only the teeth erupted at the start of the fluorine addition, probably like the

action of the topically applied fluorine, but also effected greater caries reduction in the teeth during the preeruptive period, after the atrophy of the ameloblasts. No saliva studies were made; McClure (1949) states that the fluorine content of saliva is not correlated with the ingestion of fluorine.

These preliminary results seem to demonstrate that: 1) Artificial fluoridization of drinking water is effective in decreasing the caries frequency in children; 2) The building up of the resistance of the enamel is not confined to the period of the functional activity of the ameloblasts, nor even to the preeruptive period of the tooth, but may take place also posteruptively. The posteruptive influence, however, is greatest during the first years.

The results confirm the observations of Deatherage (1943) and of Klein (1947, 1948) that the teeth of individuals after 8–10 years of age may be benefited by moving from a low- to a high-fluoride area, and those reported by Bull (1949) in children three years after fluoridation of their drinking water. No other effects of the fluoride have been detected by medical examination of the Newburgh children.

An important question is: How permanent is the effect of the fluoride in the teeth, either after incorporation through metabolism or through topical application? Knutson and Armstrong (1946) and Bibby and Turesky (1947) report a considerable difference, up to 36 per cent, between the fluoride-treated teeth and the controls after 2 and 3 years respectively. Results of longer periods of observation after topical applications seem not to have been published yet.

As to the duration of the effect from naturally fluoridized water, the report of Weaver (1944) from England and that of McKay (1948) from Colorado Springs are contrary to each other. Weaver writes: "For the investigation now described, evidence was obtained which suggests that fluoride is a caries-postponing rather than a caries-preventing factor. Examinations of older pupils and of mothers in North and South Shields studies indicated that the average postponement probably does not exceed five years." In 400 individuals, 10 years old and upwards, in Colorado Springs with a drinking water containing 2.6 p.p.m. fluoride, McKay found an average of 2.98 carious and filled teeth per person. In each of the 5-year age groups from 10 to 39, the caries experience was 5 to 7 times higher in Madison, Wisconsin, with 0.05 p.p.m. fluoride in the water. The average number of extracted teeth per person in the adult groups was from 15 to 36 times higher in Madison than in Colorado Springs. The number of individuals in each group is small, but the evidence is in favor of the caries-preventing effect of the fluoride having persisted to adult age in Colorado Springs. Nordh and Säden (1945) report, concerning a district with 1.0 to 2.2 p.p.m. fluoride in the drinking water in northern Sweden, that during childhood and adolescence there is a distinct resistance to dental caries which diminishes or disappears after the age of 20 years.

Ill Effects of Fluoride as a Caries-Preventive Measure

As fluoride is a very toxic substance, one might expect some ill effects on the body as a whole or on specific organs. The most conspicuous damage observed in human beings where intakes are high (usually above 2 p.p.m.) during the tooth developmental period is the mottling of the enamel. Besides disfiguring of the teeth, the enamel disappears more or less by attrition and abrasion. As to the possible influence on the supporting structures of the tooth, nothing definite can be stated. McClure (1949) failed to find any height-weight-age disturbances and any increased incidence of bone fracture in high school boys and in inductees. The liability of fluoride accumulation in the body is not great since the urinary excretion is roughly parallel to the intake. He concluded it unlikely that 1.0

to 1.5 p.p.m. fluoride in the drinking water presents a public health hazard.

It would not be expected that topical application of 2 per cent sodium fluoride solutions, according to the adopted methods of Knutson, should do any harm to the enamel, but applied to teeth with open deep dentin cavities, the fluoride may cause inflammation of the living pulp. The studies by Lefkowitz and Bodecker (1945) and Rovelstad (1947) indicate the possibility of an injury to the pulp. Small amounts of fluoride in contact with the gingiva for such a short time can hardly be expected to give rise to any pathological changes of the supporting structures of the teeth, but careful studies on this point are needed. Schmid (1948) reports damage to silicate fillings after topical application of sodium fluoride.

Based on the experience to date, as far as both the advantage and the disadvantage are concerned, appropriate ways of reducing caries in children's teeth through the use of fluoride may be:

(a) Adding the appropriate amount of fluoride to the drinking water. This will reach the entire population of a community in the most convenient and economical way. The concentration will depend on the climatic conditions; in the United States a concentration of 1.0–1.2 p.p.m. has been chosen. There is a question whether those parts of the teeth mineralized during the prenatal period and during the breast-feeding period will be benefited by this amount of fluoride in the water, since fluoride does not seem to pass very easily through the placental wall and into the mammary glands. Ockerse (1947), however, found that deciduous teeth of children whose mothers drank water containing up to 10 p.p.m. of fluoride during gestation and lactation were remarkably free from caries and were not mottled. These facts were confirmed by investigations on rats. If the lower caries frequency was caused by increase in fluorine, this could have taken place at a later stage as shown by the figures from Ast. According to McClure (1949), food grown in areas where the drinking water is high in fluorine does not contain more fluoride than usual. During cooking in such water it may pick up small amounts. Cows' milk does not increase in fluoride when the cow is fed a high fluoride diet.

(b) As a substitute for the fluoridized drinking water, a fluoride preparation may be given to the children during the tooth-developmental period. According to the calculations by McClure (1943), 0.5 mg. of fluoride per day may be given to children up to 3 years of age and 1 mg. daily from 3 to 8–9 years of age. However, no report has been published on the effect of this medication, and caution should be exercised if the water contains more than 0.5 p.p.m. of fluoride.

(c) The topical application of 2 per cent sodium fluoride may be used on children's teeth, preferably soon after eruption when the enamel is most permeable. As a system for mass treatment the technic advocated by Knutson (1948) specifying the application at 3, 7, 10 and 13 years of age seems logical.

Fluoride-containing mouth washes, tooth powders and tooth pastes have been widely advocated. As yet no conclusive evidence of the effectiveness of such preparations has been reported. Systematic studies to settle this point should be encouraged.

In spite of much knowledge gained during recent years on the relation of fluorine to dental caries, much remains to be learned. When fluoride occurs naturally in water, it is often associated with increased hardness of the water. However, there seems to be no doubt that the fluoride in such areas is the deciding factor in a reduced caries rate. Whether fluoride added to a soft water has the same action as natural fluoride-containing water remains to be proved. The Newburgh studies do not show any difference so far after three years.

Where and how the fluoride applied from

the outside is deposited in the enamel is not wholly clear. Neither is the role of Nasmyth's membrane in the *ad-* or *ab-*sorption of the fluoride. The mode of action of the fluoride is probably clear as far as the induced reduction in enamel solubility is concerned. Although studies have been reported suggesting increased density of the enamel in man and increased hardness in dog's enamel, further studies are necessary to establish the influence of fluoride on the physical character of the enamel. The mechanism of the assumed antienzymatic action of the adsorbed or absorbed fluoride in the bacteria-food mixture on the surface of the tooth is also still obscure.

OTHER SUBSTANCES ACTING ON THE CARIES BACTERIA OR ENZYMES NECESSARY FOR ACID PRODUCTION

Studies on bacteria and enzymes have been numerous since the time of W. D. Miller. In order to reduce the number of bacteria in the mouth, ordinary antiseptics as well as drugs with specific action on the aciduric flora have been used, and although many have been effective in reducing acid production in the test tube, most of them have failed to act the same way in the mouth.

Ammonia-urea preparations have become prominent during recent years. The background for these was the observation by Hill (1939) and by Kesel, O'Donnell, Kirch and Wach (1947) of the property of stagnated saliva from caries-immune persons to inhibit the growth of *L. acidophilus*. Earlier, Bunting, Crowley, Hard and Kellar (1929), had found that *L. acidophilus* introduced into the mouth of caries-free persons would disappear quickly. Kesel and collaborators (1949) found that the maximum inhibiting activity of saliva was reached after 1 week and that *Bacterium aerogenes* was the only one capable of inhibiting the growth of *L. acidophilus*.

The inhibiting effect of the filtrate of stagnated saliva from caries-free individuals was found to be proportional to the concentration of ammonia nitrogen. Further studies revealed that the effect was due to the ammonia nitrogen *per se* and not its alkalinity. As a result of this finding, Kesel and coworkers tried different ammonia preparations on the *L. acidophilus* counts in saliva from a number of individuals with positive results. Urea was added to prevent the formation of lactic acid in saliva from individuals with active caries, partly because of Stephan's findings (1943) of reduced acid production in saliva-glucose mixtures after a urea mouth wash. The most successful combination contained as active ingredients 3 per cent of urea and 5 per cent of dibasic ammonium phosphate. This preparation was used by 33 individuals for one year, preceded by half a year of the ammonium dentifrice alone. At the end of the experimental period, more than half of the individuals showed a negative *L. acidophilus* count. The others had a very low *L. acidophilus* count and from one to three new cavities each. The authors stress that these findings must be interpreted with caution because the experimental subjects were not well selected. Kesel (1948) writes: "The manner in which these ammonia-liberating agents work is still a mystery to us. . . . We feel that probably on the surface of the tooth in this bacterial plaque there is a continuous fight for survival by two antagonistic types of bacteria, and that it may be possible, by the application of ammonium salts, to influence this bacterial population so that we get a rearrangement with the proteolytic types in a dominant position. The ammonifying organisms are present in almost all mouths, even in caries-active plaques, but apparently they are suppressed by the presence of the acid-producing organisms and by the fact that sugar will prevent deamination process." Kesel writes further: "The inorganic constituents of saliva are probably important

for the effect they have on the end products of bacterial metabolism, but the organic composition of saliva may have a very significant influence on the types of organisms that are living in the mouth. It may be that age, nutrition, metabolic state, and so on, can influence the organic content of saliva and thereby influence significantly the bacterial population." However, no consistently higher ammonia content of the saliva has been found in caries-immune individuals, and if the ammonia is the active agent in these cases, the amount produced in the plaques must be too small to be detected in the whole saliva.

Conclusive evidence of the effectiveness of ammonia or ammonia-urea preparations on caries is lacking. The positive results reported by Henschel and Lieber (1949) are not reliable because the sampling was too heterogeneous. Further research is necessary before the effectiveness of such preparations will be known.

Antibiotics

Penicillin: McClure and Hewith (1947) reported that none of 82 rats given a caries-inducing diet and penicillin (75 units per ml. in drinking water) developed caries after 125 days, whereas 50 per cent of 82 control rats did. The *L. acidophilus* counts were much lower in the experimental group than in the control group. Webman, Hill and Kniesner (1949) also demonstrated reduced caries in rats with penicillin. Zander and Bibby (1947) gave three groups of hamsters a caries-inducing diet. The teeth of one group were brushed daily with a penicillin-containing tooth paste. The teeth of another group were brushed in the same manner with an ordinary paste. The teeth of the third group were not brushed. After 35 days the number of cavities per animal for each group respectively was as follows: 0.86, 5.87, and 19.00. Zander and Bibby found no change in pH in a saliva-glucose mixture 2 hours after rinsing the mouth with a penicillin mouth wash. Hill (1948) found that after 5 months of use of a penicillin-containing dentifrice by boys in orphanages, there was a somewhat greater decrease in *L. acidophilus* counts than in a group using an ordinary dentifrice. Hill and Kniesner (1949) reported that after one year's use of the penicillin dentifrice by 108 boys there was no smaller increment of new caries than in the 68 boys using the same dentifrice without penicillin, although there was some reduction in the *L. acidophilus* counts.

Zander (1950) reported a statistically significant caries reduction in school children after using a penicillin-containing tooth powder compared with a control group who used the same powder but without the penicillin. They could find no effect on the penicillin sensitivity of streptococci and staphylococci in the throats of the children who had used the penicillin tooth powder.

Chlorophyll: Sodium copper chlorophyllin has been reported to have a stimulating action on cell growth and cell metabolism, but an inhibiting action on the growth of aerobic microorganisms. Because of these properties, Hein and Shafer (1949) made some *in vitro* and animal studies in relation to dental caries. In a saliva-sucrose mixture with added chlorophyll to make a concentration of 1:400, no acid was produced after 24 hours, and only a faint drop in pH was noted after 48 hours. Control mixtures, without the chlorophyll, gave a pH of 3.8–4.1 after 24 hours, and 3.5–3.8 after 48 hours. The difference in the total acid produced by a saliva-carbohydrate mixture with added chlorophyll to make a concentration of 1:2000 and by the same mixture without the chlorophyll was 40–70 per cent. Female rats given a caries-inducing diet with chlorophyll in the drinking water at a concentration of 1:500 showed a 93 per cent reduction, and at a concentration of 1:1000, a 67 per cent reduction in caries as compared with the control animals. No change in the caries was observed in male rats. The authors offer no explanation for this peculiar sex difference. No difference could be ob-

served between the experimental and control groups in weight curves, blood studies, and autopsy studies. Rapp (1949) reports reduction in *L. acidophilus* counts by using a mouth wash or dentifrice containing chlorophyll (water soluble chlorophyll "A"). Guerney and Rapp (1949) found that the same chlorophyll compound had an inhibitory effect on the acid production and on the proteolytic activity in human saliva.

No clinical studies of the effect of chlorophyll on caries frequency have yet been noted. The results of the experimental studies referred to above may be said to be promising and further research should therefore be encouraged. According to Smith (1944) concentrations up to 2 per cent may safely be used in the oral cavity.

Antienzymes

Bacterial enzymes produce acid from carbohydrate; acid production may be controlled by inhibiting the growth of bacteria or preventing the action of enzymes. As previously stated, fluoride has the effect of inactivating the enolase, and thus preventing acid formation. In 1942 Fosdick, Fancher and Calandra discovered that synthetic vitamin K or 2-methyl-1,4-naphthoquinone was an excellent enzyme inhibitor for some of the enzymes involved in the anaerobic degradation of carbohydrates to acids, and when added to a glucose-saliva mixture would prevent acid formation. Burrill, Calandra, Tilden and Fosdick (1945) made some clinical studies with the compound incorporated into chewing gum and reported less caries increment in the group of individuals who used this gum after each meal compared with the group of individuals who used the gum without the quinone addition. The chewing gums contained calcium carbonate which may have neutralized some acid, and the control group also showed reduction in caries increment, making the experiment inconclusive. Fosdick and Calandra (1947) reported that many other quinones, as well as peroxides and aldehydes, have a corresponding antienzymatic action. Among the latter, racemic glyceric aldehyde added to a saliva-glucose mixture in the test tube reduced the acid production to a considerable extent. No clinical studies of its effect on caries frequency have been noted. Shaw (1950) was not able to find any effect in caries-susceptible white rats or cotton rats by adding 0.5, 1.0 or 2.0 per cent of crystalline l-glyceric aldehyde to a purified high sugar ration. However, he suggests that there may have been a difference in the dissociation of the compounds used. Turner and Crowell (1947) report an inhibiting effect of tryptophane on carbohydrate breakdown in saliva, and other amino acids have been shown to possess antienzymatic properties.

THE EXAMINATION FOR DENTAL CARIES

As emphasized in both the review on Nutrition and Dental Caries and the review on Prevalence of Dental Caries, the diagnosis of dental caries may be very difficult and hence often uncertain. This fact must be ascribed to the anatomy of the tooth (the deep fissures and pits), the inaccessibility of the contacting surfaces both visually and by the explorer, and the gradual development of the lesion, making it a matter of choice when a minor change in the surface structure will be recorded as caries. Because of these circumstances, the accumulated caries data of different observers may vary to a considerable extent, even when the same subjects have been examined. The same person examining the same subjects on different occasions may record different results. The reported number of carious lesions, therefore, as a rule will not be accurate. Accuracy will be possible only after the teeth have been extracted and viewed under the microscope. The routine dental examination, especially a field examination, comprising the use of a mouth mirror and a fine explorer, could be greatly improved by a

thorough cleansing of the teeth and subsequent drying. The use of dental floss will aid in exploring approximal enamel lesions. The most dependable tool in detecting caries on the contacting surfaces is the X-ray. However, as clearly demonstrated in an extensive study by Ström (1948), even by this procedure the caries process cannot be diagnosed unless it has penetrated to a certain extent into the enamel. The reason is that the outermost contour of a spherical body disappears in a roentgenogram, and the proximal surfaces of the teeth are more or less spherical. The roentgen findings of Bodecker and Ewen (1937) of the so-called "unilateral" approximal caries, therefore, have to be evaluated very cautiously.

In longitudinal studies intended to determine the yearly increment of carious lesions or the effect of changes in diet or other factors supposedly affecting the caries rate, the most exact results will be obtained when only one person examines and uses the same basis for diagnosis. In most of the studies this has not been the case, and the results obtained must be evaluated accordingly.

The individual, the tooth, and the tooth surface are used as units for expressing the occurrence of caries. The first is too crude to be used as the only method of expressing the caries distribution in a population. Using the tooth surface as the unit should give the most accurate figures for the quantitative destruction of tooth substance, but this method may give the largest error, especially when working with people having had dental treatment. The therapy very often necessitates extending the cavity to surfaces unaffected by caries and the restoration will represent more than the actual number of carious surfaces. Teeth with artificial crowns may constitute a similar source of error. Teeth extracted because of caries may have had from one to five carious surfaces. The counting of three carious surfaces per extracted tooth seems to be more appropriate than the counting of five carious surfaces.

During the period of exfoliation of temporary teeth the absence of such teeth makes the picture uncertain. The omission of missing teeth may give a totally different caries picture than the true one when several of the teeth have been extracted because of caries. A fairly reliable result will be obtained by counting as carious all teeth missing up to the age of 6 and the same for molars and canines up to 10 years of age. Precocious exfoliations, as of the second molar from pressure by the first permanent molar, may of course happen.

There has been found such a high degree of correlation between the DMF teeth and DMF surfaces that recording the DMF rate should offer adequate information for epidemiological studies. However, such correlation probably will not be found in all circumstances; for example, during wartime the reduction in caries in Norwegian children was almost two and a half times as great in the front teeth as in the molars. Studies on this relation are under way (Sunde, 1948).

Among the different systems for grading the extent of caries in the specified tooth, the Swedish "Moulage System" worked out by Westin (1940), Dahlberg (1940) and Lindström (1940) is probably the most comprehensive. For larger studies it seems too complicated and too detailed. However, it has been used in Sweden in epidemiological studies (Westin and Wold (1943)).

In longitudinal studies in children for the purpose of observing the eventual effect of changing different factors, one must remember that the yearly increment of caries may not always be uniform even when the dietary and environmental factors seem to be constant. This is plainly demonstrated by Boyd, Wessels and Cheyne (1950). Among others the age factor enters and necessitates age grouping within fairly narrow limits (1 year), and calls for a longer observation time (at least 2 years) than has been the case in most studies. In age groups above 15 years the yearly increment of

caries seems to be more uniform (Hollander and Dunning (1939)). Another circumstance which dominates the curve of the yearly increment of new caries is of course the number of caries-susceptible tooth surfaces. Especially in comparing the effect of a specific factor in two groups of children, the one having had regular dental treatment and the other not, this point has to be considered.

According to the well established fact that the caries distribution is almost 100 per cent symmetrical, examination of only the right or the left half of the dentition has been practiced during recent years (Westin and Wold (1943), Toverud (1949)). In large scale field studies on caries experience it would be of great advantage if one could limit the dental examination to a still smaller number of teeth. This is a problem which deserves attention.

The heterogeneity of method in conducting clinical examination for dental caries and in tabulating and expressing the data makes it very difficult to obtain a reliable evaluation of the prevalence of dental caries within a country, and even more so to get a world picture of this most prevalent of all diseases. The need is most urgent for a unification of methods for clinical examination and recording and of terms expressing the data. This applies not only to the study of caries prevalence proper, but also to the comparison of extent and value of public health dentistry. This project should constitute a most urgent one for a future Dental Division of the World Health Organization, since relationship has now been established between the World Health Organization and Federation Dentaire Internationale (Rowlett, 1949).

SUMMARY AND CONCLUSIONS

Direct tooth-attacking factors

There is full agreement among investigators of dental caries that bacteria are necessary for production of dental caries. This fact has been confirmed experimentally by caries reduction with penicillin orally as well as by failure to produce caries in germ-free rats fed a cariogenic diet.

There is, however, a controversy between investigators as to whether acidogenic or proteolytic bacteria are the primary agents. The most widely accepted view is that acidogenic bacteria are chiefly, or almost exclusively, responsible for the carious destruction of enamel. Recent studies of enamel caries, however, have provided some evidence in favor of the proteolytic bacteria. Since the enamel contains both organic and inorganic materials, it is likely that both proteolytic and acidogenic bacteria are responsible for enamel caries.

Proponents of the proteolytic theory maintain that the tooth itself furnishes sufficient nutrients for bacterial activity and no extraneous substance is necessary for the initial destruction of the enamel. Proponents of the acid theory claim that the bacteria act chiefly through their enzymes on fermentable carbohydrates constituting a part of the dental plaque, with the resulting acid responsible for the decalcification of the mineral part of the tooth; the organic material, chiefly in the dentin, is destroyed by proteolysis.

Among the investigators of the acid theory group there is agreement on the dominating role of *L. acidophilus*. In consequence of this, the *L. acidophilus* count is widely used as a "caries-susceptible" test. There is strong evidence for the validity of this test as reduced intake of sugar (total carbohydrate) reduces the *L. acidophilus* count and the number of caries-affected teeth. However, since other work does not confirm the correlation between *L. acidophilus*, sugar and caries, further critical studies are needed in this field.

Resistance to Caries

Most investigators agree on the existence of resistance or degrees of susceptibility to dental caries, developed primarily during the formation and maturation of the tooth. One group of investigators claims that the resistance to caries is a *dynamic* operation as long as the tooth has a living pulp, implying vitality of the dental tissue. The dentin is no doubt a vital tissue capable of reacting from the inside to external irritants. Recent studies of the enamel, especially using radioactive isotopes, show that the human enamel is permeable from both sides to different ions. However, no methods used up to this time have demonstrated a reaction in the enamel from the pulpal side to external irritants. Nevertheless, from the structure of the enamel and from its organic connection with living dentin, it must be characterized as a living substance. The use of topically applied fluoride solutions has demonstrated without doubt that the resistance of the enamel may be increased by action from the outside.

It has been shown that several of the vitamins and many minerals, and a normal balance of the endocrine glands, are necessary for a biologically perfect tooth structure, but the nutrients responsible for optimum resistance to tooth decay are not known. When laboratory animals are fed a "natural" diet during the development and maturation of the teeth, their susceptibility to caries is low. The same is true in man, but studies on primitive man show that teeth formed and matured under "natural" conditions will not withstand a later change to civilized food.

The marked reduction in dental caries in children during and after the second World War in war-torn countries seems to strengthen the view that a high caries rate may be reduced by substituting a major part of the high intake of sugar and other refined carbohydrates by less refined foods. Caries reduction by reducing sugar intake reported in other studies may also be interpreted in the same way. However, reduction of acid formation in one way or another without changing the diet has also proved to be beneficial. Also, a high sugar consumption at regular meal times only, together with a good protective diet, has been reported to give a low caries rate in institutionalized children.

More studies are needed before a definite explanation can be given of the role of sugar in the whole caries picture as to (a) the total amount, (b) the distribution of intake during the day, (c) the different forms of carbohydrate and (d) various dietetic conditions.

Much valuable knowledge has been gained concerning the part played by saliva in maintaining dental health, but more work has to be done in this field. The importance of its buffering capacity, concentration of hydroxyapatite, and rate of flow are noteworthy. Also, there is still much to be learned about the influence of diet and the state of nutrition on the quality of saliva.

There is no doubt about the importance of nutrition in the development and maturation of teeth and its bearing on resistance to tooth decay, but to what extent nutrition plays a role during subsequent periods is controversial and needs clarification by further studies.

Despite the fact that much has been added to our knowledge during the last decade, the words of Howe, Bessey and White (1941) are still true: "In the mouth is the handwriting of individual physiological activities. We can interpret comparatively little of what is written. Nevertheless, oral diagnosis has had a beginning and, little by little, we are adding to our store of knowledge. The next step in dentistry is to open the portfolio of facts relating systemic to oral states."

EXPERIMENTAL DENTAL CARIES IN ANIMALS

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CONTENTS

EXPERIMENTAL DENTAL CARIES IN ANIMALS

This study of the literature of dental caries in the rat, hamster and cotton rat has been concerned with etiology. A primary purpose has been to determine to what extent rodents can be used in studies to explain the causes of dental caries in man. The conclusions to be drawn are for caries in rodents and are only suggestive of what may occur in man.

The review concerned with the rat is divided into two main parts. The first treats in a chronological fashion the studies of caries made previous to the discovery by Hoppert, Webber and Canniff (1931, 1932) of the coarse particle factor in the initiation of caries-like lesions in the rat; the second examines in a topical way the use of rodents in caries studies. The early studies are further subdivided into those in which carious lesions were observed and those in which no such defects were found.

The literature concerned with dental caries in the hamster and in the cotton rat is separately considered.

This review does not cover studies in problems of amelogenesis or of mottled enamel. It is a review of reports in which caries was directly sought as the terminal condition or its progress observed after decay had been initiated.

Many reasons for using animals in the study of dental caries may be given, but in lieu of such a listing, the following quotation is given from Rosebury, Karshan and Foley (1935):

> "It is not practicable, for example, to attempt deliberately to produce dental caries in human beings, partly for humanitarian reasons, and partly because the great mass of human beings already have the disease; so that one is obliged to work backward, as it were, toward a means of prevention. In animals, on the other hand, after it becomes possible to produce a pathologic condition experimentally, the disease can then be subjected to rigorous analysis; and if the experimental disease is sufficiently similar to that in man to make it probable that they result from the same or similar causes, the experimental analysis may be expected to illuminate the problem of the human disease."

Rosebury, Karshan and Foley have contributed in an outstanding way to showing that certain lesions in rat teeth are sufficiently similar to dental caries in man that study of such lesions can illuminate the problem of human caries. It is quite worthy of attention that they do not extend their findings directly to man.

The only teeth of limited growth in rats, hamsters and cotton rats are the three molars of each jaw. They are permanent and are not preceded by deciduous teeth. In this these rodent teeth resemble the permanent molars of man. In the absence of other forms of teeth of limited growth, molars of rodents then can in a strict sense be used properly only to illuminate the etiology of caries in the *molars* of man.

Cheyne (1942) has reported the chronology of the development of the molars of the Wistar albino rat and his tabulation is reproduced in Table 1.

Orland (1946a) has given the calcification and eruption times of hamster molars, as shown in Table 2.

The data on calcification and eruption of cotton rat teeth were obtained by Shaw, Shaffer and Soldan (1950) and are shown in Table 3.

For convenience in later reference, the coarse cereal ration used by Hoppert, Webber and Canniff (1931, 1932) is described here. It consists of:

Yellow maize meal	60%
Whole-milk powder	30
Linseed meal	6
Alfalfa meal	3
Sodium chloride	1

TABLE 1

Postnatal chronological development of the fixed dentition of the rat (Cheyne, 1948)

Developmental process	Molar 1	Molar 2	Molar 3
Beginning of apposition of primary dentin (enamel apposition occurs 12–24 hours later)	20th day in utero to birth	2nd–3rd day postnatal	13th–14th day postnatal
Beginning of calcification	Birth to 1st day	2nd–3rd day	15th day
Completion of appositional growth in crown	10th–11th day	12th–13th day	19th–21st day
Beginning of bifurcation of roots	11th–13th day	14th–16th day	24th day
First appearance in oral cavity	19th day	21st–22nd day	35th day
Cessation of root elongation by means of primary dentin	80th–83rd day	90th–95th day	110th–115th day

TABLE 2

Calcification and eruption of hamster molar teeth as observed directly or roentgenographically (Orland, 1948a)

(Figures are expressed in days post-natally)

Hamster molars	Osseous crypt first visible	First evidence of calcification	Crown completely calcified	First evidence of eruption into the oral cavity	Complete eruption; teeth in occlusion	Roots completely calcified
Maxillary 1st molar.....	at birth	2	8	9	8–9	35–40
Mandibular 1st molar...	at birth	2	8	8		25–30
Maxillary 2nd molar....	2	6	13	14	17–18	42–46
Mandibular 2nd molar..	2	5	12	12		36–38
Maxillary 3rd molar....	12	20–22	30–34	32–34	42–46	90–100
Mandibular 3rd molar..	12	18	25–28	29–30		75–85

TABLE 3

Calcification and eruption times for the molar teeth of the cotton rat (Sigmondon hispidus hispidus) (Shaw, Shaffer and Soldan, 1950)

(Figures are expressed in days postnatally)

	Maxillary molars			Mandibular molars		
	First	Second	Third	First	Second	Third
First appearance of dental lamina	Before birth	Before birth	1 to 3	Before birth	Before birth	At birth to 2
Beginning of calcification	Before birth	Before birth to 3	9 to 14	Before birth	Before birth to 2	8 to 12
Completion of appositional growth in crown	3 to 6	7 to 13	26 to 34	3 to 5	6 to 11	24 to 30
Beginning of bifurcation of roots	6 to 9	14 to 18	35 to 40	4 to 8	12 to 18	33 to 40
First appearance in oral cavity	5 to 7	9 to 13	28 to 33	4 to 7	7 to 12	26 to 30
Complete eruption	7 to 12	15 to 20	40 to 54	6 to 10	12 to 19	31 to 42
Root completely formed	25 to 32	50 to 70	90 to 110	22 to 28	45 to 60	70 to 90

This ration is designated in succeeding pages as the "H-W-C ration," an abbreviation which has been often used in the literature. The "simplified H-W-C ration" is the above H-W-C ration with 66 parts of maize and no linseed meal.

Another ration used to initiate caries in rats is Ration 2965 of Steenbock and Black (1925). It is composed of:

Yellow maize	76%
Wheat gluten	20
Calcium carbonate	3
Sodium chloride	1

This is a "rachitogenic" ration, but that designation does not imply in any way that caries resulting from its use is related to rickets.

EARLY STUDIES ON CARIES-LIKE LESIONS IN RATS

Positive Findings

McCollum, Simmonds, Kinney and Grieves in 1922 were the first to record observations of dental caries in the white rat. Their description of caries, supplemented later by Grieves (1922a, b, 1923) using the same data, covers most of the gross features observed by later investigators. They noted the similarities of rat to human teeth and also the differences, and thus pointed out where caries in the two species was to be regarded as identical. Penetration of enamel first, followed by spread in the dentin, followed by fracture, are features they observed that have since been seen by others and by a few in that sequence. Grieves (1922a) said, "Fractures frequently resulted from caries-like lesions, but caries rarely invaded previously fractured surfaces or roots." Most caries was found in first and second lower molars. Interproximal lesions were found only rarely. The investigators observed secondary dentin formation, of which Grieves (1922b) said, "Caries-like lesions are so far from being an internal disturbance, or resorption of enamel and dentin by pulp function, that the pulp actually protects its vascularity by tubular calcification and secondary dentin formation." His statement condenses observation of fact and speculation on function.

McCollum and his coworkers used a stock ration consisting of 30 per cent each of wheat, maize and rolled oats, and 10 per cent flaxseed oil mixture, with whole milk, *ad libitum*, and fresh cabbage or carrots twice a week. "The seed mixture was ground together so finely as to preclude the possibility of the animals picking out and eating the components separately." There was "nearly complete absence of dental lesions in most of the animals from these controls, even though they were twelve to fifteen months old. . . ." Most of the rats which showed caries were on experimental diets which contained maize, presumably finely ground, but, as Rosebury, Karshan and Foley (1934a) have pointed out, maize retained on a 60-mesh sieve, though it may be described as "finely ground," can cause caries in rats. Also, as the rats of McCollum *et al.* were placed on experimental diets at an average age of 50 days, and hence had presumably had access to and been able to consume the maize-containing stock ration for at least 30 days, all animals can be assumed to have been exposed to a caries-initiating diet, albeit of low potency. Subsequent rations may have promoted the development of caries though lacking initiating properties themselves.

Bunting (1925) observed caries in 31 of 66 rats distributed among 10 diets. Of the 38 on maize-containing diets, at least 30 developed caries in contrast to only 1 or 2 of 28 on maize-free rations. He found no caries in rats fed McCollum's stock ration (not clearly identified) for 1½–2 years. Animals on a total meat diet for 11 months had very defective bones but no caries.

As a minor part of a study on calcium in fertility, pregnancy and lactation, Macomber (1927) found cavities in 2 of 12 rats on a ration adequate in calcium and in 7 of 9 on a deficient diet. The ration was of the casein-starch-lard type and the animals were placed

thereon at 150–200 gm. in weight. The origin of the cavities may have been in the stock ration.

Marshall (1927, 1928) described and illustrated caries in rats that had been placed on vitamin-A-deficient rations at weaning for periods generally exceeding one year. At least seven specific cases are described in text or plate. Of these, two showed cavities in the lower teeth and five in the upper. As all of Marshall's rats were placed on the low vitamin A rations at weaning and as neither of the two rations described contained cereal particles of any kind, caries initiation cannot be ascribed in this case to coarse cereal particles.

Shibata (1929) fed young rats of 30 to 60 gm. weight, and older animals 150–200 gm. in weight, rice and greens mixed with 5 to 10 per cent of sucrose, glucose, lactose and maltose. He found caries in 70 to 80 per cent of the animals, the lesions appearing as early as 20 days on the diet and "in a week the dental crown is almost destroyed." The ration was less effective in the older rats. Shibata ascribed the effects of the diets to the added sugars. Provision of bedding straw slowed the rate of development of caries.

The Japanese text of Shibata's paper gave more details of the above experiments. He observed formation of secondary dentin, the occurrence of caries in the upper molars, though with reduced frequency compared to caries in the lower first and second molars. He did not find caries in rats on "rice flour and dextrine," but the table indicated that "white rice and flour" was the diet used. He did not find caries in rats put on caries-producing rations at 300 gm. body weight. The table, which appears only in the Japanese text, shows caries appeared in two rats as early as 13 days.

Shibata (1930) added further comment on the above results. He indicated that the third upper molars are the least affected and that no caries was produced by "polished rice or dextrine."

Kesel (1932) wrote: "I have repeated Shibata's experiment, not once but on four different groups of animals. The results will be published in detail. I have fed these diets to young rats not from twenty to fifty days but to some for more than sixty days. They were markedly undernourished and weighed less at the conclusion of the experiment than at the beginning. *Bacillus acidophilus* was quite regularly found in their mouths; but not one rat developed one decayed molar. As Klein points out, caries can hardly be expected in the rat before 100 days of feeding." No further details have appeared and Kesel (1934) reviewed the papers of a number of students of rat caries but made no mention either of Shibata's work or of his own failure to find caries in rats on Shibata's diets.

Barker (1931) studied lamellae of rat teeth as the point of attack of caries, showing two views of cavity formation in the bottom of the sulcus and development along the dentino-enamel junction after penetration of the enamel via a lamella. He also showed caries of the dentin in the summit of a cusp of a rat molar, though apparently originating in the enamel since a lamella is shown. No statement was made of how the lesion was produced. Presumably, however, the rats were from two diets described by Kronfeld and Barker (1932) one of which was polished rice, three parts, and calcium carbonate, one part; the other, McCollum's rickets-producing ration 3143, containing 33 per cent maize meal. Irradiated ergosterol was given to some of the rats. The authors said, "In fact, caries in rat molars resembles histologically the caries found in human molars. In our experiments, caries was quite frequently observed among the animals fed on McCollum's diet 3143. We could find no relation between the occurrence of caries and deficiency in vitamin D." Also "we believe that the glutinous consistency of the food was more responsible for the occurrence of caries than the presence or absence of vitamin D."

Klein and Shelling (1931b) wrote: "(1) Rats fed diets high in calcium and low in phosphorus, plus large doses of viosterol (irradiated ergosterol), develop caries of the molar teeth. (2) Rats fed diets low in calcium and high in phosphorus, plus large doses of viosterol, do not develop caries of the molar teeth. (3) Rats fed diets adequate in calcium and phosphorus, plus large doses of viosterol, develop caries of the molar teeth. (4) Rats fed diets adequate in calcium and phosphorus, without viosterol, do not develop caries of the molar teeth."

Klein and McCollum (1931) studied 750 rat skulls from three Johns Hopkins University laboratories and concluded that caries did not occur under 60 days of age on a diet which would produce caries in 88 per cent of the rats in 81 to 155 days. They considered it necessary for the rats to be at least 100 days old for any significant degree of caries to appear. (This is in sharp contrast to "caries" in 20 days as found by Shibata.) They recognized different caries-causing potency in four rations, two of which were considered adequate stock rations.

Klein and Harris (1931) examined the mouths of stock rats and showed the presence of "organisms that grow well at pH 4.5 and resemble closely the aciduric organisms of the *Lactobacillus acidophilus* type."

Klein and Shelling (1931a) found caries in "the offspring of healthy females which had been receiving diets adequate in all respects." The stock ration was not disclosed and the "adequacy in all respects" of any ration is always to be challenged. The rats placed on a rachitogenic ration at weaning for 19 days and then given adequate viosterol for 87 days developed dental caries. The rachitogenic ration was not described but probably was high in maize. Conclusions concerning vitamin D and caries, to be inferred from this brief descriptive report, were repudiated by Shelling and Asher (1933).

Blackberg and Berke (1932b) used rats on three rations described as normal, excessive vitamin D, and no vitamin D. The basal ration was a modification of the Steenbock and Black rachitogenic ration No. 2965 in which yellow corn was present to the extent of 73 per cent. The rats were on experiment from 5 weeks of age for a period of 150 days. The number of animals was not stated, but certain features of their data suggest that only a few rats were used. They found most caries with excessive viosterol. They wrote "the characteristics of the carious lesions were clinically and histologically identical with the occlusal variety of human caries. They occurred in the crevices of the occlusal surfaces of the molars, destroying the enamel and mushrooming out at the dentino-enamel junction. With the progress of the destructive process, the adjacent enamel was undermined and the penetration increased. . . ."

Kellogg and Eddy (1932) fed rats lemon pulp as a supplement to the Sherman and Spohn vitamin-B-deficient ration (casein, purified by alcohol, 18; Osborne and Mendel salt mixture, 4; butter fat, 8; cod liver oil, 2; starch 68). They found carious lesions which were studied by Bodecker and Applebaum (1932). These latter authors indicated their belief that the lesions had originated at cusp summits.

Etchells and Devereaux (1932–33) using a sterilized maize-containing ration, added (1) a rat strain of *Lactobacillus acidophilus*, (2) a rough type of the same organism from human caries, (3) a cocco-bacillus from the same human source, and (4) a coccus of fowl origin. Two rats were used in each group and extensive cavities developed in all cases. Comparable cavities appeared in two rats on the coarse unsterilized ration, much less caries in two on the coarse sterilized diet and none in two on the fine. They recovered the organisms from 1, 3 and 4 but the human lactobacillus was found in only small numbers. The sera of rats on No. 1 organism agglutinated the corresponding antigen in 1–256 dilution; the titre in the other cases was of the order of 1–2.

Agnew, Agnew and Tisdall (1932) found caries in every one of 38 rats on a rachitogenic diet containing 78.4 per cent of "finely ground" maize. The test period ranged from 2 to 6 months. They found caries also on a casein-starch-fat type ration deficient in phosphorus, but they did not record the number of rats on the ration, the number with caries, or the duration of the experiment. No caries was found on a similar ration with normal phosphorus and with the starch replaced by cane sugar. They said of the lesions observed: "The histopathological examination, involving ground and decalcified sections, showed typical dental caries, backward decay of enamel, pipe stem tubules, liquefaction foci, coalescence of foci, defense mechanism of pulp (secondary dentine), etc. The macroscopic and microscopic lesions resemble so closely the recognized carious lesions in human teeth as to warrant the assumption that the processes are analogous to human caries."

Agnew, Agnew and Tisdall (1933), in full publication of the work referred to above (1932), used rats presumably all raised on a normal stock ration (Diet 1) consisting of:

Whole wheat	60.0%
Casein	14.0
Milk powder	10.0
Calcium carbonate	1.2
Sodium chloride	0.8
Alfalfa	1.0
Butter	8.0
Wheat germ	5.0

In addition, each animal received 40 gm. per week of spinach or lettuce and 10 gm. of lean meat.

There is no particularly rich fluorine-bearing constituent, though casein may be suspected of being such. (Hodge, Luce-Clausen and Brown (1939).) The lean meat portion is small. Their animals, including both albino and hooded rats, were apparently susceptible to caries, since 70 out of 71 fed modifications of the Steenbock ration No. 2965 developed caries in periods of 2 to 7 months.

Their prevention of caries by phosphorus is not convincing, having been effected in four of seven (or eight?) rats by the addition of sodium pyrophosphate to the Steenbock ration No. 2965 in a period of time not exceeding 6 months. Prevention of microscopic caries in three rats by addition of phosphorus is the bulk of the evidence for assertion that posteruptive dietary phosphorus plays a role in caries. Of this Shelling and Asher (1933) have said, "The efficacy of dietary P in preventing tooth decay cannot be evaluated from the experiments of the Agnews and Tisdall, since in their experiment, in which sodium pyrophosphate was added to an otherwise low-P ration, only eight animals were used."

They found no caries in 365 animals fed the normal diets, presumably largely the stock ration above and also Diet 8, below, with normal Osborne and Mendel (1919) salt mixture, but apparently found a few cases of splitting of cusps with production of "holes of non-carious origin."

Of ten animals fed Diet 8, below, five developed caries in 5½ to 13 months. Nine rats fed the same ration with the addition of vitamin D for a similar period showed no caries. The complete absence of macroscopic caries in rats on the ration without vitamin D indicates the low caries-producing power of the ration. The vitamin D possibly acted to arrest extensive development of the cavities. Diet 8, normal in calcium and low in phosphorus and vitamin D, consisted of:

Cornstarch	62.0%
Casein (alcohol extracted)	18.0
Crisco (hydrogenated cottonseed oil)	10.0
Yeast (dry powdered)	5.0
Salt mixture (Osborne and Mendel, without calcium or phosphorus)	2.5
Calcium carbonate	1.5
Sodium chloride	1.0

with fresh spinach, 100 gm. per animal each week, or machine-dried alfalfa, 2 per cent by weight

Brown and Tisdall (1933) said of the work of Agnew, Agnew and Tisdall, "If phosphorus be very low, vitamin D may delay but cannot prevent the carious process."

Johnston, Kaake and Agnew (1933) studied the oral flora of the rats of Agnew, Agnew and Tisdall (1932, 1933). Their method of swabbing the rat mouth could be only roughly quantitative as shown in the extreme variation in counts of *Lactobacillus acidophilus* obtained at one-minute intervals from the mouth of a single rat. However, they reported high counts in 27 of 30 rats with caries and low counts in the others. Of 153 rats with no evidence of gross macroscopic caries, high counts were found in 109 and low counts in the remainder. They said in conclusion, "The etiologic significance of *L. acidophilus* in the production of dental caries is diminished by the demonstration of a high incidence of this species in the mouths of caries-free rats, as well as in those rats in which caries was produced experimentally." The value of this statement by Johnston, Kaake and Agnew is much impaired by the fact that they did not demonstrate the absence of *microscopic* caries.

Johnston, Kaake and Agnew also reported "that in a series of eighteen animals fed, over a period of many months, a diet containing 62 per cent of cane sugar, caries failed to develop in any animal, although high carbohydrate diets are believed to foster caries. The acidophilus incidence was high in eight and low in ten."

Templin and Steenbock (1933) noted caries in 73 per cent of the lower molars and 66 per cent of the upper teeth of rats fed a ration of 79 parts yellow corn, 20 parts wheat gluten and 1 part sodium chloride. The rats were placed on the ration as adults, presumably having been raised on a ration similar to the following control ration:

Yellow corn	137
Oil meal	29
Crude casein	10
Alfalfa	4
Bone ash	2
Sodium chloride	1
Dried yeast	9
Skim milk powder	72
Butter fat	28

Rats on the control ration had 40 per cent of the lower teeth carious but none of the upper. When the 79 parts corn meal ration was irradiated, the incidence of caries was 65.0 per cent in the lower teeth and 3.3 per cent in the upper. As apparently only 10 rats were in each group, these results cannot be considered of significance. The apparent lowering of caries in this group of 10 rats is possibly because of dilution of the ration with skim milk powder, the change of texture by butter fat, and chance variation. Templin and Steenbock did not describe the lesions. The total number of rats observed seems to have been 30.

Arnim, Clarke, Anderson and Smith (1933) reported 11 cusp and 11 sulci lesions in 41 rats placed on rations that provided approximately 16 mg. of phosphorus and 1 mg. of calcium per day. The rats were on the ration from 35 days of age for 12 to 24 weeks. The authors indicated their belief that the cusp lesions originated by abrasion down to the location of dentin formed on the low salt diets, followed by fracture of this deficient tissue and necrosis of the pulp. They could not account for the sulcus lesions which originated in enamel formed before their experimental regimen.

Negative Findings

Toverud (1923) analyzed the molar teeth of rats that had experienced one or more pregnancies on low calcium diets, and presumably in such manipulation he would have observed caries if any had been produced. Six such rats were observed. They were on a diet of 92 per cent ground wheat,

5 per cent butter, and 3 per cent of Osborne and Mendel (1919) salt devoid of calcium. The ration was fed as a stiff paste made with water.

Mellanby and Killick (1926) said, "McCollum, Simmonds, Kinney and Grieves (1922) in America claim to have produced 'caries-like' lesions in the teeth of rats, but as these animals are very small, and so far we have not succeeded in reproducing the results obtained by the American workers, we sought for another experimental animal." They voiced a valid objection to the rat in that the teeth cannot be satisfactorily examined in the living animal but admitted the same to be true for the rabbit, the animal of their choice. Their use of the word "claim" is difficult to understand in the light of the photographic evidence presented by McCollum, *et al.* If they used rations of a sufficient degree of fineness it is not surprising that they failed to find caries in rats. They produced no caries-like lesions in rabbits' teeth in spite of very poor calcification obtained by dietary means.

Toverud (1926), in a study of nutrition and rat teeth, found no dental defects in over 100 animals on a normal ration containing 92 per cent of ground wheat. "In a group, consisting of 4 rats, fed bread ad libitum and 2½ cc. of calcium-poor milk daily from 3 weeks old to the age of 3½ months, all the members showed occlusal defects in the molars." "The caries-like defects observed varied from small pits in the fissures of the molars, just penetrable by an ordinary dental probe, to wide openings with exposure and decomposition of the pulp."

Maclean (1927) wrote: "Dogs, rabbits and rats were fed on cultures of both *Streptococcus mutans* and *Bacillus acidophilus*, and no caries resulted. Some of the animals were under the care of Mrs. Mellanby, at Sheffield, and were at the same time on a markedly deficient diet; but even in these no caries was induced."

Knowlton (1929–30) maintained 34 rats on a casein-starch-fat type of synthetic diet with variation of vitamin B complex and vitamin D from weaning to from 28 to 50 days. He found no caries in any rat. He considered the rats too young to have developed caries, in accord with Marshall's (1927, 1928) conclusions.

Mellanby (1930) said that she had fed large numbers of rats on varying diets but that macroscopic lesions were found in only a few and bacteria were not always seen in the dentinal tubules. Such organisms as were found were more frequent in rats on a diet deficient in vitamin D.

Krasnow (1932) undertook "to determine the role of fluorine (F) in dental caries" in rats with eight groups of ten animals each. Various additions of sodium fluoride, viosterol and carrots were made to a ration with no coarse cereal particles. No caries data were given.

Rosebury and Karshan (1931a) did not find caries in any one of 60 rats that had been on rachitogenic, scorbutic or high carbohydrate rations from 40 to 60 days. In addition, half these rats received twice a week a gum tragacanth paste bearing a human strain of oral *Bacillus acidophilus*. In a study of the incisors and molars of the rats of the preceding study, Rosebury and Karshan (1931b) found typical rachitic changes in rats that had been on the pertinent rations. There were no cereal particles in any of their rations.

Blackberg and Berke (1932a) used 35 young rats, divided into normal, rachitic and "starved" groups for 25 days. No further description of the rations was given; consequently no decision can be made as to why they found no caries. Their experimental period was very brief for caries development and none is recorded.

Lilly (1932) used rats bred and raised on a stock ration of fresh meat, whole milk, bones, bread and fresh and cooked vegetables. They were placed at 30 days of age on a

ration of:

Starch	53%
Lard	25
Casein	18
Osborne and Mendel salt	4

Cabbage (10 gm.), and brewer's yeast (1 gm.) were fed daily.

This ration was varied by (1) replacement of starch by sucrose, (2) substitution of cabbage by lettuce, and (3) half the animals had "about 1 cc. of a pure culture of *Bacillus acidophilus* forced into the mouth and rubbed over the teeth and gums with a large wire loop three times a week throughout the entire period of feeding." A total of 52 rats was used in an experimental period of 12 months. Sections were prepared of jaws suspected of caries. None was found.

The single section shown by Lilly from a rat on a high carbohydrate diet shows a well defined crack or lamella at the base of a sulcus. The illustration suggests spread of a lesion along the dentino-enamel junction but definite interpretation is not possible. Lilly did not stain the sections. However, such minor lesions would not alter the conclusion to be drawn from Lilly's work, that a plethora of carbohydrate and aciduric organisms is insufficient to produce caries in rats.

A second series of 10 rats was fed by Lilly (1932) the Steenbock rachitogenic ration No. 2965 until death (3 to 11 months) or for one year. No statement was made on how finely the maize of this ration was ground. Again no caries was found. This is apparently contradictory to the findings of others who have used Steenbock ration No. 2965 or slight modifications. An explanation may be surmised in the comminution of the caries-producing rations and in the inherent resistance of the molars of the rats derived from characteristics of (a) the strain of rats or (b) the stock ration. Lilly's stock ration provided fluorine from bones and possibly an unidentified protective agent in fresh meat. The fact that the Steenbock ration, with its content of maize, did not produce caries for Lilly detracts from the value of his conclusions with respect to carbohydrate and bacteria as an initiating combination, suggesting as it does that his rats were highly resistant to caries from any initiating factor.

Lilly's figure 1 shows the extensive attrition that occurs in rats and, in the enamel at the summit of the posterior wall, an irregularity of the enamel contour that may be a fracture which did not decay. But as the preparation is a ground section the nick may be merely an artifact. Lilly and Grace (1932–33) found no caries in groups of 10 rats that were fed rations high in carbohydrates for 6½ months. The carbohydrates used were glucose, lactose and maltose fed at a 66 per cent level; the remainder of the ration was casein 20 per cent, lard 10 per cent, Osborne and Mendel (1919) salt mixture 4 per cent, with 0.3 gm. viosterol per kg. Two groups of 10 rats did not develop caries in 5½ months on rations low in fat, namely, starch 73 per cent, casein 19 per cent, salt mixture 3 per cent and cod liver oil or butter fat 5 per cent.

Whittle, Klein and McCollum (1933) fed rats rations with approximately 78 per cent oatmeal with various supplements. "Neither macro- nor micro-dental caries was found in any rat. Cusp fractures occurred in all groups, but no cusp caries was noted."

Sharpless and McCollum (1933) raised rats on rations which deposited 6 to 25 p.p.m. of fluorine in the bones compared with 10 to 15 times that amount in controls. The fluorine of the teeth was given as less than 5 p.p.m. "The teeth showed no caries and from gross appearances seemed to be perfect." The ration was a casein-fat-starch type with no coarse cereals. It is interesting to note here that McCollum, Simmonds, Becker and Bunting (1925) in a study involving feeding 226 p.p.m. of fluorine to

rats, and in which they discovered mottled enamel in rat incisors, were following a hypothesis that "perhaps a deficiency of fluorine in the food might lead to the formation of teeth which had poor structure, and consequently possess little power to resist the agencies which lead to decay."

Discovery of the Relation of Coarse Particle to Caries in the Rat

In 1931 and 1932 Hoppert, Webber and Canniff (1932) reported the discovery that cereal particle size was a factor in the etiology of dental caries in rat molars. These authors, on failing to find cavities in rats on diets planned to produce caries, examined stock animals for comparison. They found extensive decay. They abandoned their planned studies and sought the cause of caries in the controls. They concluded that the size of maize particles in the ration was the determining factor in caries production. Rations with coarse corn meal induced caries; those with corn meal that passed a 60-mesh screen produced none.

Hoppert, Webber and Canniff (1931) apparently had effected some fractionation of maize in sifting and were accordingly criticized by Klein and McCollum (1931), who suggested that the fine corn meal rations were richer in phosphorus than those containing coarse maize. Hoppert, Webber and Canniff (1932), and also Webber (1932), replied to this criticism in an addendum to their full publication, in which they showed caries in rats on a corn meal ration high in phosphorus compared with an oatmeal-containing ration which did not cause caries. Hoppert, Webber and Canniff ascribed the origin of caries to the impaction of corn meal particles in the sulci of the rat molars, with subsequent fermentation and acid solution of enamel.

Klein and McCollum (1933) agreed that particle size of maize "plays a role in determining the occurrence of dental caries in rats" after finding caries in 22 of 34 rats on the Steenbock and Black rachitogenic ration No. 2965 but none in 7 rats on the same diet ground to pass a 60-mesh screen.

The caries-producing ration of Hoppert, Webber and Canniff, the H-W-C ration described previously, has been used by many subsequent investigators, either with or without the linseed meal. The approximate distribution of the particles of maize reported by Hoppert, *et al.* was: "On a 20-mesh screen 45 per cent; on a 40-mesh screen 30 per cent; on a 60-mesh screen 12 per cent, through a 60-mesh screen 13 per cent." In the study of degree of fineness of the cereal particles their simplified ration was employed, that is with linseed meal replaced by the cereal under consideration.

Hoppert, Webber and Canniff (1932) reported also that replacement of maize by rice induced caries in rats but that oatmeal or hard wheat was ineffective. They stated, however, that soft wheat was more comparable to maize and rice. Webber (1932) indicated that boiled, dried ground potatoes also initiated caries in rats.

It cannot be successfully argued that the H-W-C ration causes caries in rats because it is a deficient ration, because Hoppert, Webber and Canniff (1931, 1932) have clearly shown that by fine grinding alone the caries-producing property of the ration is destroyed. The fine diet is not changed in chemical composition. That a differential deficiency results from failure of digestion and absorption of the maize particles remains to be shown and identified. The whole milk constituent is not altered between the coarse and fine variations of the H-W-C rations.

That the H-W-C ration is deficient in one or more factors is indicated by Cox, Dixon, Matuschak and Walker (1938), who found that when it was used as a ration for reproduction it gave the minimum weaning weight at 21 days (31.9 gm.) compared with 6 other rations with mean weaning weights ranging up to 50.3 gm. That it is also defective with respect to tooth formation is shown by Cox, Matuschak, Dixon, Dodds

and Walker (1939) who found in rats from mothers on the H-W-C ration during pregnancy and lactation: "The incidence of both occlusal and fissure caries is the highest that we have observed on any ration, suggesting that some deficiency existed in the corn meal diet . . ."

The contention of Hoppert, Webber and Canniff "that the caries-producing process is independent of the internal structure of the tooth" is not to be interpreted to mean that alteration of dental structure would not alter the resistance to caries. They made no studies of that phase of the problem, i.e., no alterations of diet were made preceding formation of the teeth. They found "that the addition of liberal amounts of vitamin A, D or C, or of calcium and phosphorus, did not appreciably retard the decay of the teeth."

The observations of Hoppert, Webber and Canniff that the size of the cereal particle is of etiological significance in caries in rats has been confirmed by Klein and McCollum (1933), as noted above, by Shelling and Asher (1933), Rosebury, Karshan and Foley (1933a, b, 1934b, 1935), Bibby and Sedwick (1933), Lilly and Wiley (1934), King (1935), prior to 1936, and by others since.

Discussion and Conclusions Respecting the Early Studies of Caries-like Lesions in Rats

1. In studies of caries-like lesions in rat teeth before the discovery of the coarse cereal factor in the initiation of decay, the gross features of caries in rat molars were described as enamel penetration, spread along the dentino-enamel junction, pipe stem tubules, liquefaction foci, coalescence of foci, secondary dentin formation, backward decay of enamel and fracture presumably after decay was extensive. Interproximal lesions were recorded.

2. The opinion was stated that caries did not follow fracture.

3. Enamel lamellae were indicated as a point of attack.

4. Lesions were observed in both upper and lower molars.

5. The time of earliest appearance of caries was found to be variable, and even as short a time as two or three weeks after exposure to a caries-producing diet.

6. On the basis of histological studies, caries-like lesions in rat molars were considered as similar to caries in man.

7. In general, it may be said that caries was observed if corn or rice particles were present in the ration fed during a time which may be regarded as caries-initiating period. Conversely, if corn or rice were absent from the initiating ration or were finely ground no caries was observed. Oatmeal or hard wheat did not initiate caries.

8. Studies of the posteruptive influence of calcium, phosphorus and vitamins A, D and C and empirical rachitogenic diets on caries incidence were made. None of these conclusions is valid if cereal particle etiology was not considered (Bunting (1935)).

9. Acidogenic, aciduric organisms of the *Lactobacillus acidophilus* type are normally present in the rat mouth and are apparently increased in number with caries.

10. In spite of the normal presence of oral aciduric organisms, long-time feeding of rations very high in starch or the fermentable carbohydrates (glucose, sucrose, maltose, lactose), with or without feeding human strains of *Lactobacillus acidophilus*, was not found to initiate caries in the rat if coarse cereal particles were absent.

11. The ideas of age of the animal and duration of exposure to initiating rations were introduced as factors in the development of dental caries in rats.

12. The idea of the relation of fluorine to dental caries was introduced but no data were given.

The discovery by Hoppert, Webber and Canniff that coarse maize particles induce caries-like lesions in rat molars necessitates, in all studies, a consideration of particle size in matters of etiology.

TYPES OF CARIES-LIKE LESIONS IN RATS

In order to consider the etiology of caries in rat teeth it is advisable first to examine the nature of these lesions. The fact that several types of caries-like lesions in rats have been described suggests that different sets of factors have been operative in the experimental procedures of the authors. The gross descriptive features which are given for the identification of the particular lesions relate to location, tissue attacked, and general appearance.

McCollum, Simmonds, Kinney and Grieves (1922) and Agnew, Agnew and Tisdall (1932) described, as given above, the gross appearance of caries in rats without reference to specific types of lesions. Rosebury, Karshan and Foley (1933b) in particular have emphasized the difference of types of lesions, and certain of their descriptions are given in detail on other pages of this review. They described (a) "fissure caries," (b) lesions originating in the exposed dentin of the cusps and (c) fractures. Klein and McCollum (1932b) described briefly (d) interproximal caries. Cox, Dodds, Dixon and Matuschak (1939) recorded (e) an "opaque" type and (f) caries at the site of the fusion of cusps. Rosebury (1939) and Weisberger, Nelson and Boyle (1940) reported (g) gingival caries and Arnold (1942) has reported (h) cervical caries in the hamster.

A description of these various types of caries-like lesions in the rat and the hamster follows.

(a) *Fissure caries:* This lesion was described by Rosebury, Karshan and Foley at first in four stages. It occurred at the bases of sulci with the initial process either as a diffuse penetration of the enamel or "narrowly through one or more enamel lamellae." In the second stage decay spread along the dentino-enamel junction. The third stage involved penetration of the dentin. The fourth stage referred to loss of continuity of the surface of the enamel. The authors regarded rat "fissure" caries as analogous to fissure caries in man.

(b) *Cusp caries:* Rosebury, Karshan and Foley (1933b) described cusp caries as "a process of dissolution resembling caries of dentin." It was found so infrequently that no further description was given.

(c) *Fractures:* These "lesions" were described by Rosebury, Karshan and Foley (1933b) as occurring in the cusps. The lesions in the advanced state were indistinguishable from advanced "fissure" caries. All advanced decay was assigned to fracture origin by Rosebury *et al.* as a conservative measure and was not included in their index which related to fissure caries.

(d) *Interproximal caries:* As observed by Klein and McCollum (1932b) "the lesion consists of a breaking down of enamel-rod substance, showing the transverse striations of enamel rods . . . incipient enamel-caries in the rat begins very frequently just below the contact point where the approximating surfaces of the molars meet."

(e) *"Opaque" type:* According to Cox, Dodds, Dixon and Matuschak (1939): "Opaque lesions appear initially as white areas which have lost the normal translucency of the enamel. In later stages surface continuity is lost. The sites are at the buccal end of the floor of the mesial sulcus of the lower first molar and similarly in the main sulcus of the third lower molar; also on the gingival line of the buccal-distal angle of the first lower molar and similarly on the buccal-mesial angle of the second lower molar."

(f) *Fused cusp cavities:* As described by Cox, Dodds, Dixon and Matuschak (1939) these cavities "occur lingual to the anterior-posterior axis of the upper first and second molars at the occlusal edge of the mesial walls of the sulci at a line of cusp fusion. In the lower first and second molars they appear in shallow longitudinal buccal fissures in the occlusal surface of the major distal

cusps." (The "center" and "lateral cusp" lesions described by Cox, Dodds, Dixon and Matuschak (1939) may be identical with "fracture" lesions of Rosebury, Karshan and Foley (1933b).)

(g) *Gingival caries:* Rosebury (1939) described proximo-gingival lesions in rats which had been fed rations composed principally of pilot biscuit. He said: "The lesion usually starts at the junction of enamel and cementum just below the gingival termination of the contact area. The gingival papilla is depressed by impaction, in the interdental space, of a mass of debris, probably consisting of food and bacteria. The enamel is usually not primarily involved, the earliest apparent change being infiltration of the cementum and dentin; but the enamel may be undermined as the infectious and distintegrative process spreads along the dentino-enamel junction. The changes characteristic of caries appear in the dentin." Weisberger, Nelson and Boyle (1940) reported caries in rats from which the salivary glands had been removed as: "The carious process, which was possibly primary in the cementum, also involved the enamel and dentine. Secondary dentine was present as a result of the carious process." A severe xerostomia with recession of gingival tissue preceded development of these lesions, the earliest of which were observed in 22 weeks following extirpation of the salivary glands.

(h) *Cervical caries in the hamster:* Arnold (1942) described cervical caries in the hamster as "lesions which occurred on the mesial surface of the first molars, both upper and lower, near the cemento-enamel junction and extending around the cervical portion of the tooth usually more to the lingual than to the buccal." He recorded in a footnote "other questionable areas were observed on the second and third molars" but withheld judgment concerning them.

METHODS OF OBSERVATIONS OF CARIES AND ANALYSIS OF THE DATA

Observation of Caries

Most investigations of caries in rats have reported lesions in unsectioned teeth as they are observed under low power magnification. Hunt and Hoppert (1938, 1939) have examined such lesions in the living rat because they were interested in the earliest appearance of cavities.

Rosebury and his coworkers, Karshan and Foley, have generally examined decalcified and sectioned teeth, though in some studies they have prepared ground sections. They routinely used, for decalcified sections, the teeth of one lower jaw as an estimate of caries in four jaws.

Cox and Dixon (1939a, b) described a method of mounting of all the jaws of 50 or more rats in a hard plaster and grinding them by machine on a rotating carborundum plate so that successive planes could be observed at approximately 0.2 mm. intervals. The teeth were stained in these successive planes with methylene blue and examined for fissure caries as described by Rosebury, Karshan and Foley (1933b).

Gomori (1940) has described a method of staining carious lesions of rat and human teeth that was used by Miller (1938) and Norvold and Armstrong (1943). Essentially it consists of staining teeth fixed in 80 to 95 per cent alcohol or formalin with 0.25 or 0.5 per cent silver nitrate and reduction with sodium thiosulfate. Areas take an intense black stain. The teeth may be examined directly or sectioned after decalcification with sulfosalicylic acid.

Leicester and Schamp (1942) devised a method of clearing rat teeth that consists of simple immersion of the whole dissected jaw in 88 per cent phenol in water at room temperature for 36 hours. The teeth are then examined in such phenol. The various anatomical features, such as dentino-enamel junction and dentinal tubules, are plainly visible. "Carious lesions are easily differentiated since the carious material is yellowish

brown and usually clearly demarcated from the rest of the tooth."

Treatment of Data

Most investigators of carious lesions in rats have reported their quantitative results in terms of number of rats with or without caries or number of teeth decayed per rat or per 100 rats, without specification of the locality or extent of decay. Such methods give gross indications of success of various procedures in altering caries in the rat, but the loss of information obscures the effects of possibly different sets of causes acting on teeth of different ages.

Rosebury, Karshan and Foley (1934b) used an index based on lesions in separate teeth but finally expressed as a single figure which combines all fissure-type lesions. Fracture lesions were treated separately. Their index was the summation of the products of the size and number of the lesions in the teeth of one lower jaw divided by the number of rats in the group. Ten sizes of lesions were recognized. Cox, Dodds, Dixon and Matuschak (1939) evolved a system of scoring caries in rats based upon *empirical* sites of decay. They designated 17 pairs of such sites in which decay occurred which could be examined without sectioning the teeth. They described these "occlusal" cavities as to their location and appearance and numbered them from 1 to 34, inclusively. Similarly, they described 19 pairs of "fissure" caries observed by the "sectioning" method, of Cox and Dixon (1939a, b) and scored in the system of Rosebury, Karshan and Foley (1934b). The locations of the empirical sites of decay are shown in Figure 2.1. Schamp and Leicester (1943) devised an index based on modifications of the descriptions of fissure caries by Rosebury *et al.* (1934b) but giving 11 stages with two degrees of caries in stage 1. Their index was "made up by evaluating the various stages of penetration by the carious process and summating these for the twelve molars of each rat to get an index figure indicative of the total caries present in that animal."

The scoring system of Cox, Dodds, Dixon and Matuschak (1939) has been used by several investigators, but usually with undescribed modifications. Armstrong (1942) and Norvold and Armstrong (1943) have indicated a trend toward a separate treatment of certain areas. They said of the 34 areas of Cox *et al.*, "It was observed that every lesion that could be seen was apparently either a fracture or was closely associated with a broken cusp in that area." They used the Gomori (1940) stain to differentiate fractures from true caries and said, "Because of the difficulty of detection of areas of blackening of the small minor fissures, which are seldom affected, we considered only 20 major fissures of Cox. . . ." Sognnaes (1949a) devised a scoring system for caries in albino rats based on number of carious molars, number of carious areas and size of cavities. He described elsewhere (Sognnaes (1948)) lesions found in rats after three or more months on a ration compounded of sucrose, casein, corn oil, salt mixture and vitamins but devoid of coarse cereal particles.

A system of recording caries by areas, either determined on an arbitrary or empirical basis, has the advantage of directing the attention of the observer to those certain areas and insuring a uniform method of examination. It has the disadvantage, however, of inflexibility to accommodate conditions which cause caries to appear in uncharted areas. For example, the system of Cox *et al.* would probably not provide for recording the gingival caries of Rosebury (1939) or of Weisberger, Nelson and Boyle (1940). Nor does the method of Norvold and Armstrong (1943) provide for all of the "opaque" type of lesions of Cox, Dodds, Dixon and Walker (1939) if conditions are encountered to produce significant numbers of these lesions. Cox *et al.* did not describe lesions in the upper third molars because they found these so infrequently

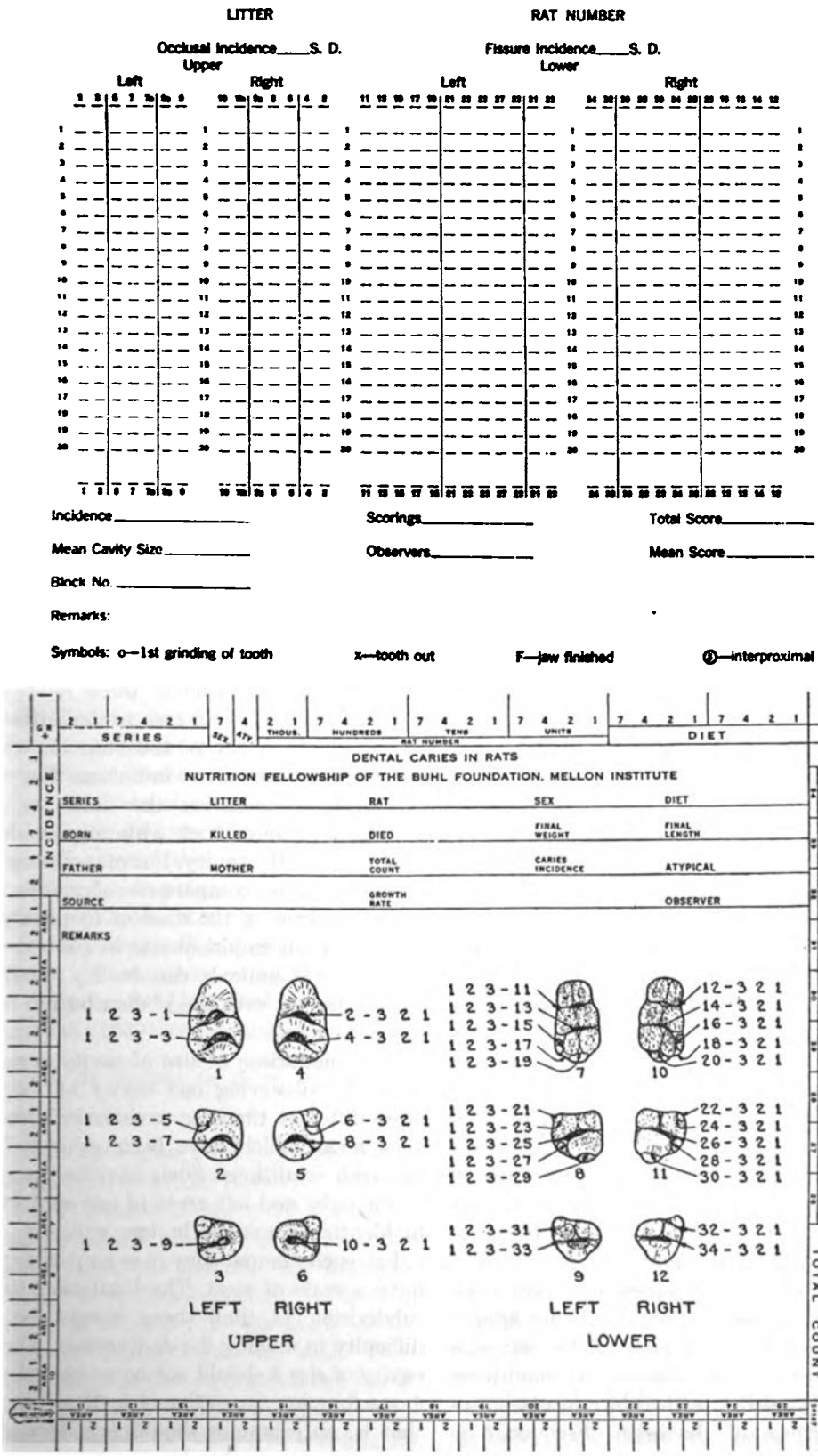

Figure 2.1. Chart for scoring caries in rats. "Occlusal" caries on face; fissure caries on back (Cox, Dodds, Dixon and Matuschak).

in their animals exposed to the H-W-C ration until 77 days of age. Schamp and Leicester (1943) found the highest incidence of caries in these molars. Their rats were on caries-producing rations from the 35th day, the time of eruption of third molars, for 100 days.

The fact that different kinds of lesions have been recorded in rat molars under varied conditions suggests that caries in any given area may have a set of causes distinct from that of caries in another area. If the data of decay in two or more different sites are combined to an index or other single figure expression, the possible differences of the system of causes of the lesions are obscured. Such combinations of data are not justifiable on *a priori* basis, as they constitute summation of unlike things. It is therefore suggested that for the study of etiology of caries the data of incidence of decay (in rat or man) for each pair of empirical areas be kept separate from those of all other areas until, for *a posteriori* reasons, they may be combined.

It would seem on first consideration of such a system that a very large number of pairs of empirical areas would need to be observed. However, this is not necessarily true. For complete coverage of all possibilities it is necessary to have *descriptions* of all lesions that have been observed by all investigators. But by a preliminary survey of his material, a particular investigator can determine what lesions he will consider. This is, in a way, what Norvold and Armstrong (1943) have done. However, if lesions which may be primary fractures are excluded on such considerations as appearance or failure to take a certain stain, there is a possibility that fractures secondary to caries may be missed.

If a criterion of *frequency* is employed, that is if all lesions of any kind that appear in, say, 5 per cent or more of the subjects are retained, some relation to conditions may be found that will yield information as to the cause of the lesion. Frequency is not a part of causation; designation of a lesion as "caries," "fracture," "hypoplasia," or other such term implies etiology, which is itself the objective of the study.

Separate treatment for each condition of an empirical site has a great advantage in the simplicity of analysis of the data. Missing, filled, deciduous, and decayed teeth in human teeth are accommodated by the description for each site, with no influence on the data for other sites, which is the difficulty encountered by all present caries indexes.

There are three different characteristics of carious lesions in teeth to be recorded: (a) description of the cavities as to location, (b) occurrence or non-occurrence, i.e. frequency and (c) size. The latter, in fact, is a part of description, being the quantitative aspect.

There are two phases of caries that require separate analyses. One of these relates to the susceptibility of an area to the initiation of decay; the other is the rate at which decay progresses after initiation. For susceptibility to initiation the data are *frequency of carious attack* without consideration of size of the cavity. For rate of progress it is necessary to compare *size of cavities*.

The analysis of the data of frequency of decay in each empirical site in control and experimental animals can readily be done by comparison with the χ^2 distribution for a fourfold table.

The comparison of size of cavity is made either by observing one cavity at two or more different times or cavities in homologous areas which have been subjected to different conditions. Such cavities may be in the right and left areas of one subject or in identical locations in two subjects.

For such comparisons it is convenient to have a scale of sizes. The limitation to its subdivision is that there should be no difficulty in making the distinctions. Thus a cavity of size 5 should not be estimated as a 4 or a 6 in, say, more than 1 in 10 estimates; that is, an examiner should not deviate in

his reestimate of a series of cavities in more than 10 per cent of his material. If the error rises above such an arbitrary value, the indication is that the scale is too finely divided.

Treatment of the data of size of cavities involves some consideration as to the meaning of numbers assigned to cavity sizes. Of two cavities in the same site, the larger one represents the greater carious attack. It is quite obvious that no series of cavity sizes can be assigned numbers that give a strictly arithmetic measure of the intensity of the caries-causing forces. For example, one cavity may be deep and another shallow, but equal volumes of tissue may have been removed. Past activity might best be expressed by volumes but present activity by the area of the inner surface of the lesion. Thus, since the numbers descriptive of the cavities cannot possibly be in arithmetic series, the operations of addition and division to derive an arithmetic mean give a value with a central tendency, but it is an arithmetic mean of numbers and not of cavity size. Derivation of standard deviations and standard errors of such means are additional questionable operations. For the purpose of curbing such possible misuse of cavity size numbers, it is suggested by the reviewer that letters such as A, B and C be used.

Analysis of the data of cavity size has been discussed by Cox, Dodds, Dixon and Matuschak (1939) as a comparison with the binomial distribution, with $p = q = 0.5$, the simplest of the operations of mathematical statistics.

These authors have considered the problem of how many subjects are necessary in the control and experimental groups for a valid comparison. They concluded that for scoring of caries and analysis of data in their system 67 rats would be required for a statement that a significant difference in caries had occurred if the mean scores differed by 2.

McClure and Arnold (1941) noted familial variation of caries incidence in rats. They estimated on the basis of their data "that roughly 35 litters per group would be required to demonstrate a 10 per cent significant difference between groups made up of litter units."

These two attempts to estimate the number of rats needed for experimentation are typical of the method of solution necessary for this problem.

For certain large differences of response to experimental conditions a small group of animals may suffice. For small differences, hundreds of animals, or empirical sites of decay, may need to be used. The only solution of the problem is analysis of the pertinent data or use of the results of a sufficiently similar experiment. It is likely that many of the investigators of rat caries have used insufficient numbers of animals for valid conclusions.

ETIOLOGY OF CARIES IN RATS, WITH SPECIAL REFERENCE TO COARSE CEREAL PARTICLES

Critical Studies

In 1933 three groups (Shelling and Asher; Bibby and Sedwick; and Rosebury, Karshan and Foley) examined coarse cereal caries in rats and generally found, with the exception of fissure caries, the condition not analogous to human tooth decay and hence of little or no value as accessory to the study of the etiology of dental caries in man. Their methods and conclusions are detailed below.

Rosebury (1932) listed the characteristics of human caries: (1) origin in enamel, (2) occurrence in the young and (3) rapid progress. He required that these characteristics be exhibited by animal caries for significant experimental use. He considered only Shibata (1929) and Barker (1931) as dealing with true caries because (a) evidence presented by other authors was for rats 100 or more days old, and (b) the lesions depicted were too far advanced to determine the nature of origin.

Shelling and Asher (1933) examined 1150 rat heads from different sources and found

generally that maize-containing rations, irrespective of calcium, phosphorus and vitamin D contents, produced caries in rats. If wheat replaced maize, no caries resulted. Three rats that received 20 per cent sand in a wheat-base diet did not develop caries. The investigators reviewed the work of preceding authors and concluded that none had shown evidence of variation in caries incidence in rats other than by variation of the maize content of the rations. It must be pointed out that in thus discarding the rat as an experimental animal in caries studies they did not consider that a preeruptive ration may alter the susceptibility of the teeth to maize-particle caries.

Lilly and Wiley (1934) found that sea sand that passed a 40-mesh sieve, but was retained on a 60-mesh screen, did not induce caries in groups of 12 rats fed at a 5 per cent level in the fine maize ration. At a 10 per cent level 3 of 12 rats had one or more carious teeth in 100 days.

Rosebury, Karshan and Foley (1933a, b) studied caries produced by coarse rice diets in the Long-Evans strain of hooded rats. The rats were bred on "a modified McCollum stock ration," given later by Rosebury and Foley (1934) as:

Whole wheat flour	67.5
Casein, Merck, technical	15.0
Klim (whole dried milk)	10.0
NaCl	1.0
$CaCO_3$	1.5
Cod liver oil	2.0

They established that the causal factor in Shibata's rations (1929) was coarse rice and not the added carbohydrates to which he had ascribed cause.

Rosebury, Karshan and Foley described three distinct types of dental lesions. "One begins in the enamel at the base of the molar fissures. A second results from fracture of the cusp, and a third from a process of dissolution resembling caries of dentin. The second and third begin in the dentin at the cusp summit. Of these lesions only the first appears comparable to human dental caries. Cusp 'caries,' the third type, occurred in only a few instances; was scattered so widely among the groups as to permit no correlation with experimental conditions; and accordingly no further mention of this lesion will be made." "Fissure caries" and "fracture lesions" were described in detail. Four arbitrary stages of fissure caries were discussed. The first consisted of penetration by crack or lamella of the enamel at the bottom of the sulcus. Stage + + showed a crescent spread along the dentino-enamel junction. Stage + + + was penetration of the dentin with liquefaction foci and pulpitis. Stage + + + + was "*loss of surface continuity and formation of a cavity*. This final stage in the process, definitely identifiable as such, has been seen relatively seldom in these preparations. When it occurs grossly, secondary fracture and loss of undermined cusps probably occur, and it is accordingly difficult to decide whether lesions such as that shown resulted from fissure caries or from cusp fracture. The lesion in question is classed in the record as cusp fracture."

Of fracture lesions, Rosebury and his coworkers say: "The primary change in this case is fracture through the dentin, sometimes also through the enamel, of the cusp, generally producing exposure and consequent infection of the pulp before other changes begin in the dentin. Subsequently the dentin undergoes carious changes, further destruction of the tooth ensues, and a large cavity forms rapidly. The most common site of these lesions, in the mandible, was the large subdistal cusp of the first molar, which seems architecturally to bear the brunt of masticating stress. Such cavities, when fully developed, may be seen to occupy a position directly under what had been the center of the subdistal cusp; otherwise, as we have noted, they cannot be distinguished from cavities resulting from fissure caries. All such gross lesions in this work have been classed as fracture cavities. Obviously, although the progress

of these lesions simulates true caries in its secondary stage, its manner of inception differs greatly, both from fissure caries in rats and from dental caries in man. It also differs in etiology from fissure caries, as will be noted below." This last sentence refers probably to the statement: "Fracture lesions . . . were not observed on the adequate diets; therefore, they may be distinguished sharply from the fissure caries on this basis."

As all the diets used by Rosebury, Karshan and Foley were fed posteruptively, the action was probably as they recognized, that of retarding progress of caries in the dentin rather than influencing the initiation of caries in the enamel. Consequently, it would be expected that extension of cavitation and fracture by collapse of unsupported structure would be diminished. Therefore, if it were assumed that all such fractures were consequent upon preceding carious attack, the fracture lesions of Rosebury, Karshan and Foley would take a normal place in the etiology of caries in rats rather than as a purely mechanical phenomenon. McCollum, Simmonds, Kinney and Grieves (1922) and Grieves (1922a) had early concluded that fracture was secondary to caries.

In the experience of Cox and coworkers (unpublished) fissure caries was very frequently observed in the sulcus mesial to the sub-distal cusp referred to by Rosebury, Karshan and Foley, and in many cases loss of enamel and formation of a cavity in the sulcus was observed without loss of the overhanging cusp. The obvious interpretation is that the loss of this sub-distal cusp is by a fissure-caries process followed by fracture.

Cox, Dodds, Dixon and Matuschak (1939) reported a correlation coefficient of +0.64 between "occlusal" and "fissure" caries scores in 443 rats and suggested a common origin of such lesions. They recorded that Rosebury had earlier found fissure lesions by his technique in rat molars in which they could find no "occlusal" lesions. Regression equations, which were not given, had suggested that "as the teeth are improved the occlusal caries disappear first."

The paper of Rosebury, Karshan and Foley (1933b) is notable in that it reports one of the first attempts to use rat caries in the study of the relation of nutrition to caries with a recognition of the coarse cereal factor. Their statement, "The occurrence of fissure caries seems to be independent of the calcified state of the tooth," is unjustified inasmuch as they did not report any modifications of diet in the formative period of the teeth, which would be expected to be the major factor in affecting the "calcified state of the tooth," particularly of the enamel.

The deficient diets used by Rosebury, Karshan and Foley were modeled after Shibata (1929) and contained 90 or more per cent of rice. The protein, mineral and vitamin deficiencies were partially corrected by various additions of wheat gluten, egg albumin, Osborne and Mendel salt mixture, cod liver oil and yeast. They said: "The reduced or delayed incidence of caries on adequate diet, as compared with deficient diets, on the other hand, suggests that dietary deficiency, although not a determining factor in etiology, may increase the rate of the caries process, perhaps by increasing the permeability of the defectively calcified teeth. Changing the deficient diet to make it adequate, however, involved alteration in physical character as well, if only by 'dilution' of the rice particles, presumably the caries-producing ingredient."

Enright (1933) summarized the work of Rosebury, Karshan and Foley by commenting on the report by Rosebury: "(1) He has confirmed the work of Shibata in producing in rat molars, carious lesions simulating typical human caries; (2) he has substantiated the results of Hoppert, Webber, and Canniff in relation to the significance of the size of food particle in caries production; and (3) he has shown that caries can be produced in the rat whether

certain deficiencies in diet are present or absent, but that there is a reduced incidence of decay on the more adequate diet." It should be repeated that the dietary deficiencies were concurrent with initiation and development of caries and not during enamel formation.

Bibby and Sedwick (1933) concluded: "Evidence in practically all lesions indicated that the cavities originated as fractures of a portion of a cusp or fissure wall. About 96 per cent of cavities were found in mandibular first and second molars. The explanation is offered that the peculiar normal morphology of these teeth permits them to be easily fractured by forces from the occlusal surface. Development of such fractures is promoted by presence in the diet of coarse hard particles. It is suggested that heretofore fracture-lesions have been confused with caries, although the conditions resemble each other only in late stages." The conclusions were reached from an examination of the literature of rat caries from their own observations of rats on the Steenbock rachitogenic ration 2865.

Bibby and Sedwick stated that McCollum, Simmonds, Kinney and Grieves (1922) "found lesions resembling caries in teeth of 31 per cent and fractures in 29.5 per cent" but did not notice that McCollum *et al.* said, "Fractures frequently resulted from caries-like lesions, but caries rarely invaded previously fractured surfaces or roots." They did not review Grieves (1922), who repeated the statement of McCollum *et al.* word for word.

There is no doubt that McCollum *et al.* observed cavities in upper teeth, as some of their figures show "caries-like defects . . . in first and second maxillary molars."

The illustrations of Bunting (1925) show such advanced lesions that no reasonable assertion could be made that they developed by gross fracture followed by carious invasion rather than by the reverse order. The same may be said of all the illustrations cited by Bibby and Sedwick (Marshall (1928), Klein and Shelling (1931a), Hoppert, Webber and Canniff (1932), Bodecker and Applebaum (1932), Blackberg and Berke (1932b), Agnew, Agnew and Tisdall (1933)) or authors cited in their addendum (Rosebury, Karshan and Foley (1934b)).

Agnew, Agnew and Tisdall (1933) say concerning their figure 2 which exhibits large cavities of the lower first and second molars, "There is an absence of cusp fracture or other artefact." They stress the undermining of enamel by spread of caries in the dentin. The illustrations of Bibby and Sedwick fail to convince that fracture of walls of sulci leads to carious attack at that site. For example, figures 9 and 10 of Rosebury, Karshan and Foley (1934b) show decay at the dentino-enamel junction originating far enough up the wall of the sulcus to account for the loss of enamel shown by Bibby and Sedwick in their figure 6. Figure 8 of Rosebury *et al.* shows spread of such decay to the summit of the cusp with the enamel still in place.

In the matter of the rare occurrence of decay in maxillary molars, Bibby and Sedwick did not review Marshall (1927), who presents five pertinent figures representing different rats. Four out of five show lesions in maxillary molars. Marshall (1928) does more than mention caries in the upper molars; he illustrates extensive decay in an upper second molar in his figure 2. In the text he describes specifically caries in maxillary molars, as found in two of the rats in his 1927 paper. Bibby and Sedwick said of Agnew, Agnew and Tisdall (1933), "all actual cavities shown in the illustrations are in lower teeth," whereas figure 3 of Agnew *et al.* shows "carious molar in upper jaw of rat. There is extensive loss of the occlusal portion of the crown uncomplicated by fracture."

It is undoubtedly true that caries is less frequent in the upper molars of rats. It is likely that the frequency depends upon the strain of rats used or upon the diet during formation of the teeth. The low incidence

of caries observed by Bibby and Sedwick may be accounted for by such variables. Bibby and Sedwick (1933) found the incidence of caries in rats associated with maize particles in the rations and not affected by cod liver oil present in a post-eruptive rachitogenic ration.

Kearney, Sognnaes and Bibby (1941) sacrificed rats at 2-week intervals in a series of animals on a coarse maize ration up to 100 days. They said: "The teeth were first examined under the binocular microscope and subsequently prepared for histological examination in decalcified and ground sections. Cusp fractures were the dominant findings during the first weeks on this diet. Grossly the typical cavity formation did not occur before the rats had been 12 weeks on the diet. Histological study suggests that the enamel fractures follow a slight undermining of the supporting dentin. Lesions typical of fissure caries were relatively late and rare in occurrence."

Norvold and Armstrong (1943), in studies of fluorine and caries in rats, used the Gomori (1940) method of staining to distinguish carious from fracture changes. They said: "By an area of decalcification is meant any lesion of the enamel of the unfractured caries-susceptible major fissures that can be detected because of the black color which it takes on during the staining process. These alterations of continuity of enamel, in our opinion, are the only lesions of rat molar teeth which are comparable to human occlusal caries." They said further: "It has been suggested that the cusp fractures in the rat molar teeth occur chiefly after the cusps have been undermined with caries and that therefore caries was the primary factor in bringing about these fractures. Our observations do not support this premise."

Rosebury (1933) in a general review of dental caries, said of rats: "At present, indications from such work in relation to etiology suggest that dental caries in rats depends on the physical character of the diet, with rachitic or other deficiency perhaps an augmenting or accelerating factor; but the findings are as yet too recent and too incomplete to warrant their unquestioned acceptance." Rosebury (1934) reiterated the above conclusions and adhered to them in his publications with Karshan and Foley and in other reviews (1935–1939).

Noyes (1933) had said: "It has seemed that much of the experimental work on diet has been too far detached from the study of caries in the human mouth. The rat is an animal normally immune to caries and to produce 'holes in his teeth' that look like caries by feeding him abnormal diets seems unlikely to give us information that will be of value in treating or combating the disease".

Cowgill (1934) in reviewing phosphorus and caries did not accept the coarse cereal etiology of rat caries but inclined to the deficiency explanation with especial reference to phosphorus.

King (1935) with a ration described as "coarse ground" found caries in 7 of 10 rats. His rations described as "fine ground" do not seem to have been very fine as reference is made only to a 20-mesh sieve and the particle size shown in his figure 8 definitely indicates the maize was not of the degree of fineness associated with freedom from caries by previous authors. He said all rations were retained in close contact to the teeth and hence this factor seemed of no significance. However, with the brown rice ration of Shibata (1929) and of Rosebury, Karshan and Foley (1933b) he found caries in 46 of 55 rats, with most of the lesions in the lower teeth. King designated a lesion as carious only when it showed the presence of gram-positive microorganisms in the dentin in decalcified sections.

King noted the forward tilt of the cusps of the lower molars and suggested that "the cusp lesions are due to fracture of the enamel in those situations where this brittle tissue is relatively unsupported by the more elastic dentine." This explanation is similar

to that of Bibby and Sedwick (1933). King does not show sections to illustrate this concept but gives only a diagram; Bibby and Sedwick likewise did not present factual evidence of *gross* fracture preceding caries. Similarly, both ground and decalcified sections purporting to show a "more or less diffuse area of enamel decalcification" show lesions so far advanced that there is no indication of their origin.

King suggested also that fissure caries of rats is initiated by fracture forces originating in wedging hard particles in the deep sulci of the teeth. He advanced theoretical reasons for these stresses being greater for larger particles and deeper and narrower sulci.

King discussed the relation of cusp and fissure caries of rats to interproximal and fissure caries in man. He suggested that "the somewhat bulbous shape of the approximal surfaces" of human teeth "tends to lessen the support given to the enamel from the underlying dentine at the contact points, and thus renders the former tissue more susceptible to mechanical injuries." He similarly related fissure caries of the two species because "it is well known that in man the shallower the fissure the less susceptible it is to decay." He stated: "It would therefore appear that the initial stages of the carious process in the rat and in man are associated with the occurrence of structural defects—developmental or otherwise—in the enamel. Moreover, in both instances enamel decalcification, cavitation with secondary fracture of the cavity walls, decalcification and bacterial invasion of the dentinal tubes, formation of liquefaction foci, and, finally, infection of the tooth pulp are characteristic sequelae." He indicates, however, that gross fracture is effective in the rat but only enamel cracks in man. King's suggestions were made as only tentative.

Applebaum and Adam (1938a, b) using the Grenz ray, found radiolucent zones near "cracks" or "lamellae" in rat teeth which had been exposed to coarse cereal rations but not in the case of similar structures in control rats. "It cannot be said that this study settles the question whether the structures seen in rat jaw sections are actually cracks induced mechanically or lamellae present as developmental structures. The presence of such structures in rat jaws without caries seems to invalidate the idea that they initiate the process."

Rosebury (1935) questioned the validity of the assumption by King that fissure caries in rats arises by traumatic forces and pointed out that King "appears to overlook in this connection his own data on the occurrence of 'diffuse' lesions, in the production of which it is difficult to conceive of the operation of traumatic influences." Rosebury commented on King's further evidence of the coarse particle etiology of rat caries and said, "In all instances with which the writer is familiar, either this mechanism is clearly indicated by the data given, or the reasonable inference that it was operative is left open by incomplete presentation of the essential facts."

King (1936) in a review stated: "In some further rat experiments, the present writer has found that food, containing particles of size and hardness incapable of producing lesions in well-calcified teeth, may yet cause the disease in animals whose teeth have been rendered developmentally defective by feeding their parents on deficient rations. Besides the mechanical factors in rat 'caries,' food stagnation and acid production also play a part (Johnston, Kaake and Agnew, (1933)). Since, however, the amount of food debris retained about the teeth and in their fissures did not materially differ in any of the diet groups referred to above, it seems probable that chemico-parasitic agencies may only take effect when the teeth have been previously injured by mechanical means."

"On the basis of this work, it is suggested that mechanical factors may perhaps play some part in the etiology of the carious process in man." "... it seems possible

that in man, too, defectively calcified enamel in the region of the approximal contact points, where less support is obtained from the dentin, and at the base of deep fissures, where the disruptive stresses are greatest, might in time become injured during the mastication of the usual soft 'civilized' food."

Rosebury (1939) described proximo-gingival caries as well as the fissure type in rats fed for periods up to 100 days with pilot biscuit. Four varieties of biscuit were used, two hard, one medium and one soft. Results were compared with those from a ration of 82 per cent coarse maize and 18 per cent saccharose. The latter amount of sugar was also used with the biscuit.

The proximo-gingival caries was initiated in the cementum at the cemento-enamel junction. In a single case "a lesion developed on the exposed mesial surface of a mandibular first molar." Finely ground hard biscuit produced the highest incidence of this type caries; corn meal, relatively very little decay. No caries of any kind was produced by the soft biscuit. Coarsely ground hard biscuit resembled corn meal in producing appreciable fissure caries but *no lesions of the cusp fracture type*. Rosebury concluded, therefore, that particle hardness is an important factor in both types of lesions but that fineness seems to operate in opposite ways.

Rosebury and Karshan (1939) had given evidence "which incriminates pilot biscuit as a cause of dental caries among Eskimos." These papers thus present for the first time an identical causal agent for caries in the two species. Previous conclusions on the similarity had been based mainly on histological evidence. Rosebury said: "On the whole the parallel between dental fissure caries in man and its experimental counterpart now seems fully satisfactory. For pilot biscuit, physically and chemically similar to the raw cereal particles, is in itself a foodstuff used by man, and may moreover be considered representative of a widely used class of human foods with similar composition and physical characteristics."

Rosebury (1938) reviewed experimental dental caries in rats in a summation of the knowledge to that time. The following is quoted from his latest statement in a review in 1939: "Dental caries in rats is not caused by a high-carbohydrate diet, by a potentially acid diet, or by deficiency of any dietary component; nor is it prevented by dietary adequacy or by feeding any of the components of an adequate diet. It is not due to soft foods, and never develops when the whole diet is of soft consistency. It need not be present in animals with severe rickets or other marked nutritional disturbances; it may be present in normal breeding stock. It is caused primarily by the presence in the diet of hard, compact particles of carbohydrate-rich food, such as coarsely ground raw corn or rice. These food particles, because of their hardness, become forcibly impacted in the fissures of the molars under the heavy occlusal pressure required to crush them. Being compact, they do not soften readily on contact with saliva. In the fissure, since they are rich in carbohydrate, they evidently undergo fermentation through bacterial action; and the rest of the process follows Miller." (W. D. Miller) "With identical diets in which the corn or rice is finely pulverized or cooked soft, without other changes, no caries whatever develops. In these instances food impaction still occurs, as can easily be determined; but in the absence of the forcible wedging which occurs with the larger or harder particles, the finely divided or soft foods are evidently readily removed from the fissures by the next food mass that comes along, or by the movements of tongue and cheeks, and do not remain long enough to do any damage. In fissure caries in rats, the wedged plug of fermenting grain is the 'plaque' of caries.

"Dental caries in rats, then, is due directly to the forcible impaction in the molar fissures of hard, compact, carbohydrate-rich

foods. This much is true quite irrespective of other dietary conditions. But these other dietary conditions have secondary effects: although in themselves they neither produce nor prevent caries, they may to a limited degree increase the amount of caries among a group of animals. Although sugar alone does not produce caries, addition of sugar to a coarse corn or rice diet usually increases its caries-producing effects. More caries develops on a grossly deficient diet than on a fully adequate diet, although caries is not prevented by the adequate diet. The limited reduction of caries which we call the 'adequate diet effect' can be produced by vitamin D alone, by fats or fatty oils alone, and probably by other single dietary factors. There is no reason to believe that any amount of any of these factors, or any combination of them, is capable of preventing caries. There is a minimal level below which caries can be reduced only by elimination of the primary cause—by grinding or cooking the rice or corn, or by replacing it with finely divided or soft foods."

McClure and Arnold (1941) said: "It is not improbable that the efficacy of the coarse particles of corn meal or rice may as well be referred to as a food impaction phenomenon. In fact the prevention of rat caries by fluorine in itself may be taken as evidence against the belief that a mechanical factor is the essential feature of these caries-producing diets. These facts, by assumption at least, relate the etiology of rat caries to that of human dental caries." As McClure and Arnold's "prevention" was by 125 p.p.m. of fluorine, it is possible that prevention was complicated by arrest of progress of caries through lessening of acid formation by bacteria.

Cox (1937a) wrote: "Experimental rat caries produced by corn meal is said to be of traumatic origin and thus of no certain analogy to human tooth decay. Unpublished data obtained to date, however, indicate that the incidence of the corn-meal type of caries in young rats can be reduced significantly by the dietary regimen of the mother. The effect may be merely a manifestation of a mechanically stronger tooth; it may be a true resistance to dental caries. It has also been found that caries in rats can be arrested or promoted at will by change of the diet. Here the findings are independent of the cause of the lesions, and, as decay is occurring in tooth substance, the results are most likely directly applicable in arrest of human caries."

Sognnaes (1941b) produced caries in rats with the H-W-C ration, used for 100 days, and then gave them a stock ration of "finely ground fox chow" for 2 months. He found "the caries process had not only stopped but . . . the exposed dentin was found hard and polished." "The dentin had a sclerotic appearance and beneath every place where the outer part of the tooth had been subject to attack a new thick layer of secondary dentin had been laid down. . . ." Sognnaes suggested use of this histological feature in the study of "dietary and other factors involved in the control of dental caries."

Van Huysen and Vincent (1945) examined caries produced in rats by corn meal which passed through a 20-mesh and remained on a 40-mesh screen. The teeth were then ground in a plane from the distal to the mesial surfaces and the nature of the carious lesions observed under 12 diameters magnification. "It was concluded from this study that this diet will not cause fracture unless the cusp is first undermined by caries."

Braunschneider, Hunt and Hoppert (1948), in a study of age in relation to caries development in rats, found that 11 of 15 of their caries-susceptible strain developed cavities in less than 150 days of age on a "fine" rice diet. When rice flour prepared by passage through bolting cloth was used, after 150 days of age in 92 animals, 6 developed caries in 91, 120, 139, 142, 147 and 147 days, respectively. The "fine" rice ration had 56 per cent of the particles

retained on a 40-mesh screen and only 10 per cent passed a 100-mesh screen.

Schlack, Gerende, Berzinskas, Taylor and Mullins (1949) concluded from a study of five litters of albino rats with 3 males and 3 females each, with one rat of each sex from each litter, on (a) Purina Lab Chow, (b) coarse corn, and (c) the sucrose ration of Shaw, Schweigert, McIntire, Elvehjem and Phillips (1945) that sex and litter differences on caries were insignificant. Coarse corn was most effective in inducing caries and the Lab Chow the least. Slight if any correlation was found with weight gain, food, and water consumption.

Sognnaes (1941c, d) studied the masticatory efficiency of rats following the observation of undigested maize particles in the feces of animals on the H-W-C ration. He fed ground maize retained on a 20-mesh screen to rats that had been fasted overnight. The rats were sacrificed and the stomach contents dried and sifted. The percentages of maize from the stomach retained on a 20-mesh sieve were as follows:

3 normal young rats	1.9
2 normal adult rats	7.6
2 rachitic young rats	33.3
2 fluorosed adult rats	5.5
2 rats on H-W-C ration 50 days	14.2
10 rats on H-W-C ration 100 days	43.3
8 rats on H-W-C ration 150 days	63.6
2 rats without maxillary molars	96.3

He said: "A few of the rats which had not developed any severity of carious lesions after 100 days on the caries-producing diet, still were unable to masticate coarse corn properly. This may suggest that the rats for some reason get tired in their attempts to masticate the coarse-corn diet over a longer period of time." He did not consider that pain may have deterred mastication and that thus less caries development by fracture would have occurred. The teeth were not sectioned to determine how much the pulps were involved. The low efficiency of the rachitic rats was explained by weakened bone structure.

The rats, given 3 mg. of fluorine daily as potassium fluoride, showed, if anything, a slightly greater strength of the gastrocnemius group of leg muscles and hence weakened muscles of the jaw could not account for reduced incidence of caries in rats fed the H-W-C ration with added fluorine (1941e).

Sognnaes (1941f) extracted maxillary molars of 50-day-old rats and placed the animals on the H-W-C ration for 100 days. Those with all upper molars removed (6 rats) developed no caries in the remaining teeth. Those which were fed the ration for 30 days (4 rats) and then were operated upon showed a few cavities. Five rats with the right upper molars removed showed moderate caries in the lower left teeth. Another group of 5 similarly treated after 30 days was similarly affected. Controls (11 rats) had about twice the involvement of the 2 preceding groups. The growth of these rats was not affected by the reduced masticating efficiency. Sognnaes suggests that growth effects might have been observed with more animals.

The indications of this series of studies by Sognnaes are that "environmental factors in the mouth arising from mastication of the coarse corn particles are important at least in initiating this type of experimental rat caries." The extraction studies after 30 days suggest that promotion of cavitation in the rat is also influenced by mastication.

Ginn (1942) extracted the right maxillary molars of rats which had been on a caries-producing diet for 100 days and continued them for an additional 60 days. "The left molars, where the opposing teeth were present throughout the experimental period, showed a much higher incidence of caries." "These results seem to indicate that local environmental factors are important in the progress of the carious lesions in the rat, and that the most important factor

concerned might possibly be mechanical in nature."

Sognnaes (1948) reared a litter of nine rats to 26 days of age on Purina laboratory chow. They were then transferred to a ration containing 67 per cent granulated sucrose, 24 per cent casein, 5 per cent corn oil and 4 per cent salt mixture with supplements of vitamins and liver concentrate. The first and second maxillary molars were extracted at 30 days of age. After 75 days on the ration five of the rats were sacrificed. Four of these animals showed cavities in the unopposed teeth and one showed decay in an opposed molar. In the four remaining rats, killed after 130 days, the lesions were about equally distributed. Coarse dextrin substituted for sucrose produced fewer carious lesions.

Hunt and Hoppert (1948) found that when the upper first molar was missing because of damage, the appearance of a cavity in the first lower molar of a rat was delayed. Following extraction of the upper molar at 35 days of age, the appearance of a cavity in the lower first molar was similarly long delayed. They began the use of a finer ground rice in their studies of heredity in rats, with the result that the time of appearance of cavities in the first lower molar rose from 18 and 13 days in the 14th and 15th generations to 43 and 32 days, respectively, in the 16th and 17th generations.

Wheatcroft, Schlack, Gerende, Taylor and Berzinskas (1949) from the examination of 153 male and 179 female albino rats on the H-W-C ration for 100 days after weaning, found that bilateral symmetry of decay occurred with a high degree of probability.

Discussion

There can be no doubt that coarse cereal particles initiate lesions in rat molars that simulate human caries. This initiation is not dependent upon adequacy of the diet since by fine grinding of the maize or rice the initiating power of the ration is lost, with no change in the chemical composition. As a matter of fact, the failure to digest much of the coarsely ground corn or rice might actually result in somewhat improved nutrition of rats kept on the coarse diets because relatively more milk would be utilized. The extent to which supplementation of coarse cereal diets retards *cavity* development will be examined in succeeding pages, but the effects are probably not related to initiation of the lesion. It is indicated (Ginn 1942) directly that particle size is also a factor in increase of cavity size by fracturing undermined structures, and there is fairly general agreement that this secondary fracturing occurs.

A factor of uncertain significance, but one which must be considered until more evidence is available, is the influence of particle size upon intestinal synthesis of nutrients that can be absorbed by the rat and utilized for protection against caries.

There should be a distinction made as to the size of a fracture in the initiation of caries. Gross fracture of teeth in man does not lead to caries, but the possibility that micro-fracture may be an initiating factor has not been eliminated. Similarly in the rat, it appears from the experiments with sand and the observation by staining methods of non-carious fractures that gross fracture does not initiate decay. Micro-fracture remains a possible explanation of the initiation of caries by coarse particles.

In a discussion of etiology of rat caries it is proper to draw upon knowledge of human caries, but, of course, the purpose of rat studies is to illuminate the etiology of caries in man. Since it is not known precisely why some people remain entirely free of caries, though it is fairly generally agreed that removal of dental tissues in caries is a result of bacterial action, it would seem that it is the mechanism of *initiation* of caries in man that is not understood. Therefore, it seems hardly likely that the initiation of coarse cereal caries in the rat can be explained by present knowledge of caries in man.

On the other hand, King's suggestion that mechanical factors may play a part in the initiation of human caries is a logical process of reasoning from the known to the unknown. For this reason it is desirable that further study be made of the mechanism of initiation of caries in the rat.

There is some suggestion that preeruptive diet may govern the extent of caries in rats subsequently exposed to coarse cereal diets. Such variations are difficult to explain on a purely local environment theory of caries initiation. In view of the dominant role of particle size in initiation of caries in rats it is obligatory in any future reports that the nature and size of particles be stated. Since coarse cereals promote enlargement of cavities, studies of progress of caries should be made with rations that are finely ground or contain no cereals.

Conclusions

1. Caries-like lesions are initiated in young rats by rations in which coarse particles of maize or rice are present.

2. If the maize or rice is fine enough to pass a 60-mesh sieve no tooth decay will be initiated in the normal young rat, under the conditions of most tests that have been reported.

3. Mere fracturing is probably not the cause of caries in rats, since coarse sand is not as effective as cereals and many fractures found in rat molars are not carious.

4. The cause of caries in rats may be by fracture or by impaction of food particles to provide a protected area for bacterial action.

5. Susceptibility of rats to coarse cereal caries varies, and it is possible that this variation may be due in part to diet during formation of the teeth.

THE BACTERIOLOGY OF DENTAL CARIES IN RATS

It has been noted that Mellanby (1930) and King (1935) considered lesions in rat teeth carious only if characteristic microorganisms were found in the fissures. Klein and Harris (1931) and Kesel (1932) found bacteria of the *L. acidophilus* type regularly present in the mouths of rats without caries. Johnston, Kaake and Agnew (1933) found the counts of *L. acidophilus* roughly proportional to caries as induced by coarse cereals. Maclean (1927), Rosebury and Karshan (1931a), Lilly (1932) and Etchells and Devereaux (1932–33) failed to induce caries in rats by feeding strains of organisms associated with human caries, with or without fermentable carbohydrates.

Jay, Crowley, Hadley and Bunting (1933) attacked Lilly's (1932) finding that cultures of *B. acidophilus* and carbohydrates do not cause caries in rats. Details of their experiment are meager but apparently they used two rats only on each of two high carbohydrate diets and gave a smooth and rough strain on each diet. The rats had previously been on an undescribed finely ground diet, presumably containing maize, for 9 months. There is no evidence that feeding of the cultures was for more than two 5-day periods followed by test 2 days later, a total of eight such tests on four rats. They criticized Lilly in that he did not prove that the bacteria he fed became established in the rat mouths. However, "In bacteriologic study of rats caries on the Hoppert diets, it was found that a type of organism was present in the mouth which was distinctly different from that commonly found in rats. The typical organism of the rat is a thick rod having colonies that are more or less rough. We have rarely recovered the characteristic slender palisaded rods usually associated with dental caries from a caries-free rat. In rats on the Hoppert diet, the flora was predominantly aciduric and *B. acidophilus* occurred in colonies more closely resembling the smooth types. The flora of the rat can be changed at will by changing the diet. All of the cultures made from rats on the Hoppert diet were positive for *B. acidophilus* and the S colonies predomi-

nated. When the rats were taken off the Hoppert diet and placed on the sugar-free diet, the flora changed decidedly within one month. Two of the rats had become negative for *B. acidophilus*, and S colonies were seen only twice in the remaining rats. It is interesting to note that the flora of the rats on the Hoppert diet is the same whether the diet is of the fine or the coarse variety, although use of the coarse diet is much more conducive to the formation of caries."

For the above experiment six rats were used. The sugar-free diet was:

Unpurified casein	74%
Lard	20
Salt mixture (McCollum)	4
Agar	2

with supplements of lettuce, yeast and cod liver oil. The H-W-C diet was fed for 2 weeks. Whether caries was observed is not stated.

These authors on p. 2140 of their paper speak of "caries-immune rats" and of "factors in immunity against dental caries in rats." To test immunization of rats against caries, they used three vaccines prepared respectively from R, S and R and S strains of *B. acidophilus*. These were injected into a total of nine rats, three for each vaccine over a period of 10 weeks. The rats were on the H-W-C ration. "Those that received the rough vaccine had no caries; whereas, the remaining six had an unusual amount of caries." But "strangely enough, the serums of all the animals were entirely devoid of agglutinins. This was certainly an indication that the vaccine had not been antigenic for the rats and that the failure of some of the animals to develop dental caries was due to some circumstance other than use of the vaccine." Jay, Crowley, Hadley and Bunting apparently considered that caries in rats was true caries.

Rosebury, Karshan and Foley (1934a) fed freshly isolated smooth strains of human oral lactobacilli to rats on Shibata's diets. They concluded that addition of human-mouth lactobacilli to deficient or adequate caries-producing diets did not in either case increase incidence of caries.

Rosebury, Foley and Greenberg (1934) injected suspensions of lactobacilli into rats for a period of 4 weeks after weaning. Fourteen injections were made. The rats were on variations of the Shibata coarse rice diets. Results of agglutinin tests "were not convincing because of apparent inagglutinability of the human strain and autoagglutination of the rat strains." Caries in the few rats used did not seem altered by the injections. They concluded: "The results do not suggest that immunization with living lactobacilli protects against experimental caries in rats."

Parsons, McCollum and Frobisher (1946a) cultured four strains of lactobacilli and five strains of acidogenic cocci from the crypts and fissures of rat teeth. From these organisms they prepared pooled cultures of lactobacilli and cocci and injected them into rats for the purpose of immunization of the animals against these types of organisms. "No evidence was found that repeated subcutaneous and intraperitoneal injections with lactobacilli and acidogenic cocci had any effect whatever on the numbers of these organisms found in the crypts and fissures of the teeth of the rats."

Parsons, McCollum and Frobisher (1946b) fed rats for 1, 11, 18 and 25 days and some for at least 100 days on 10, 20, 40 and 60 per cent levels of sucrose, lactose and raw and cooked starch. The flora of the teeth was examined for nonacidogenic cocci, acidogenic cocci and lactobacilli. They found the flora was quickly stabilized. "The addition of 10 per cent or more of any carbohydrate" to a carbohydrate-free control ration "greatly increased the numbers of acidogenic cocci in the crypts and fissures of the teeth. While lactose and raw starch favored growth of lactobacilli to some extent, these organisms were never found to an extent greater than one quarter of

the total number of acidogenic cocci, and usually in much smaller numbers. Yeasts and strictly anaerobic bacteria were not found in significant numbers." Nonacidogenic cocci remained largely unchanged in number.

Rosebury, Foley, Greenberg and Pollock (1934) found no difference in the oral flora of eight rats which for 8 weeks wore a device to prevent access to feces when compared with four litter mate controls.

Lyons (1935b) used rats bred on the H-W-C ration and transferred at an average weight of 66 gm. to a test ration consistng of:

Rice	63%
Oat meal	15
Alfalfa leaf meal	10
Dried yeast	5
Casein	5
Sodium chloride	1
Calcium chloride	1

The rice was ground until 52 per cent passed through a #20 screen. Lyons sterilized this ration and then inoculated portions with various acidogenic organisms. Two series of 16 rats were used, with generally 2 rats to each experiment. A second series of 12 rats was divided into 6 groups of 2 rats. A third series used 16 rats in 5 groups. The experimental period was 8 weeks. Some putrefactive bacteria were included. Lyons ascribed variations in dental caries to attenuation of certain cultures of *Lactobacillus acidophilus* and to antibiosis.

Harrison (1938) found that the incidence of streptococci in mouths of Miller's rats (1938) increased with increase of carious lesions, but that the incidence of lactobacilli dropped. He suggested the greater association of streptococci with caries of the dentin.

Harrison (1940a) studied the flora swabbed from the teeth of rats that were on an oatmeal-containing non-caries-producing ration (Miller, 1938). He found lactobacilli in all cultures and streptococci in about a third. The streptococci outnumbered the lactobacilli when found and about half were strongly acidogenic. Of rat caries he said: "In the first place it seems probable that, even though there may be marked physical differences in various contributory features, the fundamental bacteriological phenomena are essentially the same. Secondly, if the underlying phenomena are the same they can be studied in animals under a variety of controlled experimental conditions which can be approximated only with difficulty if at all in human populations."

Harrison (1940b) studied the changes of flora on the surfaces of rat teeth during the development of coarse cereal caries. The responses of the flora to increase in caries and to the effects of inhibitors, fluorides and iodoacetic acid suggested "(1) that the lactobacilli are associated with experimental dental caries as etiological agents, not as secondary invaders which are increased because the lesions provide a more favorable environment, and (2) experimental caries is prevented by inhibition of the growth and activity of these organisms." Harrison emphasized the fact that the method of swabbing the teeth could not effect collection of material from deep sulci or cavities. No difference of types of flora was found between rats with or without caries.

Harrison (1940c) cultured the flora from material in the cavities collected post mortem, of rats from coarse cereal diets. He found lactobacilli present in only three lesions and each of these was caries of enamel only. In dentinal caries "streptococci comprised over 60 per cent of the total flora and from 50 per cent to 90 per cent of the acidogenic flora of carious teeth. The apparent relationship of lactobacilli with initial caries and of streptococci with advanced lesions of the dentin is discussed."

Belding and Belding (1943) failed to establish human *Streptococcus odontolyticus* in rats by oral feeding. By subcutaneous

injection of the organism they claimed active caries was produced and the streptococci were regularly present.

Jay, Hunt and Hoppert (1944) found in studies of lactobacilli in rats developed by Hunt, Hoppert and Erwin (1944) that before the young rats were placed on the caries-producing ration, "Six out of 25 rats of the susceptible strain yielded negative counts. The remaining 19 ranged from 50 to 35,000. When examined for caries 100 days after they were placed on the coarse particle diet the 6 zeros showed least evidence of caries. Twenty-four rats of the resistant strain were also examined. Eighteen of these yielded zero counts. The counts of the remaining 6 ranged from 50 to 2000. None had developed caries after 100 days on the coarse particle diet. It was concluded from this study that there was a quantitative difference in the incidence of lactobacilli in the mouths of caries-susceptible and caries-resistant rats. As in humans there seemed to be a relationship between these organisms and dental caries activity."

Fermentable carbohydrates

Brown and Tisdall (1933) said "Sugar, so long blamed for caries, has been proved harmless in the rat, to which diets have been given containing as high as 62 per cent carbohydrate but including also adequate minerals and vitamins."

King (1935) found no caries in sectioned teeth of rats on rations containing 62.5 per cent carbohydrates for 396 days from 21 days of age. The carbohydrates were corn starch, rice starch and sucrose, respectively, for eight animals, each. The rations were deficient also in calcium, phosphorus and vitamins C and D.

Day, Daggs and Sedwick (1934, 1935) used a basal ration of Purina Fox Chow to which they added a commercial fudge in amounts of 20 per cent, 35 per cent and 55 per cent. Additional minerals and vitamins were provided for some of the 55 per cent group. The 213 rats ranged from 24 to 300 gm. weight on going on these rations, and the experimental period was from 150 to 213 days. Caries was found in four rats with no relation to the dietaries. More than half of each of the four teeth affected was destroyed. The authors said: "Fracture of the enamel occurred to some extent in nearly all of the rats in this series. In the great majority of the cases, this appeared only as a chipping of the enamel edges, but, in a great number of instances, the fracture reached as far as the dentino-enamel junction, thus exposing the dentin surface." They gave the composition and method of preparation of the Purina Fox Chow, indicating that it was unlikely that coarse particles could be present. The composition does not suggest that sufficient fluoride could have been present to have inhibited decay.

Lilly and associates (1938) stated that they "were unable to find a single dental caries lesion" in more than 350 rats that had been fed rations containing, respectively, glucose, sucrose, lactose, maltose and corn starch of the order of about 66 per cent of the diet for periods ranging from 6½ months to a year.

In a series of 113 rats on a deficient basal diet with either rice or maize, Rosebury and Karshan (1939a) found that increasing levels of saccharose "tended to increase the incidence of caries on both the rice and corn diets, but inconsistently and to a degree that is not statistically significant." The levels of sugar were 8, 18 and 28 per cent. Addition of calcium lactate made no difference in the caries outcome; increasing protein by 7 per cent reduced the caries index in a group of 13 rats.

Cox and coworkers (Anonymous 1937, 1938, 1941; Cox 1938) have indicated observations that sucrose and glucose do not initiate caries in rats but that these sugars promote the further development of cavities initiated by the H-W-C ration.

Hodge (1944) placed rats on the H-W-C ration for 6 weeks. They were then transferred to the H-W-C ration to which 50 per cent of sucrose was added. After 200 days the incidence of caries was the same as in the control rats but the lesions were much more extensive in the sugar-fed rats.

McClure (1945) found caries induced in 10 of 27 rats fed a ration containing 70 per cent sucrose of which 25 per cent would not pass a 40-mesh sieve. No caries developed in any litter mates on a ration with 70 per cent corn starch. He also noticed an abrasion of the dentin exposed on the occlusal surfaces of the molars of the rats fed granulated sucrose but not those fed powdered sucrose. The weights of the teeth were significantly reduced by the abrasion. In a later experiment in which the caries resistance of the rats was higher, presumably because of use of a stock ration containing 15 to 20 p.p.m. fluorine, only one rat of 20 developed caries on a ration containing powdered sucrose. In another strain, however, 2 of 11 rats developed caries on powdered sugar but none of 18 rats on granulated sugar. Only one of 34 rats developed caries in 270 days when fed 68.5 per cent glucose.

Cox, Dodds, Levin and Hodge (1948) reared rats on rations consisting of 66 per cent sucrose, 20 per cent casein, 5 per cent hydrogenated fat, 5 per cent butter fat and 4 per cent salt mixture or with isocaloric substitution of the fats for sufficient of the sucrose to reduce it to 12 per cent of the ration. Other rats were reared on rations with glucose or starch substituted for the sucrose. Carious lesions were initiated in only 2 of 76 rats when they were continued on these rations from 3 to 11 months.

Forty-eight more rats were placed for 6 weeks on the H-W-C ration. Then 13 of them were placed on a ration of equal weights of sucrose and H-W-C ration and the experiment continued for a total of 104 days. The animals on the increased sugar showed no increase in the number of carious teeth or missing cusps, but twice as many teeth were totally destroyed as compared with the rats kept continuously on the H-W-C ration.

In a series using 122 rats in groups of 12 to 20 it was shown that rations high in carbohydrates fed after a period of 8 weeks on the H-W-C ration tended to increase caries scores.

Another group of 180 rats was placed for 8 weeks on the H-W-C ration modified by use of a synthetic dry milk in which starch, glucose and sucrose were substituted for lactose. The rats were then continued for an additional 8 weeks on rations with the same carbohydrates at a level equivalent to 66 per cent sucrose. Cavity by cavity comparisons were then made as to size. It was found that the fermentable carbohydrates, glucose and sucrose, caused significantly more enlargement of cavity size than did starch. The indications were that the fermentable carbohydrates have little tendency to initiate carious lesions in rat teeth but will accelerate the carious process once it is initated by other factors.

Conclusions

1. The conclusions drawn indicate that (a) acidogenic bacteria are constantly present in the mouth of the rat, (b) they increase in number with caries and (c) bacteria and carbohydrates do not *initiate* caries.

2. Though fermentable carbohydrates do not initiate caries in rats, there is evidence that they accelerate caries activity once it is initiated by cereal particles.

3. The flora found in *cavities* in rats varies with the stage of cavitation, the lactobacilli being observed in early caries and streptococci predominating in advanced decay.

4. Oral flora varies with the type of diet.

5. It has not been shown that rats are subject to immunization against caries by vaccines.

FLUORIDES AND DENTAL CARIES IN RATS

Posteruptive Fluorides

Most of the investigations of the effects of fluoride on the course of caries in rats have been with fluorides administered to rats after weaning, that is after the first and second molars at least have erupted.

Armstrong (1933) fed rats 1000 p.p.m. NaF in the ration for three weeks and then the ration without the added fluoride. "During the three weeks that have since elapsed the upper incisors have broken off near the gum line." Armstrong and Brekhus (1938b) said of these rats, "Alternating transverse bands of mottled and normal enamel were thus produced across the incisor teeth ... the fracture line was usually transversely situated through a band of normal enamel. It thus appears that the hardness and strength of the incisor teeth of rats is improved by a moderately increased fluorine content of the mineral phase or by some change produced in the structure of the tooth which occurs during the formation of the enamel and dentin under the influence of a higher than normal concentration of fluorine."

Miller (1938) studied the inhibition of coarse cereal caries in rats by fluorides and iodoacetic acid. His premise was that these agents inhibit "the phosphorylation mechanism for carbohydrate breakdown" by bacteria, and he considered that the initial lesion in caries is decalcification. He used a ration of:

Brown rice	63%
Dried milk powder	28
Alfalfa meal	3
Crisco (vegetable fat)	5
NaCl	1

For a series of control rats, oatmeal was substituted for rice, with no caries in any group.

Sodium fluoride (250 p.p.m.), calcium fluoride (500 p.p.m.) and iodoacetic acid (200 p.p.m.) were incorporated in the respective rations. The drinking water for the fluoride groups contained 4.2 mg. of NaF per liter; that for the iodoacetic acid groups contained 20 mg. of the acid per liter. The animals were on the rations for 100 days from the age of 28 days.

Caries was recorded from both lower jaws. Teeth were also ground and "numerous planes were examined under a dissecting microscope, magnification 30×, and definite areas of decalcification or actual cavitation were recorded. Later, the other half of the lower jaw was stained and cleared by the more exact Gomori technic (unpublished) and the 3 molars examined for carious areas. The upper molar teeth, which are much less susceptible to experimental caries, were not examined."

The iodoacetic acid seemed most effective in prevention of caries, though the numbers of animals (10 in each group) were too small for definite conclusion on its relative effectiveness. The fluorides also reduced the incidence of caries, sodium fluoride apparently being the most effective.

Lilly (1938) replaced whole milk powder of the H-W-C ration with commercial casein. He found 6 carious lesions in 56 rats in 100 days (presumably in 6 different rats) compared with his previous experience of caries in 60 per cent of 200 rats on the unmodified ration. He speculated on reduced lactose, low alkaline reaction, or high protein of the ration as possibly causative.

Hodge, Luce-Clausen and Brown (1939), in a study of the effects of light from various regions of the spectrum on the well-being of the rat, employed McCollum's stock ration as one of two experimental diets. They found intense dental fluorosis and traced the source of the fluorine to contamination of the commercial casein. Presence of fluorine was further proved by analysis of the incisors. These findings suggested to Hodge and Finn (1939) that the reduction in

caries in rats observed by Lilly (1938) by replacing milk powder with casein was explainable on the basis of fluorine in the casein. Hodge and Finn used the H-W-C diet, the same with casein replacing the milk powder, and the casein ration plus a "daily oral administration of a drop of an aqueous solution of KF containing 3 mg. of fluorine." The teeth of the rats were "observed periodically using Hunt's technic" and, after a 200-day experimental period, the animals were sacrificed and their teeth examined at 15 diameters magnification. A significant reduction in caries was noted in the rats receiving the fluoride and it was concluded that "the caries reduction reported by Lilly can probably be attributed to fluorine contamination of the casein he fed."

Hodge and Finn said, "In several cases it was difficult to distinguish between true fractures and severe attrition." "In all the rats, upper teeth were more involved than lower, thus, the upper teeth had 171 cavities with 620 cusps involved, while the lower teeth had 145 cavities with 360 cusps involved." A minor variation in corn particle size was without significant effect.

Finn and Hodge (1939, 1941) amplified their discussion of their preliminary paper (Hodge and Finn, 1939) on the inhibition of rat caries by fluorides. They said, "The maxillary first molar is most frequently involved and, as before, there is evidence that the teeth of the upper jaws are more often carious." They found only seven teeth with fissure caries and regarded four of these as artifacts. They conjectured, "Perhaps the long duration of the experiment permitted other fissure caries to coalesce with or simulate occlusal cavities and thus prevent identification." They stated: "There was little, if any, qualitative difference in the appearance of fractures in the teeth of the fluorine-fed group as compared to the controls; all the rats exhibited many fractured cusps. The data given above lead to the hypothesis (1) that fluorine prevents the development of a carious lesion on the site of a fractured cusp, thus giving the caries-free mouths; and (2) that fluorine prevents the development of a small cavity into a large one, thus giving the lesser number of cusps involved and whole teeth destroyed."

Perry and Armstrong (1941) showed that the fluorine content of the enamel of rats' teeth is increased by 36 per cent, posteruptively, by giving 20 p.p.m. of fluorine in the drinking water. The effect on caries susceptibility was not studied.

McClure and Arnold (1941) confirmed Miller (1938) on the effect of fluorine (125 p.p.m. in food or water) and of iodoacetic acid (200 p.p.m. in food and 20 p.p.m. in water) in inhibiting caries in rats on the simplified H-W-C ration. But no effects were observed on using water from Galesburg, Illinois (1.8 p.p.m. F) as compared with water from Quincy, Illinois (0.3 p.p.m. F) or with the use of water containing 2 p.p.m. of fluorine.

Arnold and McClure (1941a, b) injected approximately 3 mg. per kilo of fluorine as sodium fluoride in rats on the simplified H-W-C ration. The amount of fluorine was approximately equivalent to 40–50 p.p.m. fluorine daily in water. No reduction in caries was observed. Also, no reduction in caries was produced by giving 10 p.p.m. fluorine in the drinking water of another series of rats, litter mates of the injected rats and their controls. Twenty-one litters were used in these studies. The teeth of the rats were analyzed for fluorine. The fluorine content of the enamel of both the incisors and molars was increased about fourfold by injection of the fluorine and by the 10 p.p.m. in the water. A greater elevation of dentin fluorine was found.

McClure (1941a, b) found a statistically significant reduction of caries in rats by 10 p.p.m. of fluorine in the drinking water, but no difference from controls when 5 p.p.m. of fluorine was used. The H-W-C ration was reported to contain 0.3 to 0.5

p.p.m. of fluorine. Fluorine at the 50 and 100 p.p.m. levels decisively inhibited caries. Animals from about 25 litters were distributed among these experimental and the control groups. His data indicate that the fluorine "was perhaps less effective in preventing the initiation of caries (caries incidence) than in retarding its development (caries score)."

McClure indicated belief that fluorine acts in an antienzymatic way but that bactericidal action is not eliminated. A statement, "Post-eruptive deposits of fluorine may not increase the tooth's caries resistance," was based on this and previous work by McClure and Arnold on rats.

Cheyne (1940a, b) observed 32 rats from which the principal salivary glands had been removed, in comparison with 44 normal animals, all being on the H-W-C ration for 200 days. Three mg. of fluorine as KF was administered daily in a drop of water to 12 of the operated rats. (This concentration of fluorine approximates 60,000 p.p.m.) Cheyne considered that the fluorine remained in the mouth for too short a time to have a prolonged effect. Return via the saliva was of course practically eliminated. The fluoride markedly reduced the rate of caries in the rats with extirpated salivary glands. The sums of the "fractured cusps" and of "carious cuspal involvement" for normal and for operated rats that received fluoride were 10.7 and 10.3 per rat, respectively. This suggests that the fluoride inhibited development rather than initiation of cavities.

Sognnaes (1940, 1941a) applied a solution of 60,000 p.p.m. of fluorine as potassium fluoride to the right lower molars of rats on the H-W-C ration and used the left molars for controls. "Ten rats were treated during the first days of the experimental period and subsequently at intervals of two weeks, while six rats were treated only during the middle and latter part of the 100 days' experimental period." Eleven rats were untreated controls. The number of teeth affected by caries and the number of cusps involved were reduced to about 60 per cent of those on the control side.

McClendon and Foster (1941, 1942) placed Wistar strain rats on experiment at weaning (21 days) and found caries in 40 days. They used a ration of:

Brown rice	400
Yellow corn	400
Skim milk powder	140
Corn oil	90
Sodium chloride	10

This ration contained 0.3 p.p.m. of fluorine.

They found caries reduced by 1.8 to 10 p.p.m. of fluorine in the water, by 2 per cent of bone meal, 1 per cent of horse teeth, 1 per cent of $Ca_3(PO_4)_2$, 1 per cent Na_2HPO_4, 1 per cent $CaCO_3$ or 0.2 per cent of cod liver oil. The results are stated in the number of carious teeth per rat. The number of rats per group was not given. More caries was indicated with advancing age.

McClendon, Foster, Ludwick and Criswell (1942) extended the above work of McClendon and Foster to include tests with 100, 200, 400 and 800 p.p.m. of fluorine as fluor-apatite. "At 87 days these rats had less caries than those without apatite had in 40 days." Apparently the same controls were used as above.

Powell and Dale (1943a, b) found sodium iodoacetate lowered the caries rate in normal rats if injected parenterally (1 cc., 400 p.p.m., every 2nd day). Iodoacetic acid, 200 p.p.m. in the drinking water, reduced the caries incidence in normal rats and 200 p.p.m. in food largely inhibited caries in desalivated rats.

Dale and Powell (1943) confirmed the findings of Miller (1938) and McClure and Arnold (1941) that iodoacetic acid inhibits caries in rats when administered with the caries-producing ration. As 200 p.p.m. in the H-W-C ration, or 200 p.p.m. in food and 20 p.p.m. in the water, 11 of 13 rats were caries-free. With 20 p.p.m. in

the water, 6 of 13, and 2 of 13 for the controls, were caries-free.

Dale (1948) removed the salivary glands from 28 rats. These, with 14 litter mate controls, were fed the H-W-C ration for 150 days. Half of the experimental rats received daily subcutaneous injections of 1 ml. of a solution of 400 p.p.m. iodoacetic acid neutralized to pH 7.2. Caries scores were: 14.0 in the control rats, 46.8 in the desalivated rats and 35.7 in the desalivated rats receiving the iodoacetate.

McClure (1943) gave 100 p.p.m. of fluorine as sodium fluoride in the drinking water to rats aged 40, 100 and 200 days, respectively, at the beginning of the experiments, for 85, 60 and 40 days, respectively. The rats and paired litter mate controls were on the simplified H-W-C ration with maize flour replacing the usual maize. All rats were then placed on the simplified H-W-C ration for 100 days. Caries developed in all the control rats that were placed on the H-W-C at 125 and 160 days of age and in 7 of 10 on the H-W-C ration when 240 days old. No test of the maize flour as a caries-producing ingredient was made. The posteruptively administered fluorine was shown to be assimilated by both the enamel and the dentin of all groups of rats. Caries incidence was reduced in all the fluoride-fed rats of the 14 pairs of the youngest group, in 7 of the 10 pairs of the 100-day-old rats, and in 6 of the 10 in the oldest group. The scores were even in one pair of each of the two older groups. The effect of the fluoride is significant. But if triads of litter mates had been used with the third animal receiving iodoacetic, considerably more confidence would be had that fluorine prevented caries rather than merely arrested decay possibly induced by the maize flour or by the undescribed diet that preceded the maize flour regimen.

The oldest group of McClure's rats had fewer cavities than the intermediate-aged animals in both the experimental and the control groups. This suggests increased resistance to caries with increasing age.

Thomassen and Leicester (1949) using rats, fed and injected iodoacetic acid containing radioactive iodine. They found the greatest concentration in the teeth and supporting bone to be "at the enamel surface and next to the pulp. This activity was of a transitory nature and suggested that the uptake was an adsorption phenomenon rather than an incorporation into the apatite lattice as occurs with fluorine."

Sognnaes (1949b) fed 5 female rats a synthetic ration plus 20 p.p.m. bromine as KBr from weaning to breeding age and continued to weaning of the young. In 15 of the 45 young the bromide feeding was discontinued. After 3 months there were fewer carious molars and lesions and lower caries scores in the rats continued on the bromide-supplemented ration. There was no effect of the preeruptively fed bromide by comparison with other rats reared on the synthetic ration.

Jackson, Tisdall, Drake and Wightman (1950) fed fluorine as sodium fluoride in the drinking water and as present in soft pork bone to groups of five rats. Four levels of fluorine were used, 2 to 16 p.p.m. as sodium fluoride and 4 to 32 p.p.m. as bone fluoride. These resulted in approximately the same consumptions of fluorine. The carcasses and incisors were analyzed after 5 months. The ratios of fluorine in the teeth to the carcasses were 8.7 and 8.2, respectively. The authors concluded that "there was no appreciable adsorption of fluorine by the teeth directly from the drinking water, and that the major portion, if not all, of the fluorine in the teeth was deposited there at the same relative rate as that at which it was deposited in the rest of the skeleton, whether it was administered dissolved in the drinking water or in a relatively insoluble form in the food."

Preeruptive fluorides

Cox, Matuschak, Dixon, Dodds and Walker (1939) (see also Anonymous, 1938,

1939, 1940, 1941; Hamor, 1938, 1939) bred control rats on a ration of sucrose 66, casein 20, "Crisco" 10 and salt mixture 4, with supplements of yeast, alfalfa and Haliver oil. Experimental rats received 10.3 and 41.2 p.p.m. of fluorine as sodium fluoride in the food, and 10.3 p.p.m. corresponding to the fluorine content of the Osborne and Mendel salt mixture. The offspring were placed on the H-W-C ration when weaned at 21 days of age for a standard period of 8 weeks.

The occlusal caries incidence of 33 rats derived from mothers that received 41.2 p.p.m. of fluorine in the ration during pregnancy was 5.97 compared with 10.13 for rats whose mothers received no added fluorine. The odds against chance occurrence of this difference were 1000 to 1. The incidence of fissure caries was 16.79 and 11.76, respectively, with odds of 2.5 million against chance occurrence.

Repetition with other groups of about 60 young rats confirmed these observations with significant but reduced odds. But with mothers bred on the H-W-C rations, with and without 20.6 p.p.m. of added fluorine, no difference in incidence of either occlusal or fissure caries was found. In fact, the caries incidence in these H-W-C rationed rats was stated to be the highest observed in any groups, and Cox *et al.* suggested deficiencies in the maternal diet that offset the beneficial effects of fluorine.

Other evidence of presumptive nature was cited without data. A milk concentrate, presumably with fluorine concentrated, fed to rats during pregnancy and lactation was stated to have reduced caries incidence in the young. Mothers on a ration containing commercial casein showed evidence of fluorosis (compare with Hodge, Luce-Clausen and Brown, 1939) and produced young with a reduced incidence of caries.

Cox, Matuschak, Dixon, Dodds and Walker concluded that the rat is a satisfactory animal for the study of the relation of fluorine to caries and that caries resistance can be built into enamel by preeruptive diets.

Armstrong (1942) and Norvold and Armstrong (1943) found that fracture lesions were increased in rats from mothers with 20 p.p.m. fluorine in the drinking water during pregnancy and lactation and with 100 micrograms of fluorine given directly to the young from birth to weaning at 21 days. They said, "This alteration in the quality of the teeth may have been a result of a change in their structure consequent upon an inadvertent overtreatment of the animals with fluoride." Fracture lesions were unaffected in other groups of rats fed 20 p.p.m. fluorine in the drinking water, either with the caries-producing ration or for 60 days from weaning at 21 days and followed by the caries diet. The teeth of the rats with preeruptive fluorine were found to be slightly more resistant to Gomori (1940) staining type of maize meal lesions; greater freedom from this type of lesion was conferred by fluorine given simultaneously with the maize meal ration or posteruptively for 60 days preceding the caries diet.

Cox, Matuschak, Dixon and Walker (1939) reported the production of mottled enamel in rat molars by daily feeding 128 or 256 micrograms of fluorine as sodium fluoride to suckling rats from birth to weaning at 21 days of age. Dixon and Cox (1939) observed mottling of third molars of rats fed 250 p.p.m. of sodium fluoride in the H-W-C ration from 21 days of age.

The suggestion was made that dental caries as related to teeth calcified under different conditions, including "fluorosis," could be studied in the same mouths by observing the change of ratio of incidence of caries in the third molar and in first and second molars.

McClure (1945) reported that 9 of 35 rats which had been fed a ration containing 73 per cent glucose for periods of 180 to 270 days showed carious lesions in one or two lower molars. Five of 20 rats with 65

to 72 per cent glucose showed caries in 142 to 150 days. None of 60 rats from the same stock showed caries in 1 to 1½ years when fed a ration with 65.5 per cent starch. When McClure attempted to repeat this demonstration of production of caries in rats by glucose, he found caries in only one of 17 animals. He noted that between these observations and the planned experiment the stock ration had been changed from a "home-made" stock diet to Purina Chow pellets. The Purina Chow was found to contain as much as 15 to 20 p.p.m. of fluorine. McClure remarked: "Reduced caries susceptibility . . . may possibly be related to a protective effect of fluorine acquired by the rats' teeth during growth and calcification. Although inconclusive, the fluorine data emphasize strongly the importance of controlling the fluorine in rations fed to colony stock if any attempt is made to stabilize caries susceptibility in the off-spring."

Cheyne (1942) produced severe mottling of the molars of rats by preeruptive feeding of high doses of fluoride. He noted that large areas of the teeth were denuded of enamel and that the enamel was friable. He did not study caries in such animals but in his conclusions stated, "Because mottled teeth are known to be high in fluorine content, the value of using experimentally fluorosed rat molars in studying the mechanism of action of this drug in preventing dental caries is made possible by this study."

It is interesting to note that Dale, Ginn and Volker (1942) record preliminaries "to testing the efficacy of cadmium in reducing experimental caries in the rat" but the plan involved a posteruptive administration of cadmium. This study is based on the observations of Wilson and DeEds (1939), and Wilson, DeEds and Cox (1941), that cadmium produced a mottling of rat incisors indistinguishable from that caused by fluorine. Since posteruptive cadmium would not alter enamel structure, such an experiment would not provide the crucial test of mottled enamel structure as distinct from bacteriostatic effects of fluorine in enamel. What is needed is preeruptive cadmium feeding to mottle molars and then testing of the resistance of such teeth to coarse cereal particles in comparison with the appropriate controls.

Conclusions

1. Posteruptively administered fluorides reduce the incidence of caries caused by simultaneously fed coarse cereal particles.

2. Since iodoacetic acid reduces caries incidence in a similar way, it is indicated that the effect of fluorides is, at least in part, inhibition of bacterial action in development of cavities.

3. Posteruptively fed fluorine enters enamel and dentin, and such fluorosed teeth have increased resistance to coarse cereal caries.

4. Fluorine, available during the formation of enamel, results in increased resistance to coarse cereal caries.

5. Excess fluoride given daily to young rats from birth to weaning at 21 days mottles the first and second molars; from 21 days on, it mottles the third molars.

6. Over-fluoridization of rat teeth increases the liability to fracture by coarse cereal particles.

SALIVA AND DENTAL CARIES IN RATS

Suppression of Saliva

Cheyne (1939a) described a three-stage technic of the removal of the salivary glands of the rat. By extirpation of the submaxillary and parotid glands a partial xerostomia could be produced with a mucous saliva; by removal of the submaxillary and the sublingual glands a predominantly serous saliva is produced. Except for the interference by operative measures, there seemed to be slight effect on the health of the rats.

Cheyne (1939b) studied caries in rats on the H-W-C and the Maynard stock

rations with and without various combinations of salivary glands removed. After 100 days the rats were killed and the number of carious teeth and cusps counted and also the number of fractures.

The number of teeth and cusps involved in caries was very much increased, particularly by removal of the parotid and submaxillary glands. Caries was significantly initiated by the Maynard stock ration, which in controls produced little decay. Fractured teeth were diminished and this was interpreted by Cheyne as an index of rapid development of decay.

Shulman (1939–40) removed the salivary glands of 10 rats at 22 days of age and examined them for caries after a period of 120 days on a ration of:

Whole wheat flour (fine)	67%
Casein (commercial)	14
Milk powder	9
Iodized salt	1
Calcium carbonate	2
Butter (unsalted)	5
Cod liver oil	1
Brewer's yeast (treated)	1

Sections were made of the teeth but no caries was found except "a fracture caries of the upper left third molar." "The molars of the experimental group showed dark stains in the fissures and a somewhat lighter pigmentation of the labial surfaces of the anterior teeth."

Weisberger, Nelson and Boyle (1940) removed the major salivary glands from rats subsisting on Ralston Purina Dog Chow. A gingivitis and recession of gums occurred, the latter being ascribed to impaction of food. Caries was found "possibly primary in the cementum, also involved the enamel and dentine. Secondary dentine was present as a result of the carious process. The earliest observation of caries was 22 weeks after operation. Thus far fissure caries has not been observed."

Powell and Dale (1943b) found the caries rate in desalivated rats about double that in normal animals, based on total caries scores of Cox, Dodds, Dixon and Matuschak (1939) compared with normal rats. The number of cavities was not increased so markedly for the areas affected. These findings suggest that the absence of saliva has a marked effect in accelerating the rate of caries and less or no effect in increasing caries susceptibility so far as the teeth themselves are concerned.

Astachewsky in 1877 noted that the saliva of the rat has high amylolytic activity, and this has been confirmed by Schwartz and Rasp (1926). These observations relate the rat to man in the possible relation of starch-containing rations to caries.

Cheyne (1948) produced molars in albino rats with mottled enamel by the procedure he had earlier described (1942). From 9 such animals he removed the salivary glands by his technic (Cheyne, 1939) and compared the caries rate with 9 litter mates after 104 days on a caries-producing diet. Fifteen normal and 12 rats with salivary glands removed but with no fluoride feeding were used for comparison. The fluorine content of the stock ration of these animals was not stated. No significant protection was given by the added fluoride. The observation of greatly increased caries rate following extirpation of the salivary glands of rats was confirmed.

Conclusions

1. Suppression of saliva in the rat increases the susceptibility to caries as lesions may appear without coarse cereal particles.

2. The caries pattern in rats without salivary glands probably differs from that of normal rats.

3. The rate of development of caries in rats is probably accelerated by a diminished flow of saliva.

VITAMINS AND MINERALS AND DENTAL CARIES IN RATS

Preeruptive Feeding

Rosebury and Foley (1934) bred 5, 6 and 7 rats on a stock, a low calcium normal vitamin D, and low calcium and low vitamin D rations respectively. Litters were raised on the same rations for 100 days after weaning. Four of the group of 7 did not produce litters that survived. In addition some of the animals were given gum tragacanth in the ration to increase its adhesiveness; others were given *ad libitum* a separate ration of glucose; for others, Na_2CO_3 was included to increase the viscosity of the saliva.

Caries was not found in any of the mothers or young. No coarse cereals were present in any of the rations. However, "lesions on abraded cusps of molars, which may correspond to that described by Klein and Shelling (1931) in experimental dental caries, were found in ten instances—three mothers and seven young". "It is apparent, from the distribution of this lesion, that it cannot be regarded as arising from any of the experimental conditions under control." The lesion shown by Klein and Shelling is similar to those of Rosebury and Foley, except that destruction has progressed much farther. Klein and Shelling say it "gave a picture that simulates caries found in human beings." It may be pointed out that this lesion, initiation in the dentin, had been excluded by Rosebury (1932) because it has no analogy in man.

Rosebury and Foley said, "No obvious hypoplasia of molar enamel was observed; but in general, irregularities of contour and apparent reduction in breadth at contact surfaces were more common and pronounced in the more deficient diet-groups." No separate statement is made for third molars which may have been affected by post-weaning diets. But the dentin of the molars was affected by the deficiencies in that it showed uncalcified dentinoid. The deficiencies were exhibited, however, in the roots and pulpal floors, which are far removed from the cusp summits exhibiting the destructive lesions.

King (1935) bred rats on rations deficient in vitamins A and D, but with poor success. The young were placed on rations consisting mainly of "nibbed," whole oats but with "liberal supplies of vitamins and mineral salts." Of six that survived over a month, four had caries.

Lyons (1938) attempted to obtain litters from rats on rations with 0.0066 and 0.07 per cent calcium and phosphorus, respectively. He was not successful when the rats were placed on the rations at the beginning or mid-point of pregnancy, but litters were raised when the ration was introduced at the beginning of lactation. The young rats were then placed on a rice-base caries-producing ration. There was no significant difference in the time—6 to 9 weeks—in which caries appeared as compared with rats derived from a normal stock ration. The variation of the Osborne and Mendel salt mixture used was devoid of calcium and phosphorus. Also it did not have the customary sodium fluoride addition.

Cox (1937a) stated: "Diet of mother during pregnancy and lactation influences caries susceptibility of young. Increased Haliver oil, increased Ca and P, high fat diet, or meat diet to mothers caused increased immunity to cornmeal caries in offspring." Later Cox (1938) said, "There was no change in resistance on (1) increasing the calcium and phosphorus content of the diet above the 'normal,' (2) varying the calcium to phosphorus ratio between 0.26 and 1.44. . . . Extreme reduction of calcium intake by the mother reduced the caries resistance of the young."

Cox and Levin (1944) reported that in 65 per cent of 91 rats from mothers fed a ration of the casein-sucrose-Crisco type the cusps of areas 3 and 4 (scoring system of

Cox, Dixon and Matuschak, 1939) were fused. In 197 rats derived from mothers subsisting on a ration of 90 per cent lean beef and 10 per cent beef liver, only 10 per cent of the cusps were fused.

Posteruptive Feeding

Price (1933) fed 15 different diets to 15 groups of rats consisting of 4 to 7 animals each. The fine and coarse H-W-C rations were used and various types of wheat were substituted for the maize. In addition, variables of high vitamin butter were introduced. The results were presented graphically to indicate more cavities on the fine diets and little or no caries in the groups with the high vitamin butter additions.

Brown and Tisdall (1933) said, "From the observations on rats it may be concluded that for the first time true dental caries, indistinguishable from the human, has been produced experimentally, and that it may either be cured or prevented by adequate amounts of both vitamin D and phosphorus in the diet."

Rosebury, Karshan and Foley (1934b) were not able to prevent fissure caries by adding optimal amounts of calcium, phosphorus and vitamin D to diets deficient in these factors. But they did find a retardation in the development of caries by such supplementation. They found no correlated level of calcium and phosphorus in the blood. Their data on cusp fractures did not confirm their previous finding that the incidence of this type of lesion is related to the calcifying properties of the ration.

Rosebury and Karshan (1935a) used rats raised on their modification of McCollum's stock ration and distributed at 22 days of age among 16 rations in 2 series, with 10 to 13 rats on each ration. These experimental rations were based on a control ration of 92 parts of 10-mesh brown rice and 8 parts white potato dextrin. Variations were made by substituting for part of the rice. The experimental periods ranged from 45 to 180 days; the rats were sacrificed as litter mates.

The variants were calcium, phosphorus, protein, fat and vitamin D as cod liver oil, viosterol in corn oil, or by irradiation of the rats. The investigators concluded: "Addition to the basal diet of cod liver oil or viosterol in corn oil as 2 per cent of the diet produced a definite reduction in caries, but did not prevent caries outright. The protective effect was not significantly augmented by increasing the dosage to 5 per cent of cod liver oil or viosterol in corn oil or by the further addition of calcium and phosphorus to a normal ration, with levels normal or definitely high.

"Irradiation of animals on the basal diet with ultraviolet rays in a dosage sufficient to produce improvement in calcification as great as that produced by 5 per cent of cod liver oil was less effective in reducing caries than feeding vitamin D, but this result is statistically inconclusive.

"Addition to the basal diet of 2 per cent or 5 per cent of corn oil did not remedy the dietary deficiencies but nevertheless produced a significant reduction in caries statistically not distinguishable from that produced by feeding vitamin D in oil. Thus at least part of the protective effect of vitamin D-oil preparations appears to be contributed by the vitamin-free oil."

Rosebury and Karshan (1939b) studied the effects on coarse cereal caries of the addition of varying amounts of several oils. Their basal rations were 92 per cent of rice or maize with 8 per cent of dextrin or saccharose. Oils replaced rice or maize in amounts of 0.5, 2 or 5 per cent of the total diet. Spinach, 5 gm. per day, was fed daily to each rat. The animals were killed generally at about 100 days on the diets. Groups ranged in size from 12 to 14, with one having 10 animals. Comparison was made with the pooled controls of these and previous studies.

It was found that corn oil, olive oil,

Wesson oil, Crisco and lard were effective in reducing the caries index. Paraffin oil was without effect. Vitamin D in a minimal amount of corn oil exerted an independent effect in lowering the caries index. The effects of the oils could not be ascribed to calcifying action, as the blood calcium was not changed from the controls, and bone calcification was described as very defective. The authors said, "All these data suggest that the mitigating effect of vitamin D can be expected to prevent caries; and hence that addition of vitamin D can be expected to prevent caries; and hence that addition of vitamin D to the diet does not remove the cause of caries, but rather acts as a modifying influence on that cause."

Karshan and Rosebury (1940) used 12 groups of 11 to 13 rats started at 22 days of age on rations containing 79 per cent of 30-mesh yellow maize and 13 to 18 per cent sucrose. The period of experimentation was 56 to 125 days. The rations varied in levels of Ca/P. "Reduction of caries was not effected until Ca and P contents of diet rose to 0.27 and 0.19 per cent, respectively." Also, "No simple relationship was found between Ca and inorganic P of blood and caries index."

Rosebury and Karshan (1939b) placed rats at 22 days of age on rations of:

Brown rice, 10 mesh or Yellow corn, 20–30 mesh	65%
Saccharose	18
Wheat gluten	4
Egg albumin	6.5
F.R.L. salt mixture	4.5
Calcium lactate	2

with 5 gm. lettuce and 0.4 gm. brewer's yeast per rat per day. The rats were derived from stock on their modified McCollum ration. The animals, 33 on rice and 32 on maize, were sacrificed after 50–65, 92–111 and 153–167 days in approximately equal numbers. The caries index values found were 1.9 for the rice diet and 2.5 for the maize and were not significantly different from the values obtained by Rosebury and Karshan (1935a) by the addition of vitamin D or corn oil to such diets. No correlation was apparent between the degree of calcification of bones and teeth of these rats and the caries index, nor was mortality, as an index of faulty nutrition, related to the extent of caries.

King (1935), advised by Tisdall (Agnew, Agnew and Tisdall, 1933) to use rations with maize as finely ground as possible, failed to find caries in 39 rats with any such rations with varied additions of olive or cod liver oil and with sodium pyrophosphate.

Lyons (1935a, b) studied vitamin A deficiency in 17 rats, vitamin B deficiency in 15, vitamin D in 6 and vitamin G in 4. The groups were further subdivided. Caries was found in one of the 6 rats depleted of vitamin D for 18 days on the Steenbock and Black rachitogenic ration 2965.

Lilly and associates (1938) reported that they had not found any caries in a series of some 200 rats restricted to rations deficient in vitamin A or B or both, although all the animals eventually died with classical symptoms of the deficiencies.

Lilly and associates (1938) stated that they did not observe a single carious lesion in 493 rats kept in a dark room and fed a *finely ground* Steenbock-Black rachitogenic diet 2965. Rickets developed in these rats and 94 per cent of them died within 100 days.

Lilly and associates (1938) wrote: "Diets in which all the minerals of Mendel's normal rat ration were reduced 75 per cent produced stunted rats whose maximum weight was scarcely half that of normally fed animals, and whose reproduction of young was less than one-fourth that of normally fed rats. Yet the teeth of these rats and of the second and third generations from these animals were free from dental caries."

Holmes, Parkinson, Wertz and McKay (1947) reported after feeding "over 70

different rations or supplements to 850 rats" that "on the Hoppert, Webber, and Canniff ration occlusal caries was advanced at 14 weeks and was not influenced by any of the known vitamins or by butterfat, yeast, vitab, beef muscle or liver, or *l*-tryptophane. The cooking of the corn, the addition of 16 per cent casein, and a reduction in particle size decreased decay by 64, 33 and 23 per cent, respectively, below that observed in litter-mate controls."

Conclusions

1. Conclusive evidence is lacking that deficiencies in the calcium-phosphorus-vitamin D complex in the period of formation of rat molars reduce subsequent caries resistance, though the indications are positive.

2. Adequate feeding of the calcium-phosphorus-vitamin D complex posteruptively may lessen caries in rats, as produced by coarse cereals, but has not been shown to prevent caries entirely.

3. Various oils may reduce the incidence of coarse cereal caries in rats, probably by local effect.

4. No relation of vitamin A or vitamins of the B group to coarse cereal caries in rats has been shown.

OTHER OBSERVATIONS WITH RATS

Acid-Base Balance

Rosebury and Karshan (1935b) studied the effects on caries in rats of acid-base variation produced by citrus juices and synthetic citric mixtures given in place of water. They did not find citrus juices lowered the caries index in rats. On the contrary, they found the index increased, though of doubtful statistical significance. They ascribed the increase to the sugar content of the citrus juices and synthetic mixtures. They concluded that alteration of the acid-base balance by food was without effect on caries in rats. They also attempted to substitute dry lima beans for all of the rice of their ration in order to change the acid-base characteristic of the diet and retain hard particles similar to rice. Their rats failed to survive on this latter diet, but a typical carious lesion of the fissure type was observed in one rat after seven days. With a fourth of the rice replaced by lima beans there was no change in the caries index, suggesting further that bean particles can serve to initiate caries in rats.

Forshufvud (1938) by alternate injections of 1.5 per cent ammonium chloride and 3.0 per cent sodium bicarbonate into rats on a ration of whole wheat bread, whole milk and raw carrots, produced dental caries in experiments lasting 82 days. No caries resulted from daily injection of either of the above salts alone. Alternation of dosage varied from every 5 days to daily. The most caries resulted from the daily alternation of the acid-base condition, with caries in 26 rats of a total of 12 groups of about 150 animals. Extensive caries was illustrated.

Thomas and Bodecker (1942) injected $NaHCO_3$ into 6 rats daily, and NH_4Cl on alternate days in 10 litter mates; 8 litter mates served as controls. The routine was continued for from 93 to 139 days. The ration was:

Ground yellow corn	13%
Ground hulled barley	13
Ground whole wheat	13
Ground hulled oats	13
Soy bean meal	13
Meat scraps	20
Powdered whole milk	10
Alfalfa meal	2
Sodium chloride (not iodized)	2
Calcium carbonate	0.5
Molasses	0.5

They found caries in only one rat and that one was a control animal; hence they did not confirm the findings of Forshufvud above.

Age and Endocrines

Smith, Light and McCay (1939) examined the teeth of 71 rats whose life span had been extended to as long as 1300 days by nutritive manipulation, namely, by restriction of energy intake to prevent growth until late in life. The ration fed from 3 weeks of age was:

Cooked starch	22%
Regenerated cellulose	2
Lard	10
Sugar	10
Salt mix (Osborne and Mendel)	6
Wesson oil	5
Yeast	5
Casein	40

They found 42 per cent of the mandibular molars and 8 per cent of the maxillary teeth carious or missing. Some teeth showed extreme attrition. Nine animals were free of caries at ages of 650 to 950 days. The females were more affected than the males. None had undergone pregnancy.

The casein is the only dietary constituent that presented a hard particle. The investigators did not believe the casein could have initiated caries, though 63 per cent was retained on a 40-mesh sieve and 89 per cent on a 60-mesh. The casein was not purified (McCay, Crowell and Maynard, 1935). No statement was made on the stock ration from which these animals were derived.

Smith and McCay (1947) examined the teeth at death of rats kept alive for long periods by restriction of food during the growth cycle, as compared with litter mates allowed free access to fat and carbohydrate foods. Caries was found to increase with age in both groups, though a maximum seemed to be reached in the control group at 410–600 days of age, and 801–1000 days in the retarded growth group. Among 188 control rats surviving more than 1200 days, 143 remained immune to caries; 58 of 174 of the growth-retarded rats were caries-free.

Braunschneider, Hunt and Hoppert (1948) tested the effect of age on the time of development of caries in their susceptible strain of rats. Animals were placed on a coarse rice diet after 35, 100 and 150 days on a ration containing rice that had been passed through bolting cloth, with the assumption that the latter would not initiate caries. They found the teeth significantly more resistant to caries after 100 and 150 days, but caries appeared earlier in the 150-day-old group than in the 100-day group after they were placed on the coarse rice diet.

Hodge (1943) placed 17 normal and 12 castrate female rats on the H-W-C ration at 1 year of age. There was no significant difference in caries between the two groups observed at natural death. The caries rate in all animals was much lower than that found in young rats by other workers and occurred in both upper and lower molars. Hodge did not assign a definite cause for the lowered rate of caries but suggested that processes of maturation may have operated.

In a study of hypophysectomized rats, Schour and Van Dyke (1934) found very little caries in any animals less than 200 days old but a "high incidence of extensive caries in both control and experimental animals above this age." Caries was, with one exception, confined to lower molars. The ration was not described.

Heredity

Hunt and Hoppert (1938, 1939) by selecting early and late developers of caries found: "The extremes within the fourth generation of the early line are the appearance of caries 16 and 51 days after the beginning of the caries diet. Corresponding limits for the (nearly complete) second generation of the late line are 35 and 249 days, with sibship averages ranging from 18 to 132 days." They apparently examined the teeth for caries directly in the mouth of the living rat. A later statement (Dental Caries 2nd ed. p. 129) by these authors is: "Studies in this relation for three years

have reached the fifth generation of resistant strains, and the seventh generation of susceptible strains, the groups showing striking hereditary differences in susceptibility.

"The rats have been fed the customary balanced diet for the induction of caries (coarsely ground rice, whole milk powder, alfalfa meal, and salt). Caries-susceptible and caries-resistant lines are being developed by a process of inbreeding, selection of individuals, and progeny testing of the lines to be perpetuated. The susceptible line has become progressively more susceptible and the resistant line more resistant. The differences between the family averages have increased from about 113 days in the second generation to slightly over 162 days for the fourth generation of both lines."

Hunt, Hoppert and Erwin (1944) have continued their development of caries-resistant and caries-susceptible rats, the 11th generation of the susceptible line showing the appearance of caries in 28 days and the resistant line in a mean of 240 days. The susceptible line was stated to be highly homogeneous but the resistant line variable.

Hunt, Hoppert and Erwin (1944b) described their development of a caries-susceptible line (12th generation) and caries-resistant line (7th generation) of albino rats by selection on the basis of progeny testing. The difference in days required to develop the first visible caries was 275 days in the seventh generation.

Hunt and Hoppert (1948) reported the differences in caries susceptibility in 17 generations of their caries-susceptible rats and 12 generations of the caries-resistant strain. The difference in number of days before the appearance of cavities in the first lower molars ranged from 59 for the second generation to a maximum of 370 for the eleventh.

Miscellaneous

Klein (1934) reported: "Of 69 wild rats, 39 adults showed dental decay in lower molars. The remaining 30 were young individuals (small size) and showed no gross dental decay. The rats were collected from traps placed about city dumps."

Grace (1939) fed rats 1 mg. of arsenic per kilo in a breakfast cereal (Grape Nuts) for about 3 months. Controls were used at different times in some of the experiments. Of 44 experimental rats, 11 showed a small number of caries each. None of 62 controls had decay. The Grape Nuts for the experimental animals was treated by softening with water containing arsenic and then dried, "leaving a food of coarse, flinty consistency." It is not clear whether the control ration was similarly moistened and dried.

McClure and Hewitt (1946), using litter mate albino rats from 43 litters, gave 75 units of penicillin per gram of food and milliliter of water to 82 animals and compared caries with that developed in controls in 118 days. They found caries in 41 of the control rats but none in the experimental series. In plate counts for *L. acidophilus*, generally many more organisms were found from the control rats. Since no appreciable penicillin could be found in the blood of the experimental rats, the authors concluded that the caries-inhibiting effect of the penicillin was through modification of the oral environment.

Webman, Kniesner and Hill (1947) found no reduction of caries in rats in 110 days by the addition of penicillin to the drinking water at the rate of 5 units per milliliter. They reported reduction of 26 per cent and 59 per cent by 2 and 5 units of penicillin, respectively, when the water contained 1.5 per cent of sucrose.

Webman, Hill and Kniesner (1949) used female rats in groups of 10 to 27 in a study of the effect of minimum amounts of penicillin on dental caries induced by the H-W-C ration. The penicillin was given as 1, 2 and 5 micrograms per milliliter of water with or without 1.5 per cent sucrose. They concluded: "Penicillin in water up to five

units per ml. did not reduce markedly either frequency of attack or progress of caries. However, under conditions simulating those of the human oral cavity, i.e., with a daily intake of sugar, there was a definite and constant reduction of frequency and rate of progress with each increment of penicillin to the water supply.

"As the increment of penicillin increased, *L. acidophilus* present in the oral flora decreased. In the presence of sugar plus penicillin, *B. lactis aerogenes* replaced the lactobacillus as the predominant organism in the rat's mouth."

Shaw (1948, 1949) found no caries-inhibiting effect of 0.5, 1.0 and 2.0 per cent *dl*-glyceric aldehyde in a caries-producing ration fed to albino rats in groups of 10 to 11 and to cotton rats in groups of 5. Growth was significantly retarded by the 1 and 2 per cent levels of glyceric aldehyde.

Arnim (1940) reported: "Caries was observed in rats fed adequate, low salt, sifted or unsifted diets. It occurred on cusps of teeth with no antagonists, on cusps of teeth with antagonists, at the bottom of pits, on the sides of fissures and on proximal surfaces."

McClure (1948) summarized his findings of various modifications of food and water on maize-particle caries in rats. He found no effects of 2000 p.p.m. of sulfate ion, of 500 p.p.m. of magnesium ion, of 15 p.p.m. of free iodine or 150 p.p.m. of iodide ion on the "slight inhibitory effect" of 10 p.p.m. of posteruptively fed fluorine. Sodium hydroxide, N/200 at pH 11.5 was without effect. Thioglycolic acid, silver nitrate and KSCN at 100 p.p.m. in the water and 1500 p.p.m. calcium ion, iron, copper and zinc at 250 and 500 p.p.m. were without effect on the course of caries. Urea as 1000 p.p.m. in water and 1 and 2 per cent in the food had moderate inhibitory effects. Nitrate at 1500 p.p.m. in water and 0.5 per cent in the food showed some slight reduction of caries activity. McClure's experimental groups ranged in size from 18 to 82 rats taken from 6 to 43 litters.

Schlack, Howell, Taylor, Berzinskas and Aborn (1947) and Schlack (1948, 1949) using 165 weanling rats divided into 15 groups as to litter mates, fed sodium and potassium oxalate in food and water, ranging up to 0.33 per cent sodium oxalate, and dehydrated spinach up to 8 per cent of the ration, with the H-W-C ration for 100 days. Schlack (1948, 1949) concluded: "Added dietary oxalates were found to produce no significant effect on caries incidence or extent. Although considerable calculus-like deposits occurred within the grooves of the experimental rats' molars, often immediately under the deposit, the occurrence of caries was not affected in these susceptible areas."

Hatton, Dodds, Hodge and Fosdick (1945) fed menadiones, (2-methyl-1,4-naphthoquinone) in the H-W-C ration to rats for 200 days. Levels of 0.1, 0.3 and 0.8 per cent menadione, the latter being the upper limit of tolerance by the animals, had no effect on the course of dental caries. There were 17 rats in each experimental group compared with 35 in the control group.

Karshan and Rosebury (1940) found that "silicious abrasive induced marked attrition of molars but did not reduce caries index." It is to be noted that Karshan and Rosebury confine their attention to lesions at the bottoms of sulci.

Norvold and Armstrong (1943) recorded that, in rats from mothers given 20 p.p.m. fluorine in the drinking water during pregnancy and lactation, with the young being given directly 100 micrograms of fluorine from birth to weaning at 21 days, the mesio-buccal cusp of the third upper molar failed to form in 11 of 26 animals but that this cusp was present in all of 265 animals that received no such extra fluorine during tooth formation. This is the first observation recorded of a change of rat molar form effected through preeruptive dietary influences.

GENERAL DISCUSSION AND CONCLUSIONS ON DENTAL CARIES IN RATS

A condition simulating dental caries in man can be induced in the molars of rats by the use of coarse maize or rice in the ration after the eruption of the teeth. The resemblance to caries in man is (a) in the appearance of the cavities both grossly and in detail in sections and (b) in the presence of microorganisms in the dentin.

Variation in the frequency of incidence of coarse cereal caries in rats, related to preeruptive conditions (dietary and familial), suggests that the presence of coarse particles in the ration does not constitute the whole of the conditions that govern the initiation of these lesions. Since the progress of decay in coarse cereal caries in rats can be altered by local environmental factors (promotion by sugars, retardation by oils), it is indicated that the course of caries in the rat after initiation by coarse particles may be independent of the initiating factors. These facts of variation in coarse cereal caries in rats, beyond the normal variation under uniform conditions, indicate that this type of lesion can be useful in the study of factors which have been related to caries in man either by theory or observation. If there is a difference in the findings between rat and man it may mean that there is a true species difference or that there is an error in the observations on one of them.

Cox (1937a, b) has suggested the study of nutrition with respect to caries in the rat in three phases: (a) variation of conditions during the formation of the teeth, (b) initiation of decay and (c) the development of the cavity after initiation.

The study in the formative period of deficiences supposed to be related to dental caries has been disappointing because the deficiencies impressed have largely prevented rearing of the young. If the conditions were modified so that the 50-50 point were approximated, that is, that half the young would survive the weaning period, a practical and sufficient deficiency would be present for study of its effects on caries susceptibility.

Whether the mechanism of the initiation of coarse cereal caries in rats is one of fracture or of impaction remains undetermined. Mere gross fractures, in general, do not seem to decay. Non-fracturing rations will induce caries in desalivated rats. It has been suggested that independent study of decay in each empirical site may clarify the question of gross fracture as primary or secondary to rat caries.

It is not essential to the study of the progress of caries in the rat that the mechanics of initiation be fully understood. It is, however, advisable, for simplification, that the study of promotion of decay be made subsequent to the initiation of caries, that is, the ration used for the promotion studies should not contain coarse particles.

Reports of studies of caries in rats should describe the strain of rats used, the stock ration, the cereal particles present in the initiating ration as to kind and sieve analysis, and pertinent details of chemical composition of the various diets. Uniform periods of time should be used for groups that are to be compared.

It is of interest to note, too, that the nutritional requirements of the albino rat do not correspond with human requirements in all respects, notably for niacin, ascorbic acid and some of the amino acids.

The following conclusions with regard to certain factors in rat caries seem justified:

1. Conclusions concerning the posteruptive relations to *initiation of caries* of the calcium-phosphorus-vitamin D complex, vitamin A and sugars are not valid if based on rations containing coarse cereal particles.

2. There are normally present in the rat mouth acidogenic bacteria resembling the types usually associated with dental caries in man.

3. The flora of caries-involved tissues in

the rat tooth varies with the stage of development of the cavity.

4. The fermentable carbohydrates, either with or without added acidogenic bacteria, do not initiate caries in the rat.

5. Caries in the rat, once initiated, is accelerated in its development by fermentable carbohydrates.

6. Fluorides, present in the diet in submottling levels during the formation of the crowns, reduce susceptibility to decay.

7. Fluorides, present in the caries-initiating ration in levels that interfere with bacterial action, retard the development of caries.

8. Fluoride enters enamel posteruptively, and enamel so fluorosed has increased resistance to caries.

9. Removal of the salivary glands increases the susceptibility of the rat to caries.

10. Posteruptive, adequate feeding of calcium, phosphorus and vitamin D may increase the resistance of rats to coarse cereal caries.

DENTAL CARIES IN THE SYRIAN HAMSTER

Arnold (1942) has observed caries in the Syrian hamster, and because of the resemblance in animal and initiation of caries a discussion is apropos. Arnold used the simplified H-W-C ration with coarse maize and with maize ground in a ball mill. The stock ration was commercial pellets. All animals were given carrots or lettuce once a week and distilled water *ad libitum*. Duration of the test was approximately 100 days.

The hamster has 12 molars and is thus similar to the rat but "in the rat molars narrow deep fissures are found, but in the hamster teeth the fissures are shallow and wide. There is considerably more exposed dentine on the tips of the cusps of the rat molars than on the cusps of the hamster teeth. Features such as these should be considered when comparing caries in hamsters with rat and human caries."

Arnold observed cervical caries, "those lesions which occurred on the mesial surface of the first molars, both upper and lower, near the cemento-enamel junction and extending around the cervical portion of the tooth usually more to the lingual than to the buccal." Occlusal caries was also observed with somewhat greater frequency.

About 30 animals drawn from 12 litters were on each of the diets. The fine maize produced more carious lesions of both types per animal than the coarse maize, but fewer animals had cervical lesions on the coarse ration. Four hamsters on the stock ration had occlusal caries.

When the H-W-C ration was made with cornstarch instead of corn, 30 of 52 animals had occlusal caries and 24 had the cervical type, 39 having caries of one or both types. A familial trend of caries was indicated.

In discussion of occlusal caries Arnold considered impaction as a factor, "although the facilities favoring food impaction are apparently reduced in hamster teeth. . . ." Enamel fracture was considered of little etiologic importance.

On the basis of the resemblance of the descriptions of caries in the hamster and caries in desalivated rats, as observed by Weisberger, Nelson and Boyle, it is suggested by the reviewer that the apparent difference in susceptibility to caries between these two species may be, in part, related to saliva.

Keyes and Dale (1944) have described the dentition of the hamster in comparison with that of the rat and of man. Dale and Keyes (1944) studied caries qualitatively on the H-W-C ration, the finely ground H-W-C ration, a rabbit chow diet, and the H-W-C ration with 50 p.p.m. fluorine. Caries was observed on each ration, with no evidence of fracture on the fine maize. Animals on the fluoride diet "were less susceptible." "The yellow pigmentation characteristic

of the disease in man was evident in all lesions."

Keyes (1944) described a system of recording and scoring carious lesions in the molar teeth of hamsters. The system was based on the following premises: the lesions seen at 14 to 20 diameters magnification are true carious lesions; the causes of all lesions are the same; the amount of tooth substance destroyed is proportional to the activity of the carious process. "Four values are recorded for each jaw and the entire dentition: 1. the number of molars affected (MA); 2. the number of cavities (CN); 3. the number of unit areas affected (AA); and 4. the total score (TS). On the basis that a score of 282 indicates complete destruction of the molar dentition, a caries index can be determined as the percentage of tooth substance destroyed, e.g. score 37 equals 37/282 equals 13.1%."

Keyes and Dale (1944) described the cheek pouches and dentition of the Syrian or golden hamster (*Cricetus auratus*). They expressed the opinion that "the pouches do not seem to be related to the teeth. However, possible effects of retained food particles on the oral flora and on the chemical properties of the saliva should not be overlooked." The dental formula is the same as that of the albino and cotton rat. Enamel covers the molar crowns completely. "The cusps are conical, sharply pointed, and separated by broad bucco-lingual sulci and deep occlusal fossae and grooves." Occlusion is characterized by interdigitation, a difference from the albino rat.

Dale, Lazansky and Keyes (1944) gave 64 litter mate hamsters in 4 groups (1) the H-W-C ration, (2) the same with 60-mesh corn meal, (3) the same with 50 p.p.m. fluorine as sodium fluoride in the water, and (4) Purina Rabbit Chow containing, by analysis, 56.5 to 78.8 p.p.m. fluorine. The numbers of animals remaining after an experimental period of 125 days were respectively, 16, 10, 10 and 15, with average total caries scores (Keyes 1944) of 31.2, 36.5, 5.8 and 5.2.

Dale and Keyes (1945) fed the finely ground H-W-C ration to 4 litter mate groups of hamsters with 10 females and 12 males in each group. Group I was a control group and in 125 days showed scores of 35.4 and 49.4 for females and males, respectively. Group II, receiving 50 p.p.m. fluorine in water and 200 p.p.m. iodoacetic acid in the food, 0.4 and 0.4; Group IV, 200 p.p.m. iodoacetic acid in the food, 3.7 and 9.8. In all 4 groups "males seemed more susceptible than females" to dental caries.

Keyes (1945) used 8 males and 12 female litter mate weanling hamsters in five groups fed (1) whole corn; (2) H-W-C semifine; (3) ¾ #2 plus ¼ confectioners sugar; (4) H-W-C plus 1/1000 NaF; (5) #3 plus 50 per cent urea. The experimental period was 110 days. The teeth of Groups IV and V received 2-minute topical applications of 1/1000 NaF and 50 per cent urea, respectively, once a week. The resulting average dental caries scores were 0.1, 0.3, 51.1, 5.8 and 111.7 for female hamsters and 1.8, 19.3, 64.1, 3.2 and 172.5 for male hamsters.

Keyes (1946) observed carious lesions produced in the molars of 125 hamsters on a ration of 20 per cent whole wheat flour, 25 per cent corn starch, 20 per cent confectioners sugar, 30 per cent whole powdered milk and 5 per cent alfalfa. Microscopic lesions were present in 10 days. The lesions were coronal and subgingival, the latter being associated with "plaque-like material in the gingival crevice".

Keyes and Likins (1946) found caries in hamsters "markedly increased as glucose content of diet increased," comparison being made with whole wheat flour. "Males were more susceptible than females." Five male hamsters maintained for 60 days on a ration of equal parts dry whole milk and glucose showed 11 cavities in the group compared with one "suspicious area" in 5 litter mates on the same ration mixed with 7 cc. of water for 5 grams of the dry mixture.

Keyes (1946) described the types of carious lesions produced in hamster teeth by a ration of:

Whole wheat flour	20%
Corn starch	25
Confectioners sugar	20
Whole powdered milk	30
Alfalfa	5

Fresh carrots or alfalfa once a week

Carious lesions were detectable as early as 10 days after the animals were placed on this ration. Gram-positive cocci were found in both enamel and dentinal lesions.

Orland (1946b) obtained saliva in 20 to 30 milliliter amounts from the perimolar region of anesthetized hamsters by aspirating it into a fine glass tube. He also swabbed the buccal pouch surfaces and sampled from carious lesions of the animals after sacrifice. The animals had been maintained for about 100 days on various vitamin test rations containing sucrose, casein, fat and salts, and on three commercial chow-type rations. The samples were examined for various types of organisms capable of growing on acid media. He summarized his findings: "Colonies on a total of 227 acid agar plates, inoculated with perimolar specimens from 179 hamsters which had been maintained on various diets, were observed and studied. Eight kinds of aciduric microorganisms were isolated: staphylococci, streptococci and unidentified pleomorphic organisms and 5 varieties of lactobacilli. Comparison of the caries indices with the numbers of microorganisms isolated indicated some degree of association of lactobacilli and streptococci with the development of carious lesions. There was no clear evidence of a direct relation between the number of microorganisms isolated and the vitamin content of the various diets."

Keyes (1947) using groups of 10 hamsters found in an experimental period of 133 days average caries scores of "control males 62.8; control females 20.9; castrated males 9.5; spayed females 7.2."

Keyes and Shourie (1948) fed various forms of fluorides to hamsters with a cariogenic ration; the resulting average scores were:

	Male	Female
Control	160.5	126.8
50 p.p.m. NaF in diet	0.8	6.2
50 p.p.m. NaF in water	0.6	6.9
NaF subcutaneously	"Invalidated by coprophagy"	
50 p.p.m. CaF_2 in diet	185.6	80.0
50 p.p.m. Na_2SiF_6 in diet	39.3	5.7

A total of 102 hamsters was used.

Shourie (1948) stated, "Fifty p.p.m. of fluorine as calcium fluoride in the diet did not prevent dental caries in the hamster."

Goldberg, Keyes, Gilda and Shourie (1948) found no arrest of caries in hamsters, 8 animals in each group, on supplementation of caries-producing diets with vitamin D for 45 days after an unsupplemented period of 50 days.

Keyes (1948) gonadectomized 10 male and 10 female hamsters and compared caries after 133 to 142 days with sham-operated litter mates. He confirmed prior observations of greater susceptibility of male hamsters to caries. Castration reduced the susceptibility of the male a significant degree and ovariectomy showed some reduction of caries in the female animals. Salivary glands were found to be significantly larger in gonadectomized animals, and females were found to have larger salivary glands than males.

Keyes (1949) failed to find any clues to the difference of incidence of caries in male and female hamsters in a study using gonadectomized animals, with or without implantation of pellets of testosterone propionate. "The caries incidence was so great in all animals that it was impossible to distinguish between groups." "These findings indicate that the sex difference in caries susceptibility in hamsters is best demonstrated when caries activity is not too great."

Lazansky (1946) treated the teeth of rats and hamsters with "Septochem," a silver nitrate preparation, on the 5th and 19th days of an experiment lasting 4 months. Six to 14 animals were used in each group. There was no reduction of caries by the "Septochem" and, if anything, an increase in number of cavities per animal.

Lazansky (1947) brushed the teeth of female hamsters with solutions of various salts over a period of 120 days, with the percentage change in number of cavities and in area involved as follows:

Water	−20	−38
Sodium fluoride	−60.7	−87
Lead fluoride	−36	−74
Acid sodium fluoride	+56	+95
Indium nitrate	+59	+18
Beryllium sulfate	−30	+44.4
Yttrium sulfate	−33	+51.4

Sognnaes (1947, 1948) used rats, hamsters and mice reared on a synthetic ration of 67 per cent sucrose, 24 per cent casein, 5 per cent corn oil and 4 per cent salt mixture, with a supplement of 10 vitamins and a liver supplement, and compared caries incidence with that of animals reared on Purina Laboratory Chow. The synthetic ration had 2.5 p.p.m. fluorine, and the chow ration 21 p.p.m. The synthetic ration was used to develop caries. In general more carious lesions developed in the hamsters than in the rats, and very few lesions were found in mice. Few lesions developed if the chow ration was fed through the experiments ranging from 4 to 7 months. More lesions were observed if the synthetic ration was fed beginning at birth, and the most if the synthetic ration was fed throughout the experiment.

Zander and Bibby (1947a) found marked reduction of caries in 7 hamsters after brushing their teeth once daily for 35 days with a dentifrice containing 1000 units of penicillin per gram. Comparison was made with 8 hamsters in a control group and with 8 whose teeth were brushed with a commercial dentifrice. They found an average of 0.86 carious teeth per animal compared with 3.87 in animals whose teeth were brushed without penicillin, and 8.87 in controls without brushing.

Nuckolls, Frisbie and Killian (1947) found no difference in caries in hamsters due to carbohydrates in two experiments using six hamsters in each group.

Hurst, Frisbie, Nuckolls and Marshall (1948) removed the molar teeth of hamsters by aseptic procedures before eruption and proved the sterility of the teeth by a 10-day incubation in a broth culture medium. The teeth were then incubated for three to five months in cultures of filamentous forms from a carious hamster and human teeth. Two teeth developed brown stains and filamentous invasion of the enamel resembling caries in the hamster.

Hurst, Nuckolls and Frisbie (1949) reported that 10 out of 13 oral actinomycetes produced lesions in 22 of 29 unerupted hamster molars "incubated in the broth cultures for 65 to 161 days at neutral pH. No lesions developed in 10 molars incubated 15 to 46 days under these conditions." Lactobacilli also produced lesions at neutral reaction in 4 of 9 molars in 65 to 130 days. "The enamel was almost entirely dissolved from five molars incubated in dextrose broth cultures of the same lactobacillus strains for 51 to 130 days at pH 3.4 to 4.0. Their appearance was unlike the histopathologic picture of caries."

Mullett, Hurst and Nuckolls (1949) examined six strains of oral actinomycetes, five of which had been shown capable of producing caries-like lesions in hamster molars, *in vitro*, for ability to grow at low pH values. They found inhibition of growth in the range 5.0 to 5.6 by lactic acid and between 4.8 and 5.7 by hydrochloric acid. Oral lactobacilli grew at pH 4.0 or lower.

Mitchell and Shafer (1948, 1949), using Purina Laboratory Chow with 18.2 p.p.m. fluorine as a stock ration for all animals,

transferred 11 pregnant hamsters to a caries-producing diet (CPD) with 1.4 p.p.m. fluorine at periods ranging from 12 days prenatally to 30 days postnatal age. All litters were on the CPD for 90 days or from 78 to 120 days. With the exception of the litter sacrificed at 78 days, the extent of caries varied with length of exposure to the CPD (Part 1A). In a second experiment with 40 p.p.m. fluorine as NaF added to the CPD, thus giving a life exposure to 18–40 p.p.m. fluorine, caries developed in only 2 hamsters of 46 from 10 litters, and to only a very slight extent. The transfer of these animals from the stock ration to CPD was made at 23 days. Of possible etiological significance are the authors' remarks: "One interesting observation made in the fluorine animals was the presence of a peculiar type of enamel lesion. Since practically no caries was found in these animals, small defects of the enamel surface were discerned easily. It was soon noted that in nearly every animal of this group, enamel had been fractured off or, in some way, lost from one or more of the small ledges or tubercles found at the gingival termination of the buccal and lingual sulci of the second and third molars. Such lesions, which had the appearance of fractures, were seen most frequently on the buccal (aspect of the lower second molars but also appeared in any position on any tooth which had formerly presented fine developmental ledges or tubercles.)* The dentin underlying the 'fracture' was stained various shades of brown. Subsequently, the jaws of the animals of Part 1A were rechecked, and these minute lesions were found here also, though in many cases carious lesions superimposed the areas in question. In speculation, it is possible that the coarser particles of the diet (e.g., whole wheat) may produce such fractures during mastication, and it is not inconceivable that the exposed dentin is then more readily susceptible to the initiation of dental caries. It is not demonstrated, however, that this is a major contributory factor in the development of hamster caries."

In a third experiment two litters on a CPD with 1.2 p.p.m. fluorine 15 days before birth had a low caries rate compared with two others on the stock ration with 18.2 p.p.m. until 20 and 22 days of age. The postnatal ages of these litters were respectively 75 and 110 and 112 days.

Shafer (1949) fed hamsters rations consisting of 61 per cent raw cornstarch, or the equivalent of glucose or sucrose, 35 per cent whole dried milk, 3 per cent alfalfa and 1 per cent salt. After 111 days he found significantly higher caries scores in the hamsters on the sucrose ration than in those on the glucose ration, and very low scores on starch. Shafer's experimental groups contained 12 males and 9 females each, distributed as to litters.

Hein and Shafer (1949) tested the caries-controlling properties of sodium copper chlorophylin in the drinking water of hamsters on a caries-producing ration. At 1:500 and 1:1000 dilutions scores were reduced from an average of 53 to 4 in 8 control and 8 experimental female hamsters, and at 1:1000 dilution from 62 to 20 in 10 controls and 12 experimental animals. No caries reduction by the chlorophyll derivative was found in male hamsters.

Granados, Glavind and Dam (1948) fed a fat-free caries-producing ration to 7 male and 7 female hamsters and after 105 days compared caries with litter mates on the same ration mixed with 7 per cent lard. There was some reduction of caries by the addition of the lard.

Granados, Glavind and Dam (1948) fed casein and powdered milk diets to 30 hamsters for 100 days. The animals were paired as to litters and sex, with 8 males and 7

* Correction to printed copy supplied by Dr. Shafer Sept. 16, 1949.

females in each group. The diets and results are shown below for each ration:

Yellow corn, finely ground	40	40
Sugar	20	20
Corn starch	5	5
Potato starch	5	5
Casein	16	
Powdered whole milk		19
Ether-extracted yeast	8	5
Salt mixture	3	
Sodium chloride		1
Lard	3	
Alfalfa meal		5
pH	5.51	5.55
Carious molars	5.0	8.2
Carious lesions	5.4	12.0
Average scores	1.1	2.4

The authors explained the significant differences in incidence and extent of caries as possible through three factors: "lactic fermentation of the lactose contained in the powdered milk; impaction of the casein granules of Diet I in the molars' fossae which prevented accumulation of fermentable carbohydrates in the fossae, and protective action against caries of the lard contained in Diet I."

Granados, Glavind and Dam (1949) fed three group of hamsters, with 7 to 8 males and females in each group, cariogenic rations. The rations of the groups were (1) control, (2) 0.057 ml. of 80 per cent lactic acid in the ration and (3) 0.050 ml. of 80 per cent lactic acid per 100 ml. of the drinking water. After 100 days there were no significant differences in the number of carious molars, areas or caries scores. The lactic acid in water produced some etching of the enamel. No significant difference in caries between males and females was observed.

Granados, Glavind and Dam (1949) found no effect produced on caries in 16 hamsters by added vitamin C or by removal of cheek pouches.

Twedt and Cajori (1950) used 31 hamsters in 5 groups including males and females in a test of caries reduction by 0.5 per cent sodium oxalate in the cariogenic ration of Keyes (1946). They believed that there was a reduced amount of decay in the 12 animals receiving the oxalate ration. The oxalate content of the molar crowns was increased by the dietary sodium oxalate.

Discussion and Conclusions

1. The teeth of hamsters have shallow sulci compared with the narrow deep sulci of rat teeth. There is much less exposure of dentin on the cusp summits of the teeth compared with the general exposure of dentin on the cusp summits of rat molars.

2. Cheek pouches characterize the mouth of the hamster.

3. Caries is initiated by finely ground rations in hamsters.

4. Less decay develops in female hamsters in comparable conditions with litter mate males.

5. Aciduric organisms are associated with dental caries in the hamster.

6. Dental caries is promoted by fermentable carbohydrates and retarded by fat in the ration.

7. Caries is diminished by posteruptively fed fluorides at 50 p.p.m. and by penicillin.

8. Caries is unaffected by posteruptively fed vitamin C and vitamin D.

9. No test of the mechanical factor in the initiation of dental caries in the hamster has been made.

DENTAL CARIES IN THE COTTON RAT

Shaw, Schweigert, McIntire, Elvehjem and Phillips (1944) and Shaw, Schweigert, Elvehjem and Phillips (1944) have reported a high incidence of carious lesions in the sulci of the molars of cotton rats which had been maintained with purified rations for periods of 10 or more weeks after weaning. The basal ration used in these early studies consisted (in per cent) of: Labco casein 18, sucrose 73, salts IV 4, and corn oil 5. To each 100 gm. of this ration were added: thiamine 0.25 mg., riboflavin 0.3

mg., nicotinic acid 2.5 mg., pyridoxine 0.25 mg., calcium pantothenate 2.0 mg., choline 100 mg., inositol 100 mg., and para-aminobenzoic acid 30 mg. To supply vitamins A and D, one drop of halibut liver oil was fed weekly to each rat by dropper. Later a modification of this diet was used which contained an additional 6 per cent casein and 6 per cent less sucrose and which had a supplement of 4 per cent Wilson's Liver Concentrate Powder 1–20. This ration was found to permit more uniform and more nearly maximal growth during the experimental period without altering the susceptibility of the cotton rats to dental caries.

Shaw, Schweigert, Elvehjem and Phillips (1944) stated: "The dentition of the cotton rat is similar to that of the white rat: Monophyodont, 1 incisor and 3 molars in each quadrant of the jaws, continuously erupting incisors, and molars which are more highly developed than the incisors and which are limited in development to the early period of life. The molars are similar to those of the white rat in most respects. The chief difference, peculiar to the species, which may be the fundamental difference in so far as caries susceptibility is concerned, is the extremely narrow, deep, occlusal fissures in the molars of the cotton rat."

These workers observed that tooth decay in the cotton rat occurred almost exclusively in the occlusal sulci of the molars, with only a few lesions on the interproximal surfaces and on the occlusal surfaces of the molars. Lesions were found very rarely along the gingival margin of the molars and subgingivally. No lesions were observed on the incisors. The carious process in the sulci began at such a depth that early carious lesions could not be detected in the intact tooth by reason of the narrowness and depth of the sulci. When these carious lesions had progressed further, regions of the molar cusps were broken away by the stress of mastication as the result of the weakness produced by the undermining carious process. It was then possible to diagnose these carious lesions by inspection of the teeth under low magnification without mechanical removal of any of the tooth substance. In order to study and evaluate accurately the number and progress of the carious lesions present, a procedure somewhat similar to that used by Cox and Dixon (1939) for the evaluation of fissure caries in the white rat was used. In this way carious lesions of all sizes at various depths in the sulci could be exposed systematically and an accurate opinion of the amount of tooth structure involved in each carious lesion derived as a composite expression of the various successive planes in which that particular lesion had been observed. To record the location of the carious lesions, each of the sulci was numbered in a manner similar to that of Cox, Dodds, Dixon and Matuschak (1939), which was devised for the recording of cornmeal caries in the white rat. There are 10 major sulci in the molars of each of the 4 quadrants of the cotton rat skull. The odd numbers from 1 to 39 were assigned to the sulci in the left molars and the even numbers from 2 to 40 to the right molars.

A system for the evaluation of the extent of tooth substance involved in the carious lesions required the formation of an arbitrary schedule in which the symbols 1+ to 5+ were assigned to represent the increasing extent of the lesions, as follows: "1+ – a lesion where there was a small, dark, definitely softened decayed region in the enamel without any penetration of the dentin; 2+ – a lesion with a more widespread softening of the enamel and a slight penetration into the dentin; 3+ – a lesion with widespread decay of the enamel and deep penetration of the dentin but without fracture; 4+ – a lesion with widespread decay in both the enamel and dentin and a small amount of fracture of the undermined cusp; 5+ – a lesion which has progressed so far that a large amount, or all, of the undermined portion was broken off."

(Shaw, Schweigert, McIntire, Elvehjem and Phillips, 1944.)

For each cotton rat the evaluations assigned for the individual carious lesions were totalled and the sum used as an index of the extent of tooth substance involved by the processes of tooth decay in that rat and as an index of the cariogenic properties of the experimental regimen on which that animal had been maintained. This type of evaluation was at best an arbitrary one. However, it was fundamental that some such expression of the amount of tooth structure involved by the carious process be recorded in addition to the number of carious teeth or the number of carious lesions for each cotton rat. Not infrequently in later studies it was found that with various experimental manipulations of the diet, the number of carious teeth and the number of carious lesions were not altered appreciably, while the extent of tooth substance involved by the carious process had been significantly changed by the experimental variations in the diet. However, it is obvious that no true numerical relation can be assigned between the various symbols from 1+ to 5+ as long as methods for studying initiation and progress of carious lesions remain so primitive.

Shaw, Schweigert, McIntire, Elvehjem and Phillips (1944) studied the effect of experimental periods varying from 10 to over 14 weeks upon the incidence and progress of carious lesions. Fourteen weeks was found to be the optimum experimental period, since the carious lesions had progressed to the point where they were easily visible when exposed by grinding, and yet little fracturing of the cusps had occurred. When the experimental period was extended beyond 14 weeks, fracturing became so extensive that individual lesions lost their identity.

The incidence of carious lesions was much higher in the molars of the lower jaw than in the molars of the upper jaw, even though there were the same number of sulci present in each quadrant. The rate of progress of carious lesions was about the same in the sulci of the lower molars as in those of the upper molars, but the time of initiation appeared to be somewhat later in the upper sulci than in the lower ones.

Very different susceptibilities to dental caries were observed in the various molars of the cotton rat within each quadrant. The first and second molars were fully erupted at the beginning of the experimental period. However, the rate of incidence of carious lesions in the second molars was much greater than in the first molars. The lower third molars were exposed to the oral environment for only one-half or less of the experimental period, yet the incidence of carious lesions in the lower third molar was even higher than the incidence of carious lesions in the second molars. The upper third molar was exposed to the oral environment for about one-third of the experimental period and yet had a high caries incidence. These data may indicate that the position of the teeth and their general contours were determining factors in the caries suceptibility. However, in the case of the third molars, which are only partially formed at weaning, their very high caries susceptibility may have been partially a result of some deficiency or deficiencies in the experimental ration during this period of tooth development which were not otherwise detectably expressed in the well-being, growth and appearance of the cotton rats.

When the same strains of cotton rats were fed rations composed of natural foodstuffs instead of the purified ration described, the caries incidence was very low. Likewise when the sucrose of the purified ration was replaced by dextrin, the incidence of carious lesions was very low. The particle size of the stock rations and of the dextrin rations did not seem to play as important a role in initiation and development of the carious lesions as was the case with the white rat. The caries-preventing action of the dextrin

could not be attributed to its fluorine content, which was lower than the amount of fluorine required to give a similar reduction in the incidence of carious lesions (Shaw, Schweigert, Phillips and Elvehjem, 1945).

Shaw (1947) extracted the right first and second maxillary molars of 25 cotton rats at approximately 14 days of age. In most cases the third molar, in the same jaw, was probed to the surface and extracted. The animals were then maintained for 14 weeks on the caries-producing ration similar to that of Shaw, Schweigert, McIntire, Elvehjem and Phillips (1944). In 21 of the animals there were more carious lesions in the opposed molars of the left mandible than in the unopposed teeth of the right jaw; in three there were equal numbers; in one there were more lesions in the unopposed teeth. The extent of tooth substance involved was greater in 23 of the animals in the opposed teeth and greater in two animals in the unopposed.

A further test of the effect of particle size on the initiation and progress of carious lesions has been made by the complete substitution of finely ground sucrose in the form of confectioners sugar for the granulated sucrose used in previous experiments. Shaw (1949) observed that the incidence and rate of progress of carious lesions in cotton rats fed the ration containing the confectioners sugar were the same as for the litter mates which received the ration containing granulated sucrose, if they ate comparable amounts of the two rations. Frequently the difficulty described by Schweigert, Shaw, Phillips and Elvehjem (1945) was encountered, with many of the cotton rats maintained with the finely ground ration scattering and wasting a large portion of the daily feeding with a resultant low daily food intake. In those cotton rats which had a restricted food intake, there were greatly reduced incidence and rate of progress of the carious lesions. When the intake of cotton rats receiving the ration with coarse granulated sucrose was restricted to a similar degree, the incidence and rate of progress of carious lesions were reduced to about the same extent as prevailed with their litter mates which had voluntarily restricted their food intake.

The study of carious lesions in various stages of development from ground sections provides further evidence that the initiation of these lesions occurs deep in the sulci of the molars prior to any visible fractures in the enamel (Shaw, 1947b).

A wide variation in the susceptibility of various cotton rats and various strains of cotton rats has been described by Schweigert, Shaw, Elvehjem and Phillips (1945) even though the animals were maintained on strictly standardized regimens. For example, after 14 weeks on the caries-producing dietary regimen, the 18 offspring of parent stock no. 5 had an average incidence of carious lesions of 31.3, with a range of 25 to 39 lesions for each animal. In contrast, after 14 weeks of the identical regimen, the 13 offspring of parent stock no. 12 had an average incidence of carious lesions of 19.8 with an individual range from 0 to 33 lesions. Analysis of variance indicated that the difference in dental caries suceptibility between these 2 strains and others was highly significant. However, individuals from caries-resistant strains responded in the same fashion to diets which reduced the incidence of carious lesions as did the individuals of the caries-susceptible strains. Of the members of the second generation, preliminary data indicated that the caries susceptibility was similar to that of their parents. Attention was drawn to the necessity to distribute litters among the control and experimental groups of each experiment in order to reduce the variation in caries susceptibility to a minimum.

In all the following experiments, the control and experimental groups each had one or more representatives of each litter. In addition, the cotton rats were placed on experiment at weaning about 2 weeks of age, when they weighed approximately 20

gms. At this age and weight, the first and second molars are fully erupted but the third molars are in an early stage of development. Unless otherwise stated, all experiments were continued for a period of 14 weeks after weaning.

The effects of different dietary carbohydrates on the incidence and extent of dental caries have been investigated by Schweigert, Shaw, Phillips, and Elvehjem (1945) and Schweigert, Potts, Shaw, Zepplin, and Phillips (1946). A very high incidence and extent of carious lesions were noted when glucose, fructose, dextri-maltose, or maltose was used in place of sucrose in the basal caries-producing ration. The replacement of one-half of the sucrose by lactose did not alter the progress of tooth decay. However, when fine dextrin and coarse dextrin were used instead of sucrose, incidence and extent of tooth decay were very low. There was no appreciable difference in the results obtained with coarse or fine dextrin. The replacement of one-half of the sucrose with fine dextrin did not reduce the severity of tooth decay as compared with that in the cotton rats which received the sucrose diets. The replacement of three-quarters of the sucrose with fine dextrin reduced the incidence and extent of carious lesions by about 30 per cent.

The inhibitory effects of fluorine as sodium fluoride in the ration were studied by Shaw, Schweigert, Phillips and Elvehjem (1945). Amounts of fluorine were tested from 22.5 to 450 p.p.m. No inhibition of the incidence and extent of carious lesions was observed when 22.5 p.p.m. of fluorine were present in the ration. When 45, 90 and 135 p.p.m. of fluorine were included in the ration, there was a progressive decrease in both the incidence and extent of carious lesions. The ingestion of 225 or 450 p.p.m. of fluorine produced the maximum inhibitory effects, with a 90 per cent reduction in the incidence and extent of carious lesions. A slight inhibitory effect of fluorine on the formation of pigment in the enamel of the incisors was observed when 45 p.p.m. of fluorine were fed. With 90 p.p.m. or more of fluorine, the incisors were pale, but no chalkiness was observed until 450 p.p.m. of fluorine were ingested in the ration. Mottling of the third molars was observed when 135 p.p.m. of fluorine or more were fed. No indication of a greater percentage reduction in the dental caries incidence was observed in the third molars than in the first and second molars, even though the third molars were partially formed during the period of fluorine ingestion.

Schweigert, Shaw, Zepplin and Elvehjem (1946) observed that cotton rats which were maintained from weaning for 14 weeks with a diet consisting solely of mineralized whole milk or mineralized whole milk plus Wilson's 1:20 Liver Concentrate developed no carious lesions. The incidence and extent of carious lesions were very low when a ration was fed which approximated the composition of milk solids in its fat-protein-carbohydrate-salts distribution. Anderson, Smith, Elvehjem and Phillips (1947) studied the effect of milk rations in which 5 and 10 per cent of various carbohydrates were incorporated by a Waring blender. The carbohydrates studied were starch, sucrose, glucose and dextri-maltose. Milk supplemented with minerals and 1:20 Liver Concentrate as above, plus additions of starch, produced animals which were free from carious lesions at the conclusion of the experimental period. The addition of 5 per cent sucrose to the milk diet did not disturb the caries-protecting properties of the milk diet.

However, the addition of 10 per cent sucrose or 10 per cent glucose resulted in a low incidence and extent of carious lesions. The addition of 5 or 10 per cent dextri-maltose did not appear to increase the susceptibility to caries development on the milk ration. Experiments were carried out to determine the effect of giving milk supplements to animals being fed the caries-producing basal ration. In order to induce

the animals to consume the milk, the available basal ration was limited to about two-thirds that of the controls. With this procedure, the cotton rats drank sufficient milk to produce a total caloric intake approximating that of the litter mates receiving the basal ration. Variation in the data on carious lesions was fairly wide, but the averages indicated that the milk supplement reduced the caries experience to approximately one-half that of the litter mates with the unsupplemented basal ration.

Schweigert, Shaw, Zepplin and Elvehjem (1946) studied the effect of various levels of fat and protein on the incidence and extent of carious lesions. Because of the higher caloric value of fats than of carbohydrates and proteins, it was judged wisest to make all replacements of carbohydrate on an isocaloric basis. Since the caloric value for fats is 9 and that for proteins and carbohydrates is 4, whenever 1 gram of additional fat was introduced into the basal ration, 2.25 grams of carbohydrate were removed, but when 1 gram of additional protein was introduced into the basal ration only 1 gram of carbohydrate was removed. Therefore, the ratio of energy to vitamin and mineral composition of all rations was constant.

The isocaloric substitution of 10 parts of lard for sucrose resulted in a 50 per cent reduction in the average incidence of carious lesions and a 75 per cent reduction in the extent of tooth substance involved in the carious processes. The total fat content of this ration was 16.4 per cent, while the sucrose content was 48.6 per cent, in contrast to a fat content of 4.8 per cent and a sucrose content of 64.4 per cent in the basal ration. When 20 parts of lard were substituted isocalorically for 45 parts of sucrose, there was a 90 per cent reduction in the incidence and extent of carious lesions in comparison with the litter mates maintained with the basal ration for the same length of time. The total fat content of this ration was 31.7 per cent and the sucrose content 27.8 per cent.

When the casein content of the ration was increased from 24 to 50 per cent at the expense of sucrose, without any change in the amount of fat present, a 50 per cent decrease in the average number of carious lesions and 70 per cent decrease in the average amount of tooth substance involved were observed. The sucrose content of this ration was 40 per cent. When a ration with 50 parts of casein and 10 parts of lard replacing appropriate amounts of sucrose was fed, there was almost a complete prevention of carious lesions in comparison with the control litter mates.

Anderson, Smith, Elvehjem and Phillips (1947a, b) fed pairs of cotton rats a caries-producing ration containing, as percentages, sucrose 67, casein 24, corn oil 5, salts 4, and milk with and without 5 to 10 per cent sucrose, dextri-maltose and glucose. Milk alone in 15 animals produced no dental caries in 14 weeks. Some decay resulted from addition of the sugars. When approximately two-thirds of the ration was the caries-producing diet and one-third liquid milk, the caries rate approximated about half of that produced by the caries-producing ration alone. The numbers of animals used in these studies ranged from 3 to 15.

Anderson, Smith, Elvehjem and Phillips (1948) fed the caries-producing ration they had previously used in comparison with (a) dried whole milk (b) the same with added butter (c) dry reconstituted whole milk (d) the same with butter and (e) the caries-producing ration homogenized in water. The number of pairs of animals ranged from 10 to 15. In general much less decay resulted from ingestion of the liquid rations, and butter in a solid ration reduced the incidence of caries.

Zepplin, Smith, Parsons, Phillips and Elvehjem (1950) fed 10 rations featuring natural foods to cotton rats in groups of 3 to 10 animals for a period of 14 weeks. They found very little dental caries in a ration with no added sucrose, but comparable

high rates at 17, 32, 47 and 66 per cent levels. Replacement of dried milk by liquid milk showed no effect on number or extent of carious lesions. There was no effect of substitution of dextrin for enriched white bread.

Wakeman, Smith, Sarles, Phillips and Elvehjem (1948) estimated the numbers of microorganisms in cotton rat molars in carious and non-carious teeth by grinding the extracted and weighed teeth in measured amounts of water and culturing from the diluted suspensions. They compared the counts also with washings from unground teeth from the same animal. The data from four cotton rats on a low caries ration and from two on a high caries ration indicate much higher counts in anaerobic than aerobic culture medium, marked increase with dental caries and many more organisms in the ground than on the unground teeth. They concluded: "Bacterial counts made by this method show conclusively that the greater numbers of organisms in carious teeth are located in the deep-fissure lesions and may only be determined by grinding and culturing the entire tooth."

Wakeman, Smith, Zepplin, Sarles and Phillips (1948) examined the flora of the teeth of cotton rats by the method of Wakeman, Smith, Sarles, Phillips and Elvehjem (1948). "Of sixty-five cultures isolated from ground teeth, 77 per cent were identified as enterococci and 16 per cent as lactobacilli." They found association between increased extent of caries and counts of organisms grown anaerobically and aerobically. They found "an increase in the incidence and extent of caries, and bacterial shake tube counts resulted when honey at 2 to 10 per cent replaced sucrose in the cotton rat's diet." The numbers of animals used were not stated.

Howell, Schlack, Taylor and Berzinskas (1948) found no effect on the incidence or extent of dental caries in cotton rats from potassium oxalate in the water supply. The animals were in three groups: controls, 0.02 per cent and 0.22 per cent potassium oxalate, with 30 of 51 animals surviving the experiment. However, 27 of the cotton rats died because of trichobezoars. In a second experiment with 3 groups of 5 animals, with 0.0002 and 0.0002 per cent potassium oxalate and 10 per cent cellulose added to prevent the formation of trichobezoars, no effect on caries was found.

Shaw (1949) found no effect of the substitution of raw for refined sucrose on rates of initiation or development of caries in cotton rats.

Shaw (1949) found no difference in the number and extent of carious lesions in the cotton rat between rations containing granulated or powdered sucrose. Isocaloric replacement of 18 or 27 of each 67 per cent sucrose in the ration by lard resulted in a significant reduction of the caries attack. Replacement of sucrose by dextrin resulted in reduction of caries.

Shaw (1949) found similar postdevelopmental effects in albino and cotton rats of various dietary variations. Restriction of caloric intake reduced the initiation and development of carious lesions. "Essentially no carious lesions developed in a 5-month experimental period" in which the diet consisted solely of mineralized whole milk, the same with 10 per cent by weight of added sugar, or when evaporated whole milk was used. When fat or protein or both were increased at the isocaloric expense of sucrose, there were large decreases in dental caries.

Shaw (1949) gave 0.5 per cent crystalline dl-glyceric aldehyde (dimeric form) in the ration to 9 rats, 0.2 per cent (monomeric form) in the drinking water to 14 litter mates, and 12 animals were used for controls. No differences in number and extent of carious lesions were found in the molars of these 3 groups. Similar results were obtained from 3 cotton rat controls and 3 given glyceric aldehyde in the water at 0.2 per cent concentration.

Shaw (1949) found no diminished fre-

quency or extent of carious lesions in cotton rats following the posteruptive addition to his caries-producing ration of various vitamins and combinations of vitamins. The additions were: (a) vitamin B complex constituents as pure substances (b) ascorbic acid (c) vitamins A, D, E and K (d) menadione (e) vitamin K, and (f) liver concentrates and folic acid. No effects were produced by use of crude casein, blood fibrin or lactalbumin in place of vitamin-free casein. Addition of dl-tryptophane to the ration was without effect. There was no effect of change of salt mixture or of the addition of sodium oxalate to the ration. The experimental groups ranged in size from 3 to 12 animals.

Shaw (1949) found fewer and smaller carious lesions in the teeth of 10 cotton rats kept in cages with bedding of wood shavings than in 7 animals maintained on a bedding-free woven wire cage bottom. A similar but lesser effect was produced by paper bedding. Accumulations of bedding material and hair were found in the sulci of the teeth of cotton rats kept on bedding.

Shaw and Weisberger (1949) reported significantly increased numbers and sizes of carious lesions in cotton rats after removal of the salivary glands at 14 days of age. No difference in distribution of the lesions was noted. No cavities developed under heavy deposits of debris that formed on the teeth.

Conclusions

It has been demonstrated that the cotton rat is susceptible to the development of carious lesions when maintained with purified rations. The initiation of these carious lesions appears to be much more independent of mechanical factors and fractures than the commonly described lesions in the white rat. Replacement of sucrose in the cariogenic diet by starch or dextrin, or feeding a mineralized milk diet or a stock ration composed of natural foodstuffs, result in a very low incidence and extent of carious lesions. Replacement of part of the sucrose in the purified diet by fat results in a great decrease in incidence and rate of progress of carious lesions, while replacement of part of the sucrose by protein results in a smaller reduction. Thus various manipulations exist for the establishment of assays for the caries-producing or preventing properties of a diet, provided careful evaluation of strain and individual animal variations are considered in the evaluation of the results.

PREVALENCE OF DENTAL CARIES

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CONTENTS

PREVALENCE OF DENTAL CARIES

A review of the dental literature impresses upon one the universal distribution of dental caries throughout the world. It is found in all nations without regard to geographic barriers or climatic variations. It is common to both sexes, to all races and at all economic levels and occurs in all age groups. Actually, dental caries presents a problem from preschool age to old age. Without a doubt, it is the most prevalent chronic disease affecting the human race today. It is a progressive disease. It has its incipience early in life and if not checked prevails and progresses until irreparable damage is done and an accumulation of dental pathology results which presents an ever-increasing problem. Even with regular dental care, the occurrence of new lesions is not prevented. It is in fact the most neglected of all correctable conditions. There are, perhaps, several basic reasons to account for this neglect: First, ignorance as to the value of dental care and minimization of the sequelae of dental neglect; second, apprehension and fear of pain associated with many dental corrections; third, cost of dental correction. With the continued expansion of dental public health programs, many of the objections cited above can be eliminated or greatly minimized.

No attempt will be made in this section to discuss the cause or methods of controlling dental caries. These subjects will be considered in subsequent papers of this series. This paper will be a review of methods and results reported in studies of the prevalence of dental caries and will present this aspect of the epidemiology of dental caries, incorporating the findings of investigators throughout the world. As far as feasible, repetition of information contained in subsequent papers will be avoided. Such factors possibly influencing caries prevalence as heredity, hardness of drinking water, fluorides, sunlight, nutrition and oral environmental factors will not, therefore, be discussed fully in this paper.

For purposes of discussion, the material has been divided into a number of categories. Since many of the articles reviewed and incorporated into this review cover the subject in a general way, repetition of certain portions of these articles has often been necessary in order to maintain the several categorical divisions.

This review is written with the realization that there is a wide variation in the methods employed by different investigators in presenting the data. Certain observations are comparable, others are not. The chief difficulty appears to be the lack of pertinent information or the grouping of information so that it cannot be analyzed. For this reason, comparisons cannot always be made between papers on the subject. Nor can this review present all the papers appearing in the literature or discuss each fully. It is intended primarily as a ready reference for those seeking information falling into a specific category.

METHODS USED IN CONDUCTING DENTAL EXAMINATIONS

In reviewing the prevalence of dental caries, one cannot interpret the findings of the many investigators unequivocally, since the definition of dental caries varies. Unquestionably, many of the differences found in the literature can be attributed to this variation in definition. For example, in a survey of school children in 26 states conducted by local examiners, Messner, Gafefer, Cady and Dean (1936) learned that more than half the examiners who returned questionnaires were designating pits and fissures as carious lesions. The majority of authors recognize, however, that in mirror and explorer examinations carious lesions should show either actual evidence of decalcification with a concomitant stagnation of debris

on the surface attacked or the penetration of the tine of an explorer into soft carious dentin within the suspected pit or fissure.

Even with this latter criterion fairly well standardized, and taking into consideration chance variation, marked differences in the number of cavities reported by different examiners exist and perhaps must be attributed in part to the fallibility of human perception and judgment. This discrepancy in examinations has been demonstrated by Radusch (1941). Each of 8 dentists examined the same 33 patients. The average difference between the greatest and the smallest number of cavities and carious surfaces observed was 4.2 carious teeth and 5.8 carious surfaces per patient. When bitewing X-ray films were used, less difference was noted in the average number of carious teeth and carious surfaces reported by each examiner.

Deathrage, Wilson and Ledgerwood (1939), observing this discrepancy among staff dentists who were giving 5- to 7-minute examinations on the same 63 patients, formulated a simple statistical device for evaluating this variable.

The training of the person conducting the examination is an important variable. Obviously, if one is not trained to delineate lesions, many will be overlooked. Jones, Larsen and Pritchard (1930) illustrate this fact by pointing out that at Oak Park, Illinois, only 2.7 per cent of the 6- to 14-year-old children had caries-free mouths as found by dentists. Examinations of these same children performed by a physician and a nurse disclosed that 67.1 per cent and 66.2 per cent, respectively, of the children were found to be free from caries.

Various aids have been utilized in making the examinations. Wiazemski (1907) used none in examining adolescents in Bulgarian colleges. He merely enquired of the students as to the number of carious teeth they had. Steggerda and Hill (1936) among others used tongue blades. Most studies have been carried on with the aid of the sharp explorer and mirror under good light. In several studies, the explorer has been augmented by an initial drying of the teeth and/or by radiographs. The value of each type of examination has been assessed by Sognnaes (1940b). His study was undertaken in order to secure information regarding the extent to which certain clinical findings may vary according to the different types of examination procedures employed, and to determine for each type of examination the type of lesion most likely to be overlooked. To obtain this information, Sognnaes studied 32 out-patients at Forsyth Dental Infirmary, ranging in age from 4 to 13 years, with an average age of 9 years. Each child received clinical examinations progressively utilizing (1) mouth mirror and explorer, (2) aided by an initial drying of the teeth, (3) aided by a cleaning and drying of the teeth and (4) aided by roentgenographic diagnosis. The examination of each child took 1½ hours. Out of a total of 757 teeth examined, utilizing mirror and explorer alone, 38 per cent of the teeth were carious. Utilizing all four procedures, 54 per cent were found to be carious, a difference of 16 per cent. When considering the 3,407 tooth surfaces, 651 or 19.1 per cent were found by mirror and explorer alone to be carious. By supplementation with an initial drying of the tooth surfaces, 773 or 22.7 per cent of the surfaces were found to be decayed. By augmenting the previous two methods with a cleaning, 837 surfaces or 24.6 per cent were found to be carious. By combining these procedures with X-rays, 879 or 25.8 per cent of the surfaces were found to be carious. Nearly one-fourth of the total number of surfaces would have remained undetected had not the mirror and explorer been augmented by the other three procedures. Sognnaes found that the pit and fissure cavities were easily detected by the explorer and the approximal cavities easily observed in the radiographs, but the remaining lesions (20 per cent of all found) located on the

smooth surfaces of the tooth were disclosed only after a detailed study of the cleaned and dried surfaces.

Dunning and Ferguson (1946) tested the efficiency of explorer examinations on 100 naval reserves. Explorer examination located 3.6 lesions per person and 1.3 proximal lesions (from the distal of the cuspids on back). Roentgenograms by themselves showed 3.4 posterior proximal lesions per person. Both types of examination gave 6.4 lesions of which 4.0 were posterior proximals. In the entire mouth, bitewings increased dental caries findings by 78 per cent over explorer alone. Posterior proximal cavities increased from 100 to 315, an increase of 215 per cent.

Arnold, Dean and Singleton (1944) in an examination of 188 Coast Guard cadets with a mean age of 19.5 years found that by augmentation of the explorer examination with 4 posterior and 3 anterior bitewings the films added 1.3 teeth (3.7 areas) in one group and 1.2 teeth (3.4 areas) in another.

White (1944) located 4.4 new carious areas per subject by X-ray examination after all necessary dental work as found by mirror and explorer examination had been completed in a group of 800 Naval Aviation cadets.

Thompson (1943) at Boystown found an average of 3.1 more cavities per boy by X-ray examination than with mouth mirror and explorer.

Delabarre (1933) found that among 18-year-old patients X-ray examinations added 1,960 cavities to 1,842 already found by mirror and explorer. X-ray examination disclosed 51 per cent of the cavities found.

Cheyne and Horne (1948) examined 429 children from 3 to 19 years of age. By employing bitewing films in conjunction with a meticulous explorer examination on 329 of these children, they found that 120 had additional cavities shown by the films. Of the 100 children who had full mouth surveys in addition to explorer examination, 56 had additional cavities disclosed by the full mouth survey. In the bitewing group, 5.1 per cent of the DMF surfaces were disclosed by bitewings and 6.1 per cent of the DMF surfaces were disclosed by the full mouth survey.

Hadjimarkos and Storvick (1948) found among 505 native born and reared Oregon school children that, of a total DMF rate of 9.9 teeth, 2.5 or 25.3 per cent were detected by X-rays.

Watsky (1949) examined 100 high school children in New York City, averaging 16.1 years of age; 988 DMF teeth were revealed clinically, of which 475 were cavities; 131 additional cavities were observed on bitewings taken of the anterior and posterior teeth. More than 21 per cent of the cavities, therefore, would have been overlooked without the employment of X-rays.

Shourie *et al.* (1948) examined both clinically and by X-rays 622 14-year-old males in Puerto Rico. There were 7.1 DMF teeth per boy found clinically, and 8.6 DMF teeth found using both explorer and X-ray. The use of the X-ray, therefore, increased the average DMF score by approximately 17 per cent.

Among 500 13-year-old persons, Day and Sedwick (1935) found 419 new cavities by X-ray examination in addition to 7,335 found by mirror and explorer. The authors attribute the difference in cavities revealed by X-ray examination in their study and in that of Delabarre (1933) (5 per cent as against 21 per cent) to the difference in the ages of the groups.

In the study of 883 students at Drexel Institute, Arnett and Ennis (1933) reported that, of the total carious teeth found, 1,662 were detected clinically and 1,372 by X-ray examination. There was agreement on only 237 teeth. If teeth had been examined without the employment of X-rays, 40.6 per cent of the cavities would not have been detected.

Burkett (1941) pointed out that 24 per

cent of the cavities found on histologic sectioning were missed by both explorer and X-ray examinations.

Hadjimarkos and Storvick (1948) found among 582 freshman students at Oregon State College that of an average of 5.50 cavities requiring fillings per student, 1.67 or 30.36 per cent were detected by X-ray examinations.

Chilton and Greenwald (1947), examining 729 children from 5 to 18 years of age, identified by X-ray films 0.79 cavities per child that were not evident in an oral examination. They concluded, however, that X-ray examinations were not necessary for large surveys of dental needs of a community.

Dahlberg (1940) has suggested that standards of tooth destruction be established so that each degree of involvement of a tooth will come under a certain category. Patterns of tooth disintegration are so variable, however, that the number of standards necessary to fit all categories would be tremendous.

In studying the rate of progression of a lesion, Shepherd (1945) plotted the area involved from bitewing films onto graph paper. The number of new squares included represents the rate of growth of the lesion. Such a procedure, although an excellent one for research studies, would not be applicable in broad prevalence studies.

Summary and conclusions: In surveying the dental status of a community, a procedure for examination should not be so laborious, costly or time-consuming that it is prohibitive. Since it is expedient to have comparable procedures, mouth mirror and explorer examinations when carefully conducted under good light are usually considered adequate if one is limiting the field of investigation to comparison of large groups. This is especially true when the examinations are used to show life caries experience in terms of decayed, missing and filled (DMF) teeth. It is presumed that by this method certain incipient interproximal lesions may be undetected that would be found by X-ray examination. If standard procedures and methods of examination are established, however, and factors such as identification of the lesions, time for completing the examinations and the necessity for satisfactory equipment are made constant, then the number of lesions found only by X-ray examination can be minimized. Our most imperative need at present is a uniform and universal standard of what is or is not to be considered a lesion. Such standards must be established if the confusion so evident in the literature of the past is to be avoided in the future.

It is recognized that for certain types of studies such as the surfaces involved in caries, where greater detail is desirable, mouth mirror and explorer examinations would not be adequate. In selecting the type of examination to be utilized, an evaluation of the goals and criteria to be employed should first be made.

METHODS USED IN RECORDING DATA OF DENTAL EXAMINATIONS

Opinions vary greatly as to the most effective method of tabulating and presenting the data on the prevalence of dental caries. Clune (1945) and Turner, Howe and Dick (1942) review a number of these methods and conclude that the majority are too cumbersome or too limited in the amount of information given. Several methods now utilized have much to recommend their adoption, however, and all have inherent disadvantages.

Group surveys of prevalent dental conditions, according to Boyd (personal communication) can serve any one or all of several purposes:

(1) To indicate the net effectiveness of the masticatory apparatus for the given mouth, or for the group collectively. This information is directly available from the careful mouth survey.

(2) To compare the amount of reparative service the child or the community has received, as compared with the residual

needs. This information too can be gained through comparison of filled teeth with teeth in need of fillings or restorations.

(3) To provide a measure for estimating past caries experience.

Actually there are only four units that can be utilized for this last purpose. These are (1) the individual, (2) the tooth, (3) the surface and (4) the lesion itself. All four units have been employed in the past. The most widely accepted index, which has been used by Munblatt (1933) and others and popularized by Klein, Palmer and Knutson (1938) is the DMF rate per 100 children. The decayed, missing and filled (DMF) rate for each tooth is an expression of the total caries experience, past and present, of that individual and is based on units of 100 individuals. Although frequency of attack on any specific tooth surface is an important consideration in many research studies, it is not essential in broad epidemiological studies where caries prevalence rates and community needs are generally the facts desired. The DMF rate is sufficiently comprehensive and yet simple enough to be readily applicable to large population groups.

The DMF rates, however, have definite disadvantages which have been recognized by Klein, Palmer and Knutson (1938) themselves. Individual teeth are considered as the unit, and hence no provision is made for multiple cavities or fillings on the same tooth. DMF surfaces are employed by these writers to eliminate some of the objection in reporting multiple cavities on the same tooth, although this practice offers no information as to the number of initial lesions on the tooth.

Another frequently overlooked objection to the employment of DMF surfaces can be raised. Since the number of involved surfaces may vary with the type and extent of dental corrections, great care must be exercised in employing this criterion as a measure of contrast between population groups. The number of involved surfaces may vary because:

(1) Fillings are frequently larger than the carious area they restore. They may include surfaces other than the one on which the original lesion existed, as for example proximal fillings which generally include the occlusal surface.

(2) Frequently non-carious pits and fissures on the occlusal, buccal and lingual surfaces of the teeth are filled which would not have been listed as carious prior to filling.

(3) The number of surfaces counted in a missing tooth may be far in excess of the number of surfaces actually involved in caries at the time of extraction. In some instances, teeth are extracted for reasons other than caries.

The number of DMF surfaces attacked in any tooth is indicative of the size or extent of the lesion or that multiple lesions exist on that tooth. The DMF rate, therefore, will give an indication of the average number of teeth attacked and the average size of the lesions. It gives no indication as to the site of attack.

Another objection to this method of tabulation as pointed out by Klein, Palmer and Knutson (1938) is the difficulty in utilizing this index for deciduous teeth, since it is impossible in many cases to determine whether the tooth missing has been extracted or exfoliated. Only the DF rate is considered in the tabulation of deciduous teeth, missing teeth being excluded. Since these teeth may be lost through extractions, the child with many broken down teeth extracted will present a mouth with less active caries experience by the DF method of tabulation than the child with many small cavities. Gruebbel (1944) mentions the system adopted by the American Association of Public Health Dentists in 1943 for summarizing caries experience in deciduous teeth. Under the term "observable dental caries experience (def)" are included carious and filled teeth and teeth indicated

for filling or extraction. Even with this modification, there is still room for improvement in tabulating caries experience in deciduous teeth. Since the important variable, extractions, is omitted from tabulation of the "def" rate some formula should be devised for estimating from the number of absent teeth at each age level what percentage was lost by extraction of carious teeth. Such a formula could be derived from longitudinal studies of tooth exfoliation on sufficiently large and representative population groups. Even with this modification in the DF rate there is still room for improvement for determining DF surfaces *per se*. The same criticism of Klein, Palmer and Knutson's index is applicable to DMF rates among adults, since it is difficult at times to establish the reasons for extractions in old age groups where periodontal disturbances are a potent factor in the loss of teeth.

The DMF rates also fail to give information about the caries-free percentage in the population. East and Pohlen (1941) state that a true dental health index for a population should be based on the number of individuals with DMF teeth subtracted from 100. This will be the percentage of the population which is caries-free. The population attack rate is an important index and is reported in a great many studies.

Knutson (1947) has established a standard curvilinear relationship between the percentage of children who are caries-free and the average DMF rate at each specific age. One can estimate, therefore, from the DMF rate at any specific age approximately what per cent of the population will be caries-free at that age.

Gafafer (1942) objects to the DMF rate because no differentiation is made between persons with 1 or 10 carious teeth. In certain studies the intensity of attack among individuals would be important, but in prevalence rates the DMF rates would offer sufficient information.

East's (1942c) criticism of the DMF rates per 100 persons appears to be a valid one, for teeth cannot be compared with persons since the number of teeth among different individuals may vary. DMF rates should be based on the number of teeth present in the mouth or on the basis of 100 teeth examined. Not only is the number of teeth in the mouth important, but so also is the length of exposure of these teeth to the oral environment, since the attack rate of a tooth is directly correlated to the length of exposure in the mouth, all other things being equal. A term suggested by East (1942a) to incorporate these various factors is the "exposure unit", defined as one year of exposure in the mouth for one tooth.

Palmer, Klein and Kramer (1938) have established a direct correlation between the number of erupted teeth in the mouth and the eruption age of the teeth. Therefore, from knowledge of the number of teeth in the mouth, the number of exposure units can be calculated. In large population groups, the number of teeth erupted at a certain age in a specific location is a constant, and the number of exposure units is therefore a constant for each age but would be an important variable when comparing areas where there is delayed eruption or comparing rates in different sexes or in special studies.

Many other indexes for determining prevalence rates have been suggested. Most of these, such as those suggested by Morelli (1924), the American Child Health Association (1930), Mellanby (1934), Jensen (1940), either are too cumbersome or involved to be readily applicable to large population studies, or do not include sufficient information to be of value to all types of studies. Clune (1945) suggests use of the dental health capacity of the first permanent molars, since they are generally the earliest and the most frequently involved permanent teeth in the mouth and would give an indication of the amount of involvement of the entire mouth. However, in comparing individuals where the reduction in dental caries is greater in some specific area in the mouth,

it is questionable whether a molar index would represent a true picture of the prevalence of caries for comparative purposes.

Although Bodecker and Bodecker established a practical caries index in 1930, Bodecker modified this index in 1931 (a) to include all teeth in the mouth. The modified dental caries index of Bodecker (1939) is similar to the DMF surfaces of Klein, Palmer and Knutson (1938) with the exception that not all teeth are considered as having five susceptible surfaces. Bodecker feels that many of the surfaces present two independent areas of attack and should be counted as two surfaces instead of one. These surfaces are the buccal of the lower molars, the occlusal of the upper molars, the occlusal of the lower bicuspids and the lingual of the upper first and second molars. Extracted teeth and crowns are counted as three involved surfaces rather than five as counted by Klein, Palmer and Knutson (1938). Three was selected as the number of surfaces from examination of a large number of extracted teeth. Wessels and Cheyne (1947) found approximately four surfaces involved, however, in 475 extracted teeth of children under 15 years of age.

Either the DMF index or the Bodecker modified index will give satisfactory and comparable results. As Sandler (1940) has pointed out, there is a high degree of correlation between the DMF rate and the Bodecker index. In large dental surveys, the more expedient method should be employed if results are comparable. Sandler indicates that recording the DMF rate requires less time than the Bodecker notations and yet offers adequate information for epidemiological studies.

The lost-permanent-tooth index of Wisan (1937) or that suggested by Turner, Howe and Dick (1942) are reflections of dental care rather than dental health, since the loss of teeth is the direct result of lack of dental care.

Knutson and Klein (1938) illustrate this fact by comparing the tooth mortality of 2,175 children with caries experience but showing no fillings with that of 987 children with caries experience and one or more teeth filled. Although the group with fillings averaged approximately one DMF tooth or more per child than the group without fillings, they had lost through extraction only 38 teeth per 100 children as compared with 54 teeth per 100 children in the group with no evidence of fillings.

Dunning and Klein (1944) believe that a tooth fatality rate, which is the number of teeth lost divided by the number of teeth attacked by caries or DMF teeth, is more satisfactory than the index of Wisan (1937). The tooth fatality index also has definite advantages over the tooth mortality rate (Knutson and Klein, 1938) which is based on the number of lost teeth per 100 individuals, since it better shows the effectiveness of dental care and allows for regional and other differences in caries experience.

Summary and conclusions: The various technics for reporting caries rates in a population have been discussed. It is realized that the tabulation and presentation of data in the literature may be arranged to summarize certain specific observations which the authors wish to emphasize. With this fact well in mind, however, it is still believed that there exists in the literature a great deal of unnecessary confusion which makes comparisons of the various papers difficult and does not permit general deductions to be established.

The simplest, and an entirely adequate, method appears to be the DMF rate for the permanent teeth, since it includes the entire caries history of the individual. From this information, the per cent of the population which is caries-free can be determined as well as the post-eruptive tooth age and the DMF rate per 100 teeth.

There is some objection to the DF rate in considering deciduous teeth, and some provision should be made to include extracted teeth in the tabulation. It should not be too difficult to derive a formula to de-

termine from the number of deciduous teeth lost at each age level the percentage due to extractions.

Because of the large number of teeth in a population group and because of the rampancy of dental caries, this disease is ideally adapted for statistical studies, yet there is still great need for statistical methods for analyzing dental data. The marked individual variations in caries rates result in wide standard deviations in any survey of dental caries. This fact is not particularly well recognized. Yet with such resultant great standard errors, any difference in average DMF between groups must be large to be significant.

DENTAL CARIES IN ANCIENT PEOPLES

A study of the dentition of ancient man presents a striking contrast to the teeth of modern man. The teeth of ancient man were relatively free from dental caries. Because of their physical structure and chemical composition, the teeth are generally the best and frequently the only preserved remains of ancient man. While the soft tissues may be autolized or eaten by predatory animals and the bones frequently crushed into dust or dissolved by action of chemicals in the earth, the teeth are not digestible nor are they acted upon by chemicals to a degree where their morphology is lost. It is true that fossil teeth frequently have deposited in them, from the surrounding soil, greater concentrations of certain chemicals such as fluorides (see Roholm 1937). These depositions, however, do not alter the form of the teeth nor diminish their value as indelible evidence of the antiquity of man. A study of these dental remains and jaw fragments often gives a clue to the nature and condition of the dental armamentarium of these individuals. They frequently afford pertinent information for the anthropologist as to the degree of advancement of these individuals in the human scale, as well as evidence of their diet and mode of habitation.

In the realm of time ancient man occupies only the quaternary period of animal existence, yet he extends over a temporal span of almost a million years. Remains of ancient man have been found as early as the middle Pleistocene or glacial period. Evidence has been uncovered in skeletal remains of that period showing the existence of dental caries, pyorrhea and alveolar abscesses. Fortunately for ancient man, the existence of oral pathology was the rare exception rather than the rule.

An estimate of the prevalence of dental caries has been made from the study of the unearthed teeth, skulls and jaw fragments. Frequently, valuable information as to location of the initial lesions and their progress can be established on the tooth surfaces with certainty. There are a few disadvantages inherent in the study of such material. Frequently the teeth, particularly the single-rooted anteriors, drop out of their sockets and are lost so that relatively few skulls contain their full complement. Lack of evidence of bone healing, however, clearly indicates when such teeth have been lost postmortem, as pointed out by Lenhossek (1919), Ruffer (1920) and Krogman (1935). When teeth obviously have been lost prior to death, as evidenced by new bone formation obliterating the alveolar sockets, the specific cause of the loss of these teeth remains uncertain. These teeth may have been lost through periodontal disease or through trauma as well as through dental caries. Because of the difficulty of determining the specific cause of the loss of such teeth, investigators such as Mummery (1870) have entirely omitted from consideration the gaps caused by these losses. There is evidence that periodontal disease must be considered in evaluating the dentition of ancient man. The existence of periodontal disease in the alveolar bone of 422 Peruvian skulls was found by McCurdy (1923) to be 13 per cent and was as high as 36 per cent in 165 skulls of Pecos Indians examined by Nelson (1938). It is unsound, therefore, to consider all teeth lost as due to caries, although un-

doubtedly a large percentage of tooth losses showing pathological alveolar changes were produced by dental caries.

That alveolar abscesses did exist in these skulls has been found by many investigators (Sedwick 1936). Pickerill (1924) was of the opinion that in most cases they are the result of excessive attrition, with the resultant mechanical opening of the pulp without evidence of caries. Lenhossek (1919), on the other hand, observed in the skulls of prehistoric peoples missing tooth spaces together with bony defects resulting from periapical abscesses. Since the teeth present in these skulls frequently showed caries and no marked attrition, he was of the opinion that lost teeth in such skulls were probably due to caries.

Regardless of interpretation, it becomes strikingly apparent from studies of the vast amount of material collected on ancient man that he was, with but few exceptions, almost completely or completely free from dental caries. This fact has been substantiated by many investigators such as Lenhossek (1919), Szabo (1935) and Krikos (1935). There are a few exceptions to this rule. These are the Rhodesian skull mentioned by Hooton (1937), skulls of the Dolmen period as reported by Krikos (1935) and the Nagysap skulls mentioned by Lenhossek (1919). To these might be added the Neolithic skulls from western Germany and from Greece as reported by Greth (1939) and Angel (1944). The limited amount of caries that did occur generally occurred in older individuals and was the result of marked attrition followed by the impaction and retention of food at the cervix of the tooth crown subsequent to recession of the gums. Both Leigh (1925) and Marston (1936) among others have reported this observation. As stated by Sarkar (1939), even the deciduous teeth were subject to marked attrition in fossils excavated in Punjab, India.

Early man possessed large well developed jaws with ample room for well spaced teeth and good occlusion. Such information has been contained in the reports of Rihan (1932), Krogman (1935), Sedwick (1936), Rabkin (1937) and Angel (1944). In contrast, modern man has a reduced tooth size, shrunken jaws and less capacious oral cavity because most of the jaw functions in modern man have been taken over by the hands and extra-organic objects used by him as implements (Hooton 1937). With the development of man has come a degeneration of the masticatory apparatus (Hooton 1937).

Lenhossek (1919), Chappel (1925), Stewart (1931), Goldstein (1932) and Krogman (1939) have observed, as has been found in modern man, that males had fewer carious teeth than did females of comparable age, and, as has been reported by Klatsky (1937) and others, the anterior teeth were almost completely free of dental caries. Lenhossek (1919) reported that when caries was present in the anterior teeth, the upper anterior teeth were more susceptible than the lower anterior teeth.

Bentzen (1929), Leigh (1930), Klatsky (1937) and Krogman (1940) report that caries of the deciduous teeth was rare. In the papers by Mattingly (1915), Christopherson and Pedersen (1939) and Pedersen (1939) caries of the deciduous teeth was completely absent. Reports in disagreement are those of Ruffer (1920) who found caries not rare in the deciduous teeth from Egypt of 1900 years ago. Rihan (1932) reported that 12 per cent of relatively recent skulls from New Mexico of individuals up to 16 years of age contained carious teeth, some of which were presumably deciduous teeth.

A definite variation in caries susceptibility has been noted in different localities of the same country and among various countries. This variation was found in England by Mummery (1870), in United States by Leigh (1925) (1928) and by Krogman (1939), in Egypt by Ruffer (1920), in Russia by Lukomsky (1929) and in Germany by Greth (1939).

A number of less uniform findings might

be added to this list. Among these is the report of Mummery (1870) that dolichocephalic skulls were relatively resistant to caries as compared to brachycephalic skulls. Similarly, Lenhossek (1919) claimed that caries was introduced into Europe by round-headed men from Asia. Lukomsky (1929) also reported that dolichocephalic skulls of a pure race showed no caries, while caries increased in regions of Russia where the cranial index showed a transition from mesocephalic to brachycephalic. Krikos (1935) found that caries among the ancient Greeks increased greatly coincident with an influx of northern peoples, and Ruffer (1920) reported the same phenomenon in Egypt with an immigration of Negroid peoples in Ptolemaic times.

Two theories concerning the cause of caries are based on this observation of the increase in dental caries with the intermixture of races. Lenhossek (1919) regarded dental caries as an epidemic introduced into Europe by Asiatics in the same way as cholera and the plague were introduced. The second theory is based upon human genetics. Hrdlicka (1911) and Hooton (1934) state that isolated groups of primitive peoples depended on the efficiency of their masticatory apparatus to a much greater extent than do urbanized modern peoples. Great variations in tooth or jaw size or abnormalities of occlusion were not advantageous for survival and were eliminated. The intermixture of racial stocks with different tooth and jaw sizes made possible the inheritance of these unharmonious characteristics, with resultant crowding of teeth and predisposition to dental caries. Support is gained for this idea from the experimental work of Johnson (1933) and Ritter (1937), which indicates that a mixture of genetic types may be responsible for abnormalities of the dentition in dogs.

A number of investigators including Mummery (1870), Bodecker (1930) and Klatsky (1937) have stressed the importance of function in the development of the jaws. But whether the function led to the development of the excellent dentition or whether the excellent dentition permitted the function seems worthy of consideration. Pickerill (1924) believed that at least among recent primitives the food is relatively soft. Support can be found for this belief in the observations of Leechman (1934) on the diet of Indians of Quebec and Ontario. One must bear in mind, however, that the teeth of American Indians are relatively susceptible to tooth decay.

Diet as an explanation for immunity or susceptibility to caries has been discussed in numerous articles concerning the teeth of primitive man. Diet must be considered in regard to its effect on tooth structure during the period of tooth formation. Vitamins and minerals must be included in this category. Sedwick (1936), however, observed among pre-Columbian Indian skulls unearthed in New York State that developmental hypoplasia was more common than it is today. French *et al.* (1939) however, could find no difference in the mineral phase of pre-Columbian enamel and dentin as compared to these tissues in both modern and fossil teeth. Diet must also be considered in regard to its possible effect on fully formed and calcified tooth structure through its stimulation of the supporting tissues, its cleansing properties and its possible lack of refined carbohydrates. These factors will be discussed in subsequent papers in this series.

Summary and conclusions: Studies of skulls and jaw fragments indicate a very low prevalence of dental caries in ancient man. The little caries that did occur appears to be related to attrition resulting from the type of diet. What factors may have operated in producing this low prevalence cannot be evaluated completely. Whether it was the result of influences operating locally within the oral cavity or systemically during tooth formation, or a combination of both, has not been established conclusively. Certainly the diet of ancient man, as the diet of modern

primitive, must have varied considerably when one considers the span of time and geographic and climatic conditions under which he existed and managed to survive.

Ancient man did possess large, well developed teeth and jaws but whether this development was due to function or a true genetic characteristic is a moot question.

DENTAL CARIES IN MODERN PRIMITIVE PEOPLES

It has been established by many investigators that the dental caries prevalence rates among modern primitives resemble to a great extent those of early man. In general, the teeth are resistant to decay or decay occurs only after excessive wear. Dental caries is, therefore, a disease affecting the mature or elderly person. As in most mouths with little caries, the prevalence of decay is confined almost without exception to the posterior teeth where it involves either pits or fissures, worn occlusal or proximal surfaces or cervical areas exposed by recession of the gingival tissues.

It has been further established, from diverse parts of the world, that upon contact with civilization these primitive peoples show a marked increase in dental caries. Mellanby (1934) illustrated this observation very well among the native attendants at the missionary schools in Southern Rhodesia. Among the adults, who were exposed to civilization for only a short period of their lives, only 5 per cent had dental caries. Among the adolescents who had been exposed to civilization over a greater period of their lives, 20 per cent had evidence of decay. Among the children who had been exposed to civilization the major portion of their lives, 50 per cent had developed dental caries. Other investigators reporting similar findings among the Eskimos have been Mummery (1870), Waugh (1937), Rabinowitch (1936), Krogh-Lund (1937) and Pedersen (1938). Among the African natives, similar findings have been reported by Colyer (1923), Orr and Gilks (1931), Oranje, Noriskin and Osborn (1935), Staz (1938) and Jones (1940). Among the New Zealand Maoris, this increased prevalence of caries has been reported by Pickerill (1924); among the Hawaiians by Jones, Larsen and Pritchard (1930); and among the Samoans by Ferguson (1935). It has been found to hold true among the American Indians by Price (1936) and by Arkle (1943); and in the Malayans by Laband (1941). The role of the change of dietary habits in the increased incidence of dental caries will be discussed more completely in the section on Nutrition and Dental Caries.

Among the Eskimos living in primitive native villages, Rosebury and Karshan (1937) reported from one village that 1.2 per cent of the teeth were carious as compared to 18.1 per cent in another village where a trader resided.

Restarski (1943) examined 1,970 pure-blooded Samoans aged 2 to 21 years and found a marked increase in dental decay in both dentitions among those living in villages near the naval station as compared with those living in outlying districts. For example, children 5 to 6 years of age living near the naval station had an average of 2.3 carious teeth per child, while those living in the outlying districts had an average of only 0.5 of a carious tooth per child.

Mellanby (1934) reviewed the literature on this subject and tabulated the percentage of skulls of existing primitive races showing evidence of caries. In all these races as presented by Mellanby, the numbers of skulls and individuals showing caries are lower than those found in modern civilized man, although there is a marked variation in prevalence among these tribes (Table 1).

Pickerill (1923) observed that Maori skulls showed that less than 1 per cent were carious. Among the Maori children under civilization, 90 per cent had some decayed teeth.

Among the Melanesians, Kirkpatrick (1935) found that only 2.9 per cent of 1,976 New Guinea natives showed dental caries,

with an average of about 2 carious teeth per carious mouth.

Clawson (1936) found that caries was almost non-existent among the Shammar Bedoins under 20 years of age. In a large group employed for several years by a western company, 1.5 per cent had carious teeth at the beginning of the employment while 18 per cent had decayed teeth at the end of the period.

TABLE 1

Dental caries in existing primitive races

(Mellanby 1934)

Location or race	Author	Number of skulls	Per cent with caries
Eskimos	Mummery	69	2.9
No. Africa Coast	"	56	3.6
No. Africa Interior	"	23	8.7
South Americans (Chile)	"	19	15.7
Fiji Islands	"	38	5.2
Polynesians (various)	"	79	10.1
Maoris (Sandwich Islanders)	"	21	14.3
New Zealanders	"	67	2.9
Australians	"	132	20.4
Malays	"	24	12.5
East Indians (N)	"	152	5.9
East Indians (S)	"	71	14.0
Caffres	"	49	12.2
Africans (W)	"	236	28.0
Ashantees	"	92	11.9
Maoris	Pickerill	260	0.76
Central Africans	S. Colyer	1,000 persons	9.8

Steadman (1939) found carious teeth in only 2.3 per cent of 156 aboriginal Australian skulls, while Campbell (1925) had found 94 per cent of 1,490 aboriginals living in contact with civilization had dental caries, with an average of 4.93 carious teeth per individual.

Friel and Shaw (1931) examined 600 government school children in South Africa and found 93.3 per cent of the children with caries, with an average of 4.82 carious teeth per child.

Schwartz (1946) found caries negligible among the Masai peoples of Africa. He observed only 38 cavities among 408 individuals, an average of only 0.09 of a cavity per mouth.

Till (1927) examined 247 children and young adults living in kraals in South Swaziland; 25 per cent were caries-free.

Ockerse (1945) found that 85 per cent of the school children in South Africa showed dental decay. The highest rate was 96 per cent in Natal. In urban areas, the average was 93 per cent, while in rural areas it was only 79 per cent.

Brown (1924) compared the percentage of the population caries-free among the Europeans and among the natives in South Africa. Among the natives in the 7- through 8-year age group, it varied from 20 per cent in Port Elizabeth to 85 per cent in the North West District. In the 14- to 15-year age group, it varied from 30 per cent in Kimberley to 100 per cent in the North West District.

Colyer (1917) examined 1,000 natives of Northern Rhodesia 10 to 45 years of age. Approximately 10 per cent were caries-free. Colyer (1919) also found a great deal of caries in the natives of the Transkeian territories on the southeast coast of Africa.

Suk (1919) examined 1,008 Zulu natives of South Africa. These natives were from 5 to 20 years of age and were from the missions; 0.68 per cent of the male teeth showed cavities, while 1.18 per cent of the female teeth were similarly involved. Among these natives, 6.3 per cent of the males and 14.6 per cent of the females had decayed permanent teeth.

Among the Finnish Lapps (Inari), Mellanby (1940) reported that the ancient Lapp skulls of children showed less caries than is found in today's Lapp children. Among the present Lapps all children over 4 years old were affected with dental caries. Of 70 children between 2 and 14 years of age, 55 per cent of the deciduous teeth and 44.5 per cent of the permanent teeth were

carious. In 16 ancient skulls, only 5.7 per cent of 52 deciduous molars, and only 21.8 per cent of 51 permanent posterior teeth, were carious. This compares favorably with 73.3 per cent of the deciduous molars and 74 per cent of the permanent posterior teeth among the present Lapp children.

Among the aboriginal tribes of the Tibetan borderland, Agnew and Agnew (1931) found the total caries prevalence to be 37 per cent, while among Chinese living in the same region the prevalence was 43 per cent. In a subsequent study, Agnew (1941) described three dietary and geographic groups from the west borderland of China and eastern Tibet. In two tribes subsisting entirely on unrefined foods the caries prevalence was 36.5 per cent, with 2.92 cavities per mouth with caries. In a third tribe living in a lower altitude, with polished rice providing part of the diet, the prevalence of caries was 52 per cent.

Anderson (1932) found no decayed teeth in the mouths of 25 Mongolians, while among the Chinese in the province of Shensi he found 25.5 per cent with caries, and in Peiping 43.4 per cent had caries. He states that the prevalence of caries decreases progressively as one travels from the port cities to the interior of China.

Wright (1941) examined two groups of Indians of Ecuador. One group lived under primitive conditions, the other in a missionary settlement. Of the 56 adult males living under primitive conditions, 32 were free from dental caries; of 11 preadolescent males, 9 were caries-free. Of those living in the missionary settlement, 3 among 34 adults were free of caries, and among 46 preadolescents, none was caries-free.

Price (1939) found the skulls of pre-Columbian Indians of Florida free from dental caries, while Hamlin (1933) reported that 29.7 per cent of 3,488 teeth of "pure" Seminole Indians were carious.

Klein and Palmer (1937) examined 7,574 Indian children from the age of 7 to over 16 years from various sections of the United States, and observed a dissimilarity in the rate of increase in caries as well as a difference in caries prevalence among the different tribes. Among the various age groups, the southwestern Indians had both the lowest DMF rate (decayed, missing and filled) and the largest percentage of children with no decay experience. Caries in the permanent teeth was most prevalent in the northwestern tribes.

Arnim, Aberle and Pitney (1937) examined 204 Indian children between the ages of 7 and 11 years from New Mexico and Arizona. Thirteen per cent were caries-free and 64.2 per cent had no caries of the permanent teeth; 24.1 per cent had white spots on the enamel suggestive of dental fluorosis.

Steggerda and Hill (1936) examined 1,861 Navajo and 944 Maya Indians. Among the Navajos, the percentage of persons having caries varied from 5.3 in the 6- to 10-year age group to 50.0 in the 30- to 35-year age group. Among the Maya Indians, it varied from 3.2 in the 6- to 10-year age group to 72 in the 30- to 35-year age group. The average number of cavities per mouth was 2.8 in the Navajo and 2.3 in the Maya. The per cent of teeth carious in the Navajo varied from 0.3 in the 6- to 10-year age group to 9.4 in the 30- to 35-year age group. Among the Maya, it varied from 0.2 to 8.9 per cent. The authors state that the Mayas have a high carbohydrate diet while the Navajos have a high protein diet. Although the caries rates did not differ significantly, the types of cavities showed marked differences.

In 34,003 dental examinations in 24 Indian reservations, Mountin and Townsend (1936) found 10,747 dental defects.

Foster (1942) found that the DMF rates among 274 northern Wisconsin Indians varied from 0.33 teeth at 6 years of age to 8.0 teeth at 16 years of age, with an average of 2.21 DMF teeth for children from 6 through 16 years of age. White children living under similar environmental conditions had an average DMF rate of 2.68 teeth per

child. This difference, however, was not statistically significant.

Summary and conclusions: Modern primitive peoples show less dental caries than do most civilized peoples. Whether environmental factors such as diet exert a more important influence, or whether an inherent racial resistance is of prime significance, cannot be ascertained with certainty. Evidence indicates, however, that primitive peoples have an increased caries attack rate when brought into contact with modern civilization and a civilized diet.

DENTAL CARIES IN MODERN CIVILIZED PEOPLES

Geographic or Temporal Variations in Caries Prevalence

There is a significant difference in the prevalence of dental caries among many of the countries scattered over the world and even in specific areas within a country. Hagan (1947) for example, has demonstrated how the DMF rate may vary within narrow geographic limits, while Neumann (1946) has considered the wide differences among the large geographic areas. This difference in some instances may be as great as, or greater than, has been reported between some groups of civilized and primitive peoples. Schour and Massler (1947) in a dental study of 3,905 individuals between 11 and 60 years of age from 4 Italian cities, found them to have from two to seven times less dental decay than was observed in sections of eastern United States. There appear to be not only geographic variations but also fluctuations in dental caries prevalence within the same area from time to time. These differences must be ascribed to environmental changes which operate among the individuals of a locality either systemically or within the oral cavity or by a combination of both. Although it is not the purpose of this section of the monograph to enter into a lengthy discussion of these observed modifiers, some mention of them must be made at this time in order that the reader will not be confused by the differences in dental caries prevalence rates reported from different areas. Many of these modifiers of dental caries prevalence have been studied rather extensively, some have been studied only cursorily and perhaps there are others entirely unexplored by the dental investigator. Certain of these variables have been pointed out in a number of papers. Those that have received the greatest attention are:

1. Hours of sunlight.

From statistical data analyzed by Mills (1937), East (1942), Kaiser and East (1940), East and Kaiser (1940), East (1941), among others, an inverse relationship has been observed between the number of hours of sunshine and the dental caries prevalence in an area.

2. Constituents of the drinking water.

From the data of Röse (1908), Förberg (1901), Mills (1937), Ockerse (1944) and others, a relationship between the hardness of the drinking water and the prevalence of dental caries has been postulated. Dean (1938) however, could find no such association. On the other hand, evidence of Dean and associates (1946) leads to a correlation between the fluorine content of the drinking water and dental caries: within specific limits, the fluorine content of the water was found to be inversely proportional to the prevalence of dental caries. Confirmation has come from many countries and from widely scattered areas in the United States. The role of fluorides in dental caries prevention in natural fluoride areas cannot be doubted.

3. Mineral content of soils and their crops.

Albrecht (1947) has associated fertility of the soil with high protein crops and low caries prevalence in the various geographic areas, as contrasted with leached and exhausted soils which yield chiefly carbohydrate crops and where the areas show a higher prevalence of dental caries. This speculative hypothesis needs confirmation.

4. Economic status.

A relationship between dental caries prevalence and economic status has not been clearly established. (Reiser 1931) (Greenwald 1939) (Klein and Palmer 1941a) (Klein 1941) (Wilkins 1941)

5. Racial differences.

The prevalence of dental caries among races will be discussed later in this paper. The evidence indicates that there are racial differences in prevalence rates.

6. Restricted diets during wars.

Evidence assembled from European countries during the first and second world wars indicates a steady decline in the incidence of dental caries during the war years, when many constituents of the diet were limited or completely unavailable. Whether this perceived reduction was due to a decrease in the amount of available carbohydrate or to inclusion of more desirable foods as a replacement for sugars and other refined carbohydrates is open to debate. Sognnaes (1948a) has reviewed the available literature on the subject. The evidence indicates that the reduction in dental caries among European children is an actuality and worthy of further study.

Summary and conclusions: Some possible variants affecting dental caries prevalence have been mentioned at this time to acquaint the reader with the presence of these variables. Although they may be potent factors affecting the caries picture, these influences are merely mentioned in the text of this paper. A more complete discussion of these subjects will be found in other papers of this series which deal more specifically with these factors. The chief reason why these modifiers of caries rates are presented in this section of the review is to indicate to the reader that, disregarding chance variation and differences due to different examiners, there still exist differences in dental caries rates among geographic areas which are the result of modifications in the diet or in the environment under which these individuals exist. These factors account for many of the differences in caries prevalence reported from various geographic areas.

Prevalence Rates in Preschool Children

There is a paucity of surveys on the incidence of dental caries in the preschool child. The apparent reason for this lack of information is the difficulty of assembling any sizeable representative population group for examination. Most of the preschool studies have been done on selected groups from hospitals or institutions or controlled for some special research problem, or on children seeking dental attention at a clinic. These children have not always been representative of a cross section of the general preschool population and, as Hollander and Dunning (1939) have observed, in most cases clinical material cannot be employed as a general measure of caries incidence.

McCall (1934) in studies conducted at the Guggenheim Dental Clinic on 1,089 preschool children found them to be very susceptible to dental caries. At 4 and 5 years of age, more than half of the children examined had 7 or more cavities per child. At 2 years of age and under, 17 per cent of the children had 7 or more cavities per child, and over 58 per cent had already experienced dental decay. These studies employed both clinical and X-ray examination, and in addition prophylaxis was given before each examination. This may account for the seemingly high prevalence rate. The percentage of mouths with carious teeth as found in these preschool children is included in table 3.

Munblatt (1933) in a study of 455 institutionalized school and preschool children found that the percentage of caries-free children varied from 100 at 2 years of age to 54.54 at 5 years of age, and the percentage of teeth carious varied from 0 at 2 years of age to 13.9 at 5 years of age. The original article describes the 125 preschool children as being divided into half-year age groups, with a resultant great variation in rates between the half-year ages because of in-

sufficient numbers in each group. It has been observed by many investigators that institutionalized children average fewer teeth with dental caries experience than the general population of corresponding age (Hewat 1931–32). The percentage of caries-free children as found by Munblatt (1933) is presented in table 9 and the percentage of carious teeth in table 10.

Cohen (1936) (1937) reporting data from 2,554 children between the ages of 2 and 15 years in Minneapolis, found that children from higher socio-economic groups had better deciduous teeth. He also found better deciduous teeth in non-Jewish children up to 6 years of age, but the reverse was true for the permanent teeth after 12 years of age.

In a study made by Finn (1947) on a group of 938 preschool children in connection with the fluoride study being conducted by the New York State Department of Health, he found 24 per cent of the children were caries-free at 5 years of age, with 4.67 DF deciduous teeth and 8.42 DF surfaces per child as determined by mouth mirror and explorer examination under good light. Table 2 presents the findings in both the deciduous and permanent teeth.

Hamill and Sausser (1933) examined 2,982 children between 1 year and 14 years of age. Of these, 2,165 were white, 650 were colored and 150 were unclassified patients

TABLE 2

Dental caries experience in 938 preschool children in Newburgh and Kingston
(Finn 1947)

Age	Children with Teeth		Teeth per child		% of children caries-free	DF deciduous teeth per child	DF surfaces per child	DMF permanent teeth per child	DMF surfaces per child
	D	P	D	P					
2	119		18.80		89.60	0.31	.35	.00	.00
3	127		19.80		69.30	1.13	1.37	.00	.00
4	158	2	20.00	1.00	45.60	2.58	3.58	.00	.00
5	534	186	19.10	3.30	24.30	4.64	8.33	.35	.24

The "average" child, 3 years of age, showed 70 per cent of the lower second deciduous molars carious and about 60 per cent of the upper second deciduous molars. About 30 per cent of the upper central incisor teeth and 20 per cent of the upper lateral incisor teeth were carious, while less than 10 per cent of the remaining teeth had been attacked.

Jones, Larsen and Pritchard (1930) in a study of 1,610 preschool and school children of various races in Honolulu found 91.5 per cent of the children at 5–6 years of age affected with dental caries. One-third of the children between 6 months and one year of age were affected with odontoclasia, as were 76.9 per cent at 5–6 years of age. The percentage of carious teeth and mouths with carious teeth for the various ages is included in table 3.

with heart disease. The children were well distributed among all age groups. Two-thirds of the white children were under 6 years of age. All were from low income families. At 1 year of age, there was an average of 0.95 cavities per child; at age 2, 1.98; at age 3, 3.75; at age 4, 4.80, and at age 5, 5.7 cavities per child. The per cent of mouths with carious teeth at various ages is included in table 3.

Jensen (1941) among 471 Copenhagen children under 6 years of age found that 16 per cent of the boys and 21 per cent of the girls had good teeth.

Jessen (1906) among 2,269 Strassburg clinic children between 3 and 6 years of age found 84 per cent with caries. Approximately 7.8 per cent had 10 or more cavities.

Doherty (1931) states that in medical examinations conducted in Birmingham, England, of some 7,000 children examined, 2.3 per cent of those from 18 months to 2 years of age showed defective teeth. At age 4–5 years, the percentage had increased to 52.3.

Pitts (1927) found that slightly over 26 per cent of 84 English children under 3 years of age coming to a dental treatment clinic showed dental caries.

Grandison (1937) examined the elementary school children in Cambridge and found that the 5-year age group suffered most from dental caries; over 20 per cent of the deciduous teeth, and 26.2 per cent of the permanent teeth, were carious. At 14 years of age, only 5.8 per cent of the teeth were carious.

In 1937, Henderson found among 1,530 preschool children that at one year of age 5 per cent of the teeth were carious, while at 2 years of age, 37.3 per cent of the teeth had become involved.

Among 1,197 Kansas City white preschool children, Rypkins (1922) found by tongue blade examinations conducted by physicians that 27.2 per cent of the children showed dental decay averaging 1.07 cavities per child and 3.95 decayed teeth for each mouth with carious teeth. In the 3- to 6-year age period, 44 per cent were carious with 8.9 per cent of all teeth carious, mouths with carious teeth averaging 4.09 cavities per child. The findings for individual ages are presented in table 3.

An analysis by Ast (1944b) of 544 dental records from upstate New York Child Health Conferences revealed that 39.8 per cent of the children between the ages of 1 and 5 were free from dental caries. Under 2 years of age, 5.3 per cent showed cavities; at 2 years of age, 34.7 per cent; at 3 years of age, 63.2 per cent; at 4 years of age, 85.4 per cent; and at 5 years of age, 87 per cent of the children had cavities. Of these 544 children, 18.7 per cent had over 5 cavities per child.

Roos (1944) examined 2,573 children from the area around Malmö, Sweden, who were between 1½ and 7 years of age. He found that 23 per cent of the children 1½ to 2 years of age had evidence of caries, and at 5 years of age 95 per cent had already been attacked. In considering a total of 41,258 teeth at age 1½ years, 4.4 per cent were carious; at age 2, 13.9 per cent; at age 3, 29.7 per cent; at age 4, 40.1 per cent; at age 5, 49.2 per cent; and at age 6, 52.2 per cent. Among all ages, 42.7 per cent of the deciduous teeth were carious. Roos has done a most complete study of the teeth of the young child and has presented a review of the caries-prevalence findings of other investigators among preschool children. His data with additional information are presented as table 3.

Summary and conclusions: A summary of the various papers on the prevalence rates in preschool children indicates that caries of the deciduous teeth frequently begins soon after eruption of the teeth and increases dramatically thereafter. Roos (1944) found that at 1 year of age 4.4 per cent of the teeth were carious. Henderson (1937) found 5 per cent of the teeth carious at this age. Jones, Larsen and Pritchard (1930) found one-third of the children affected with odontoclasia at age 1 and under. In comparing these rates with those found in 5-year-old children, the rampancy and rapid progress of dental caries in the preschool child becomes obvious.

At age 5, 49.2 per cent of the teeth were found by Roos (1944) to be carious; Leuthold (1922) found 30.6 per cent affected; Jones, Larsen and Pritchard (1930) reported 33.3 per cent affected. McCall (1934) found 97 per cent of the children at 5 years of age to have experienced dental decay; Jones, Larsen and Pritchard (1930) 91.5 per cent; Hamill and Sausser (1933) 82.1 per cent; Finn (1947) 74.6 per cent; Doherty (1931) found over 50 per cent of the children 4 and 5 years of age with cavities. It is evident from these figures that caries in the preschool child is a serious problem and one which sooner or later must be controlled.

Prevalence Rates in School Children

By far the greatest number of dental studies have been conducted with school children, both elementary and high school. Children between the ages of 5 and 18 years are conveniently assembled in school buildings where they can be easily examined. Because of the vast amount of available material on caries rates in school children, a portion of this material will be presented in the form of composite tables. The Caucasian race only is considered in this portion of the paper. A comparison with other races is discussed under a subsequent heading.

Odenthal in Germany in 1887 examined 987 school children and found that 43.5 per cent had cavities.

A report of the School Committee of the British Dental Association (1891) on the condition of the teeth of school children in England, states that 84 per cent of the child population had some decayed teeth.

Fenchal (1893) examined 335 "State" school children in Germany, 135 of whom were females; 323 children of this group had 2,471 carious teeth, or an average of 7.65 carious teeth per child.

Besten (1894) found in villages near Würtzburg, Germany, that among 3,347 children between 6 and 14 years of age, 17 per cent were free of caries. Of 78,348 teeth (deciduous and permanent), 12,015 or 15.3 per cent were carious. Of the 48,180 permanent teeth, 5,090 or 10.3 per cent were carious. Among the 30,168 deciduous teeth, 6,935 or 22.9 per cent were carious.

Guillermin (1895) examined 2,222 school children of Geneva, Switzerland. Carious teeth were found in 1,538 or 69 per cent. A classification according to ethnic stock revealed that the Swiss-French had the highest caries rate, with 72 per cent of the children having caries, while the least carious teeth were among the Italians, with 60 per cent of the children having cavities.

Fricke (1900) reported on the examination of 19,725 school children between 6 and 15 years of age in the German province of Schleswig-Holstein and observed that 95 per cent of the children had carious teeth. There was no difference in the caries rates

TABLE 3

Dental caries in the preschool child, adapted from Roos (1944)

Author	Country	Year	Per cent of mouths with carious teeth				Per cent of teeth carious			
			Age				Age			
			3	4	5	6	3	4	5	6
Rypkins	U. S. A.	1922	30.4	38.0	62.5	—	5.7	7.7	13.1	—
Leuthold	Switzerland	1922	50.0	77.6	91.8	97.6	9.3	18.0	30.6	34.3
Jones and coworkers	Hawaii	1930	86.2	90.9	91.5	—	26.8	35.4	33.3	—
Hamill & Sausser	U. S. A.	1933	72.1	78.0	82.1	86.0				
McCall	U. S. A.	1935	81.5	92.0	97.0	—				
Backhaus	Germany	1935	24.3	43.0	63.0	76.7	2.9	6.8	11.8	16.1
Suss	Germany	1937	61.1	75.6	84.0	87.9				
Willeke	Germany	1937	24.9	49.3	64.0	80.0				
Oravecz	Hungary	1937	30.0	55.0	78.0	80.0				
Ormeloh	Germany	1938	57.1	70.5	69.5	90.0				
Limbach	Germany	1938	40.0	57.1	80.0	81.8				
Müller	Germany	1938	37.2	64.3	76.0	72.2				
Schlosser	Germany	1938	73.5	64.4	74.7	77.9				
Sensen	Germany	1939	26.5	48.4	58.1	60.9				
Stein	Germany	1941	26.2	56.5	89.0	—	3.4	10.0	15.4	—

of children living in the highlands and in the lowlands.

Billeter (1901) conducted examinations on 300 children of Schaffhausen, Germany. These were 6th grade children, 12 and 13 years of age; 10 per cent were caries-free.

Müller (1901) examined 2,902 teeth among 116 secondary school children in Wadensweil in Switzerland and observed that 35 per cent of these teeth were carious; 100 per cent of the children displayed decay.

Worm (1903) found among 3,183 Gleiwitz children that only 86, or 2.7 per cent, were caries-free. There was an average of 5.23 carious teeth per child, almost half of which were deciduous teeth.

An anonymous editorial published in 1903 reported the findings on 1,931 school children in the primary grades in Zurich; 3.11 per cent of the children had no caries. Of 47,204 teeth examined, 13.7 per cent were carious and 1.3 per cent had fillings.

Greve (1903–1904) examined 4,522 school children of Magdeburg and found 3.07 per cent with no caries.

Cunningham (1908) analyzed 1,403 dental examinations from among those conducted on all Cambridge school children for caries experience. The decay prevalence of these children expressed as the number of extracted and carious teeth per child is presented in table 8. There was an average of 3.44 carious and extracted teeth per child; 3.1 per cent of the children had no decay in the permanent teeth.

Kloser (1913) tabulated the findings of investigators in a vast number of German communities; 212,987 children had 3,619,664 sound teeth and 1,617,206 carious teeth. Approximately 30.9 per cent of the teeth were carious, while 96.05 per cent of the children showed some decay. There was an average of 7.6 decayed teeth per child. The number of children showing dental decay varied from 71.4 per cent in Magdeburg to 99.97 per cent in Augsburg and 100 per cent in 300 Berlin children. Krumhermersdorf's 430 children showed 100 per cent of the individuals with decay, as did the children of Kongsmark. Kloser has presented a table showing the findings of investigators throughout the world.

Babini (1913–14) examined 408 children in Italian schools. Caries appeared in 70 per cent of the mouths, divided as follows: 63 per cent in the first grade, 73 per cent in the second grade, 70 per cent in the third grade and 58 per cent in the fourth grade. Of a total of 9,402 teeth, 9.45 per cent were carious. One per cent of the permanent teeth and 16 per cent of the deciduous teeth were carious.

Isacsson (1917) in Stockholm examined 283 children 7 years of age. Of an average of 14.8 deciduous teeth remaining in the mouth of each child, 8.4 or 56.8 per cent were carious.

Suk (1919), comparing the caries incidence among various races, presented the following figures for the percentage of mouths with caries-free permanent teeth in 694 Prague, Czechoslovakia, school children from 5 to 19 years of age (see table 8). It will be observed that 74.3 per cent of the children had evidence of decay in permanent teeth. The total number of decayed teeth was 1,764 of 16,567 teeth present, or 10.64 per cent.

Bloch-Jörgensen (1920) found in 1,023 school beginners in Aarhus only 7 with perfect teeth. Of 15.3 deciduous teeth per child, 9.7 were carious.

Ainsworth and Young (1925) in examinations of 4,600 children between the ages of 5 and 13 years (a few being older or younger) attending grade schools scattered throughout the British Isles, found that 43.3 per cent of all upper deciduous teeth and 13.1 per cent of all permanent teeth were carious.

Schwarz (1930) examined 19,027 school children in Germany from 43 primary schools and found that 93.9 per cent had caries; 26.8 per cent had caries in the permanent teeth and 26.9 per cent had caries only in the deciduous teeth.

Campatelli (1931) observed among 4,640

6- to 12-year-old patients in Italy 10,758 carious teeth, or 2.3 cavities per child.

Stoughton and Meaker (1931) have tabulated the percentage of children of ages varying from 6 to 19 years with 1 or more cavities and with 5 or more cavities. In all, 12,435 children who had received mirror and explorer examinations in Hagerstown, Maryland, and from areas in Georgia, Illinois and Missouri were considered. The percentage of children having at least 1 cavity is presented in table 8; 59.7 per cent of the 6-year-olds had 5 or more cavities in the deciduous teeth, while 0.3 per cent had a similar number in the permanent teeth; 61 per cent had 5 or more cavities in both dentitions. At 19 years of age, 83.3 per cent of the children had cavities in 5 or more teeth.

Reiser (1931) presented the results of examinations on 3,975 Zurich school children. These children were from the first, third and sixth grades. Yearly dental care was provided in the school clinic. In the first grade, 10.5 per cent of the children had very good teeth (1 or no cavities), 27.8 per cent had 2 or 3 cavities, 49.5 per cent had 4 or 5 cavities and 12.2 per cent had 8 or more cavities. In the third grade, 5.5 per cent had very good teeth, 49.7 per cent had 2 or 3 cavities, 38.2 per cent had 4 or 5 cavities, and 6.6 per cent had 8 or more cavities. Among the 6th graders, 7.2 per cent had very good teeth; 51.7 per cent had 2 to 3 cavities, 35.5 per cent had 4 or 5 cavities and 5.6 per cent had 8 or more carious teeth. These children were further subdivided into social classes and a comparison made on that basis.

Franci (1932) tabulated results of 1,758 dental examinations conducted in the schools of Siena, Italy. Of the children examined, 1,588 presented carious teeth (89.25 per cent), while only 170 were caries-free. In a similar examination conducted by Franci in Siena in 1913 among 1,266 children, 89.79 per cent showed caries. Of a total of 38,786 teeth examined, 10,017 were carious, or 25.8 per cent of all teeth observed. Franci mentions the caries rates as found by investigators in other Italian cities: Milan, 92 per cent; Genoa, 73 per cent; Bologna, 69.79 per cent; Rieta, 64 per cent; Livorno, 55 per cent; Torino, 54 per cent; Rome, 50 per cent; Brescia, 46 per cent; and Parma, 40 per cent. He also discusses a comparison between the hardness of the drinking water and the caries rates.

In school children in Ecuador, Toro-Freile (1933) found that 89 to 93 per cent of the school children presented dental caries. Cavities were more frequent in the city children than in the rural children.

Munblatt (1933) conducted a study of the dental caries incidence in children who were institutionalized. These 455 children ranged in age from 2 to over 12 years, although the majority ranged from 5 years upward. The percentage of children with decay as reported by the author is presented in table 8. The percentage of incidence of caries is presented in table 9.

Day (1934) examined 227 males and 87 females averaging 12 years of age from 5 villages around Hissar City in India. Of a total of 8,312 teeth, 196 were carious with 0.62 cavities per child; 80 per cent of the cavities were merely pits and fissures; 74.2 per cent of the children were caries-free. Mottling affected 73.57 per cent of the children, indicating that fluorine may be a factor in the low caries prevalence.

Mellanby (1934), table 4, summarizes the findings of investigators in England and in other sections of Europe.

Krohn (1935) found that 98 per cent of 7,000 Copenhagen school beginners had caries.

Day and Sedwick (1935) X-rayed and examined 433 13-year-old children of both sexes and of various nationality stocks in Rochester, New York. In this group of children they found that 11,317 erupted teeth had a total of 9,423 defects for a caries index of 0.83. Only one mouth in the 433 was caries-free.

Steggerda and Hill (1936), comparing the caries incidence among various races, examined 1,358 children of Dutch ancestry in Holland, Michigan. These examinations were made with the use of tongue blades only. The percentage of children with decay is reported in table 8. The percentages ranged from 46.9 in the 6- to 10-year-old group to 96.7 in the 16- to 20-year-old group. The percentage of teeth attacked by caries is reported in table 9. The DMF rate is presented in table 7. The DMF rate in children with caries varied from 2.4 to 6.9, with an average of 4.53 DMF teeth.

Messner, Gafafer, Cady and Dean (1936) tabulated the dental caries prevalence rates in a study of approximately 2.5 per cent of the school population of 26 states as reported by local examiners. The survey included both rural and urban areas. Because of the large number of examiners employed and the variety of interpretations as to what constituted a carious lesion (more than half the examiners classified pits and fissures as caries), it is difficult to draw comparisons for statistical evaluation. Some of their figures, however, have been tabulated by East (1941), East and Kaiser (1940), and Kaiser and East (1940). Because of the vast amount of material presented, the reader is referred to the original article.

Holz (1937) found among 285 7-year-old children from Sweden that 56.6 per cent of the teeth were carious.

Humphreys (1937) examined 94 refugee Basque children from 5 through 15 years of age. Of a total of 1,700 teeth present in these children, 185 or 10.88 per cent were carious. The author compares this figure with that resulting from the examination of English children by Ainsworth (1925). Among the British children, 13.1 per cent of the teeth were carious.

Mansbach (1937a) found among German Jews immigrating to Palestine that only 10.5 per cent of the 6- to 12-year-old children were caries-free, while the average Palestinian born in rural districts was from 43 to 95 per cent caries-free.

Clements and Kirkpatrick (1938) found in 802 children from New South Wales, Australia, that 97.5 per cent of the children who were 5 years of age and over suffered from dental caries. There was an average of 12.75 carious tooth surfaces for each child who had been attacked by caries. There was an average of 6.13 carious teeth and 0.96 extractions per child.

TABLE 4

Caries in European children

(Mellanby 1934)

Location	Author	Number of individuals	Per cent of individuals with carious teeth
Cambridge Dental Clinic	Cunningham	1,403	96.5
Shropshire Poor Law cases	Salop	7,374	95.9
British Dental Association Investigation (1891–97)	—	12,318	84.0
Birmingham School children	Richards	1,424	94.7
Kettering School children	Leydon	5,724	93
English Public School children	—	560	87
North German School children	Schleswig-Holstein Dental Assoc.	19,725	95.0
Baden-Hamburg residents	Röse		98.7
Würzburg children	Berlin		83.0
Hungarian children	Unghrari		87.2
Finland children	—	5,903	95.3

King (1938) reported on the incidence of dental caries in the primary and secondary schools of the Isle of Lewis and in parts of West Ross-Shire, which is on the mainland. There were 1,030 primary school children examined in the Lewis country schools and 250 in the town school. On the mainland, 96 pupils were examined in 4 West Ross-Shire country schools. The children ranged

in age from under 6 to 15 years of age. In the Lewis country primary schools, 7.9 per cent of the permanent teeth and 24.2 per cent of the deciduous teeth were carious. In the Lewis town schools, 19.6 per cent of the permanent teeth and 52.2 per cent of the deciduous teeth were carious. In the West Ross-Shire country schools, 20.9 per cent of the permanent teeth and 53.3 per cent of the deciduous teeth were carious. In the secondary schools of Lewis, 110 pupils in the 11- to 20-year age group were examined; 27.4 per cent of the teeth were carious. These were in the country schools. Of the 140 pupils from the town districts of Lewis, 15.2 per cent of the teeth were carious. The Isle of Lewis is a relatively isolated area, and this factor may bear on the lower prevalence of caries on this island, especially in the rural areas.

Mashankar (1938) examined 4,365 students between 5 and 25 years of age, of all castes and natioualities, in Ankola, India. Dental caries was present in around 15 per cent of the Ankola high school students and in 24 to 47 per cent in the primary schools.

Perhaps the most revealing studies of the school child were conducted by Klein and associates with the United States Public Health Service in the community of Hagerstown, Maryland. Klein, Palmer and Knutson (1938) carefully examined the entire elementary school population of Hagerstown and thoroughly analyzed the data. Among 4,416 children from 6 through 15 years of age, 71.5 per cent showed a history of caries in one or more of the permanent teeth. The percentages of children with caries for the various age groups are presented in table 8, the DMF rates in table 7. For the various ages, the DMF rate was 2.78 teeth per child.

Schiötz (1939) examined 127 children averaging 10 years, 7 months of age in Hedmark County, Norway. Among 2,136 teeth present, 580 had experienced caries; 17 per cent of the children were caries-free. The area is rather isolated and the residents live on natural foods.

Taylor (1939) reported that in 2,268 children between 5 and 6 years of age examined in various parts of New Zealand, 48.6 per cent of the teeth present were carious.

Read (1939) reported examinations of 2,894 school children aged 6 to 13 in Leeds, England. At age 6, 6 per cent of the children were caries-free; at age 9, 0.5 per cent were caries-free; and at age 13, 4 per cent were caries-free.

Sarkar (1939) in 1930 examined 18,445 school children up to 16 years of age in Bengali; 13.3 per cent had defective teeth. In 1931, he examined 20,000 children, of whom 14.4 per cent had defective teeth.

Klein and Palmer (1940) conducted examinations on 1,841 high school children at Hagerstown. Among the 13- through 19-year age group, only 63 of the 1,841 children examined were DMF-free. The authors present the number of DMF teeth and tooth surfaces and break the data down into the number of filled teeth and surfaces, the extracted teeth or remaining roots and the number of untreated carious teeth. The number of DMF teeth per student found in the various age groups is given in table 7. For all age groups, the DMF rate was 7.1 teeth per child.

Sloman and Sharp (1940) in an examination of 46,275 San Francisco elementary school children, reported that among the 11- to 14-year age group between 8.9 and 11.2 per cent of all the permanent teeth were found to be carious. Approximately 80 per cent of the 10- to 12-year-old children had dental defects. Of the 6,923 high school children examined, from 12 through 18 years of age, it was found that only 6 per cent were DMF-free. Nearly 64 per cent had one or more actively carious teeth, and 76 per cent had one or more filled teeth. The average number of decayed, missing or filled teeth for the various age groups is presented in table 7. For all ages, there was an average of 6.3 DMF teeth per child. The percentage of teeth attacked by caries is presented in

table 9 and averages approximately 20 per cent of all teeth.

Kaiser and East (1940) and East and Kaiser (1940) have analyzed the data of Messner *et al.* (1936) in 358 counties containing rural and semi-rural areas in the United States. Among the 6- to 8-year age group, they found 103.7 cavities in the permanent teeth per 100 children. In the 9- to 11-year age group, there were 229.0 cavities per 100 children and in the 12- to 14-year age group, 366.2 cavities per 100 children.

Lathrop (1940–41) at Letchworth Village in New York State reported on the dental caries incidence of 144 institutionalized females from 12 to 15 years of age. He found 10.59 carious teeth per person and 18.2 carious surfaces, or 1.7 carious surfaces per carious tooth. The decayed, missing and filled rate for each age group is given in table 7. The DMF rates ranged from 6.7 at 12 years of age to 8.6 at 15 years of age.

East (1941) tabulated the figures from Messner *et al.* (1936) for 528,842 children in 156 United States cities. He found that between 6 and 8 years of age there was a mean caries rate of 124.4 per 100 children. For the children 9 to 11 years of age, it was 270.5 per 100 children and for children 12 to 14 years of age, it was 435.5 per 100 children. The city children had higher caries rates than those found by East and Kaiser (1940) among the rural children.

Ockerse (1941) found that of 4,955 South Africa school children, 97 per cent showed carious teeth. The caries rate was 99 per cent among the urban born children and 97 per cent among the rural born.

Sloman (1941) compares the caries attack rate among girls and boys and compares the percentage of the population with one or more carious teeth as found by him with those found by Stoughton and Meaker (1931) in the 12- through 18-year age group. The figures for the percentage of children with caries experience in each age group and in both sexes are presented in table 8. There were 96.2 per cent of the males 18 years of age and 98.4 per cent of the females, with dental caries experience.

Shourie (1941) examined 6,866 children from 5 through 17 years of age from various rural and urban sections of India. He found with mirror and explorer examination that, of a total of 38,670 deciduous teeth, 10.8 per cent were carious and, of 139,679 permanent teeth, 4.0 per cent were carious. The percentage of children with caries at various age levels is presented in table 8. The percentage of deciduous and permanent teeth attacked by caries at various age levels is presented in table 10.

Chapin and Mills (1942) offer evidence that Panama-born Americans and those who have lived for some years in the Canal Zone have a higher caries experience rate than Americans who have resided in Panama for short durations. Panama-born Americans of 12 to 14 years of age have a caries experience of 7.85 teeth per child, while those who have resided less than 2 years there have a caries experience of 6.02 teeth per child.

East and Pohlen (1942) considered the dental status of 1,005 children between the ages of 5 and 17 years in Bergen County, New Jersey. At 6 years of age, 59 per cent of the boys and 48 per cent of the girls had DMF teeth, and at this same age 21 per cent of girls and 15 per cent of boys had as many as 4 DMF teeth per child. At age 8, only 4 per cent of children were DMF-free, while 11 per cent of the girls and 6 per cent of the boys had 5 or more DMF teeth. The number of children with 4 DMF teeth increased rapidly until children reached 8 to 10 years of age. The number of children with only 4 DMF teeth reached its peak in girls at 8 years of age and in boys at 10 years of age. The "health index of the biodenture" as designated by East decreased from year to year.

Foster (1942), comparing the caries prevalence among white and Indian children in northern Wisconsin, found an average of 2.68 DMF teeth per child in 188 white

children aged 6 through 16 years. The DMF rate for the white children is presented in table 7.

Blackerby (1943) reported the findings of dental examinations of 26,576 white children from 6 through 15 years of age in the eastern, central and western parts of Tennessee. There was a difference in dental caries experience between the various areas in the state. The DMF rate was highest in the 8-year age group. The DMF rate for the entire dentition, both deciduous and permanent, by age groups is given in table 7.

Berk (1943) reported on 198 children of low income families. At 5 years of age, 94 per cent of the children presented evidence of caries experience. Among 106 first-born children, the caries attack rate was 8.82 teeth per child. Among 92 children of fourth pregnancy or later, the caries attack rate was 10.42 teeth per child.

Brucker (1943) compared the caries incidence among white and Negro children in Newark, New Jersey. Among 7,447 white children between the ages of 6 and 16 years, he found 737, or 9.8 per cent, caries-free. Among a total of 9,269 Negroes and whites examined, 12.3 per cent were caries-free.

From examinations of 3,867 children 12 through 14 years of age in fluoride-free midwestern cities, Dean (1944) found DMF rates ranging from 5.56 in Marion, Ohio, to 10.37 in Michigan City, Indiana. The entire group of cities averaged 7.40 DMF teeth per child.

Wilzbach (1944) examined 5,623 junior and senior high school students in Cincinnati, Ohio, and found caries present one or more times in 4,698, or 83.3 per cent, of the students.

Gruebbel (1944) examined 3,250 children aged 6 through 12, in four Missouri towns; 118 of the charts of the permanent teeth were omitted. The DMF rate and def (decayed, filled and known extracted) rate is presented in table 7. The percentage of children with DMF and def teeth is shown in table 5.

The mean DMF rate for the permanent teeth was 1.04 teeth per child. The mean def rate was 1.56 teeth per child. The percentage of children with DMF teeth varied from 62 in Valley Park to 32 in Clayton. The percentage of children with def teeth varied from 61 in Brentwood to 38 in Clayton.

TABLE 5

The per cent of children with "def" and "DMF" deciduous and permanent teeth in four Missouri towns

(Gruebbel 1944)

Town	No. of children	Age														Total	
		6		7		8		9		10		11		12			
		def	DMF	def	DMF	def	DMF	def	DMF	def	DMF	def	DMF	def	DMF	def	DMF
Brentwood	482	56	3	79	12	72	24	72	43	50	44	53	69	34	67	61	35
Clayton	970	30	—	56	8	62	24	51	39	36	46	26	62	10	38	38	32
Kirkwood	1,442	45	1	65	12	61	28	61	32	49	61	32	61	13	67	48	34
Valley Park	356	90	22	95	40	96	61	87	61	50	81	15	78	—	71	57	62

Krohn and Pedersen (1945) tabulated the findings from the dental examination of 57,000 school children between 6 and 7 years of age in Copenhagen, from the years 1936 through 1943. They report a steady rise in the number of caries-free mouths from 2.25 per cent in 1937 to 5.36 per cent in 1943. The rise was most marked during the latter war years. There was also a marked drop in the percentage of carious deciduous molars and permanent first molars.

Bhat and Shelty (1947) examined 1,988 students, ranging in age from 3 to 32 years,

in Bombay, India, schools and colleges; 73.65 per cent had carious teeth.

In four Italian cities, Schour and Massler (1947) examined 3,905 individuals from 6 to 60 years of age. The number of DMF teeth per person for the various age groups was as follows: 6–10 years, 2.41 (includes carious deciduous teeth); 11–15 years, 1.05; 16–20 years, 2.02; 21–30 years, 2.31; 31–40 years, 6.96; 41–50 years, 9.26; and 51–60 years, 10.80. The percentages of caries-free dentitions in the various age groups are: 6–10 years, 29.6; 11–15 years, 53.4; 16–20 years, 38.4; 21–30 years, 16.7; 31–40 years, 8.4; 41–50 years, 6.1; 51–60 years, 9.0. These figures indicate less caries than found in the American population.

Finn (1947) examined 5,824 children between the ages of 6 and 14 in the cities of Newburgh and Kingston, New York. The DMF and DF rates for the permanent teeth and the deciduous teeth are presented in table 7. The percentage of children with decay experience is given in table 8. It will be observed that the DMF rate is 4.20 permanent teeth per child and the DF rate is 3.42 teeth per child; 77.6 per cent of the children had one or more teeth with caries experience.

Knutson (1947) analyzed the data of examinations of 2,627 Nicollet County, Minnesota, white school children between the ages of 6 and 18 years. The percentage of children revealing caries experience as presented in table 8 varied from 24.3 at 6 years of age to 100 per cent at 18 years of age. The mean number of DMF teeth per child in each of the age groups is presented in table 7. At 18 years of age there was an average of 11.21 DMF teeth per person.

A dental survey by Wisan and Chilton (1948) on 61,612 school children between 4 and 20 years of age revealed a def rate as high as 4.30 teeth per child at 7 years of age and a DMF rate as high as 10.86 at 19 years of age. The def rate and DMF rate for each age are presented in table 7. A comparison of the dental caries experience reported by Messner, Gafafer, Cady and Dean (1933) on 259,307 New Jersey children and those by Wisan and Chilton in 1944–46 is shown in table 6.

The yearly increment of new cavities changed from 0.64 to 0.91 DMF teeth per year, indicating an increasing prevalence rate of caries.

Hagan and Cook (1949) using tongue blades, examined 7,500 children from divergent areas of Louisiana. Some areas had lower caries rates and evidence of mottling.

Shourie *et al.* (1949) examined 1,050 males and 1,049 females from 6 to 18 years of age from diverse areas of Puerto Rico. Using mouth mirror and explorer, they found the DMF rates for permanent and deciduous teeth as presented in table 7.

TABLE 6

Wisan and Chilton (1948)

A comparison of dental caries experience in New Jersey children between 1933–34 and 1944–46

Age	def teeth per child		DMF teeth per child	
	1933	1944	1933	1944
6–8	3.61	4.14	1.37	1.49
9–11	1.79	1.98	3.22	3.63
12–14	0.32	0.22	5.18	6.96

Hadjimarkos and Storvick (1949) found among 419 children, ages 14–16 years, from coastal areas of Oregon that the number of DMF teeth per child varied from 14.4 in one town to 12.7 in another. From several areas in central Oregon, 322 children of similar age showed a variation from an average of 11.3 DMF teeth per individual in one town to 9.0 DMF teeth in another. These examinations utilized both explorer and X-rays.

Sognnaes (1949C) reports that 2,025 children 6 years of age from 71 schools in various districts and cities of Greece had 25.2 per cent of all teeth carious; 73.6 per cent of the children had one or more cavities. In the Athens public schools, among 206 10-year-old children, 86 per cent had cavities

TABLE 7

DMF rate of school children

Author	Location	Method*	No. Exam.	Sex	Age																	Mean
					3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Permanent teeth																						
Steggerda & Hill (1936)	Michigan	3	1,358	M, F				2.4						4.3						6.9			4.53†
Knutson (1947)	Minnesota	1	2,627	M, F				0.51	1.43	2.30	2.86	3.39	4.16	5.50	6.32	7.69	9.62	9.58	10.84	11.21			
Klein & Palmer (1940)	Maryland	1	1,841	M, F											4.8	6.1	6.7	7.2	7.7	8.7	9.3		7.7
Sloman & Sharp (1940)	California	1	6,923	M, F										3.8	4.8	5.6	6.5	7.2	7.7	8.3			6.3
Lathrop (1940–41)	New York	2	144	F										6.7	6.3	8.7	8.6						‡
Foster (1942)	Wisconsin	1	188	M, F				0.09	.65	1.32	1.75	3.17	2.15	3.62	4.44	6.0	6.14	5.67					2.68
Klein, Palmer & Knutson (1938)	Maryland	1	4,416	M, F				0.28	.72	1.19	2.02	2.52	2.83	3.66	4.54	5.23	6.43						2.78
East & Kaiser (1940)	358 U. S. counties	1	581,708	M, F					1.03			2.29			3.66								
Dean *et al.* (1944)	Midwestern cities	1	3,867	M, F											7.4								
Finn (1947)	New York	1	5,824	M, F				0.44	1.07	1.98	2.90	3.86	4.85	6.36	7.83	8.55							4.20
Gruebbel (1944)	Missouri	1	3,132	M, F				0.07	0.32	0.91	1.23	1.66	2.07	2.28									1.04
Cunningham (1908)	England	1	1,403	M, F		0.08		0.74	1.74	2.18	2.92	3.22	5.26	6.58	8.25		May not include fillings						
Wisan & Chilton (1948)	New Jersey	1	61,612	M, F			0.12	0.64	1.54	2.32	2.90	3.62	4.42	5.68	7.11	8.12	9.17	9.86	10.45	10.76	10.86	10.67	
Shourie *et al.* (1949)	Puerto Rico	1	1,050	M				0.7		2.1		3.8		6.0		7.1		8.7		8.1			
			1,049	F				0.8		2.3		3.3		5.5		7.0		8.1		8.4			
Deciduous teeth																						
Finn (1947)	New York	1	5,824	M, F	4.67			5.48	5.72	5.18	4.22	3.06	2.18	1.89	1.66	1.46							3.42
Gruebbel (1944)	Missouri	1	3,250	M, F				1.34	2.02	2.07	1.60	1.08	0.82	0.23									1.36
Cunningham (1908)	England	1	1,403	M, F	5.30	7.01		7.49	7.10	6.03	5.23	4.49	2.65	1.57		0.67		May not include fillings					
Wisan & Chilton (1948)	New Jersey	1	61,612	M, F		3.10	3.62	4.24	4.30	3.89	2.95	1.97	0.99	0.44	0.15	0.08	0.05	0.02	0.01				
Shourie *et al.*	Puerto Rico	1	1,050	M				6.1		7.3		3.9		0.6		0.1							
			1,049	F				3.0		6.5		2.6		0.6		0.1							
Deciduous and permanent teeth																						
Sebelius (1944)	Tennessee	1	5,845	M, F	4.67				4.87			4.02			3.15			3.62					4.05
Blackerby (1943)	Tennessee	1	26,576					4.33	5.44	5.65	4.69	3.73	3.3	2.96	3.27	3.26	3.47	No deciduous missing teeth included					

* 1 = Explorer; 2 = X-ray and explorer; 3 = throat stick.

† In children with caries.

‡ Institution.

TABLE 8

Per cent of children with decay experience

Author	Location	No. Exam.	Sex	Age																	Mean
				3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
Permanent teeth																					
Suk (1919)	Czechoslovakia	694	M					4.0	25.0	40.0	67.9	67.9	74.2	84.5	86.1	88.5	86.5	96.5	84.6	90	74.3
Stoughton & Meaker (1931)	Md., Ga., Ill. and Mo.	12,435	M, F				20.5	47.1	63.1	68.5	73.4	75.9	81.8	84.9	87.6	91.4	92.0	94.5	99.2	98.8	
Steggerda & Hill (1936)	Michigan	1,358	M, F						46.9					88.2				96.7			
Klein, Palmer & Knutson (1938)	Maryland	2,232	M				12.3	28.9	50.2	68.8	78.9	83.2	88.0	85.8	94.5	98.8					69.9
Klein, Palmer & Knutson (1938)	Maryland	2,184	F				19.9	43.2	56.3	72.5	77.6	80.7	87.5	95.7	96.4	93.1					73.0
Knutson (1947)	Minnesota	2,627	M, F				24.3	53.6	75.7	84.0	86.2	89.8	92.7	95.2	94.3	98.6	97.8	93.7	100.0		
Finn (1947)	New York	5,824	M, F				25.3	48.1	71.1	83.5	90.3	92.4	93.6	96.1	97.8						77.6
Shourie (1941)	India	6,866	M, F			41.5	44.6	55.7	61.0	60.8	55.7	53.9	57.2	52.8	52.3	55.3	58.7	54.3			
Sloman (1941)	California	6,923	M										86.7	89.7	90.8	94.6	95.7	95.5	96.2		
Sloman (1941)	California		F										88.7	92.1	92.1	95.8	96.3	97.8	98.4		
Deciduous teeth																					
Stoughton & Meaker (1931)	Md., Ga., Ill. & Mo.	12,435	M, F				87.4	90.8	91.0	86.9	71.9	48.2	29.4	14.5	5.9						
Deciduous and permanent teeth																					
Stoughton & Meaker (1931)	Md., Ga., Ill. & Mo.	12,435	M, F				87.7	92.2	94.3	92.7	91.7	87.4	87.9	88.3	88.7	91.2	92.6	94.5	99.2	98.8	
Munblatt (1933)	Institution	455	M, F	22.8	26.3	45.5	63.2	80.8		92.7	84.5	100.	93.3				94.65				
Sarkar (1939)	India	20,000	M, F					39.1	26.8		28.2	21.3	17.1	11.9	10.8	7.0	7.3	5.8	8.9	8.0	

TABLE 9

Per cent of teeth attacked by caries

Author	Location	No. Exam.	Sex	Age																		Mean
				2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Permanent teeth																						
Steggerda & Hill (1936)	Michigan	1,358	M, F					4.2						12.8						24.1		Explorer not used
Munblatt (1933)	Institution	455	M, F					4.2	8.3	14.1	13.9	14.4	14.5	15.0				22.7				
Sloman & Sharp (1940)	California	6,923	M, F											11.9	14.9	17.5	20.4	22.5	24.0	26.1		19.8
Shourie (1941)	India	6,866	M, F					2.5	3.5	3.6	3.0	2.9	3.5	3.9	4.0	4.3	4.4	5.1	4.5			
Deciduous teeth																						
Munblatt (1933)	Institution	455	M, F	0	5.4	5.0	13.9	26.3	28.6	38.7	46.9	43.3	53.8	36.7								
Shourie (1941)	India	6,866	M, F				6.0	7.6	9.7	11.7	13.8	12.6	12.7	8.3	11.3	16.8						

with 17 per cent of the permanent teeth carious and 2.6 carious teeth per child.

Summary and conclusions: The dental caries experience of school children varies with geographic location, although in no area were the children free of caries to any marked degree. The percentage of children showing evidence varied from around 70 to 100 per cent, depending upon the age of the children and the geographic location.

The total caries experience per individual and the percentage of teeth attacked by caries, as well as the percentage of children with evident caries experience, is considerable. This holds true for both the deciduous and permanent teeth.

Because of the difference in examiners and method of examinations, it is difficult to draw conclusions about the attack rate in various countries. Until these variables in dental examinations are better standardized, comparisons will remain difficult.

Prevalence Rates in College Students and Military Personnel

Brekhus (1931) over a 3-year period examined 10,445 students at the University of Minnesota and found 106,364 affected teeth for an average of 10.2 cavities per student. These students averaged 18 years of age. In the examination of the 1939 freshman class (Brekhus 1941) there were 11.08 affected teeth per person as compared with 9.95 in 1929. In 1931 there was slightly less than one extracted tooth per student.

Arnett and Ennis (1933) examined 883 college students attending Drexel Institute. The average age of the students was 18.75 years, with 56.9 per cent males and 43.1 per cent females. Clinical examinations were augmented by full mouth X-ray examinations; fillings were not considered. Eighty-three per cent of the students showed caries, with 10.3 per cent of the teeth carious. The average number of carious teeth per student was 3.2. A total of 896 missing teeth was found in 419 students.

Healey and Cheyne (1943) compared the caries rates among those examined by Brekhus of Minnesota and those examined by the authors at the University of Indiana. The 4,348 students at Minnesota, averaging 18 years of age, were compared with 3,234 students at Indiana averaging 19+ years of age. The Minnesota students had a higher DMF rate per person and fewer DMF-free mouths (table 10).

Among 582 freshman students at Oregon State College, Hadjimarkos and Storvick (1948) found, by the employment of both explorer and X-ray, an average of 14.12 DMF teeth and 33.40 DMF surfaces per student. By age groups the DMF rates were as follows: at age 17, 13.0; at age 18, 14.28; at age 19, 14.50; and at age 20, 14.68. The group included 235 males and 347 females. The author also reported geographic variations in the DMF rates.

TABLE 10

Comparative summary of caries prevalence in two midwestern universities

(Healey and Cheyne 1943)

	DMF rate per person	DMF-free mouths
University of Minnesota....	11.08	1.17%
University of Indiana.....	10.12	3.22%

TABLE 11

Per cent of teeth showing past dental caries experience on initial examination of Navy personnel

(Schlack 1940)

Age.....	17–19	20–24	25–29	30–34	35–39	40–61	All ages
Per cent.	16.1	20.8	25.5	32.3	41.5	43.5	24.1

Among the military personnel, Schlack (1940) found 24.1 per cent of teeth with past caries experience in 1,047 officers and enlisted men in the United States Navy between the ages of 17 and 51 years (table 11).

Klein (1941) reported on the dental status of 642 West Virginia and Maryland young adult males appearing before the Selective

Service Board (table 12). For all ages, there was an average of 16.8 DMF teeth and 56.4 DMF surfaces per individual. The DMF rates for the various ages are given.

East (1942b) compared the DMF rates among 4,602 white naval recruits during 1934. Connecticut recruits had the greatest caries experience and averaged 12.54 DMF teeth per person, with the New England States showing the highest rates and the southern states the lowest. Arkansas, with only 3 DMF teeth per recruit, had the smallest amount of caries experience. East relates caries incidence to hours of sunshine.

On the other hand, Senn (1943) examining aviation cadets between 18 and 27 years of age states that the Atlantic and Pacific coast areas have high mean caries averages, although they have plenty of sunshine. He correlates caries incidence with fluoride areas.

White (1944) reported that among 800 naval aviation cadets examined, 25 or 3 per cent were caries-free.

Dunning (1944) in examining 1,208 midshipmen averaging 21.6 years of age found that each sailor averaged 9.78 DMF teeth, of which .58 was decayed, .68 missing and 8.52 filled, indicating good dental care. Dunning also noticed that the midshipmen from southwest U. S. had only 59 per cent as much caries as the New England group.

Miller (1943) reported on the dental condition of enlistees in the British Army. There were upwards of 100 men in each age group, ranging from 18 through 30 years of age. The findings are reported in table 13. It will be observed that at age 18 approximately 25 per cent of the teeth had experienced dental caries and 11.5 per cent of all teeth had been extracted, and at 30 years of age 57.5 per cent of the teeth had dental caries experience with an average of 37.4 per cent extracted.

Schlack, Restarski and Dochterman (1946) state that the geographic differences in dental caries rates among military personnel tend to level off as the individuals get older. This is readily understandable, since those having a great number of filled and missing teeth have fewer surfaces available for attack as they grow older.

Nizel and Bibby (1944) compared the mean caries rates (decayed and missing teeth only) of soldiers from various states and geographic areas stationed at a New England camp. The New England States had

TABLE 12

Average DMF permanent teeth and tooth surfaces per individual in adult males appearing before the Selective Service Board

(Klein 1941)

Age	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
DMF teeth	14.5	15.05	16.5	17.7	17.0	17.0	16.6	16.5	18.8	21.5	21.6	19.5	16.1	18.1	21.4
DMF surfaces	48.2	32.1	51.9	55.2	57.9	61.0	56.2	56.2	63.5	75.9	81.6	62.2	57.3	70.8	81.3

TABLE 13

Dental findings in British Army enlistments

(Miller 1943)

Age	Missing teeth per person	Teeth indicated for extraction per person	Teeth requiring fillings per person	Filled teeth per person	Sound teeth per person
18	3.7	1.2	2.3	0.6	24.2
19	3.5	1.3	2.3	0.7	24.2
20	6.6	2.0	2.0	0.4	21.0
21	6.0	1.7	2.4	0.6	21.3
22	7.3	1.9	2.0	0.3	20.5
23	8.7	1.7	2.1	0.7	18.8
24	9.6	1.3	1.8	0.6	18.7
25	8.3	1.8	2.2	0.7	19.0
26	12.3	1.4	1.9	0.7	15.7
27	9.7	1.4	1.6	0.8	18.5
28	13.3	2.1	2.0	0.7	13.9
29	12.7	2.7	2.5	0.1	14.0
30	11.1	4.8	2.1	0.4	13.6

the highest prevalence rates, with 12.29 DM teeth per soldier. The Middle Atlantic States averaged 11.88; the Southeast States, 9.32; the Central States, 10.31; the West Central States, 9.19; the Southwest Central States, 6.89; the Northwest States, 11.02; the Southwest States, 8.63. Approximately 90 per cent of the men examined were born in the state in which they were resident.

Summary and conclusions: From the studies reported on college students and military personnel, one can conclude that the caries experience among late teen-agers and men in their early twenties averages between 9 and 16 DMF teeth per person. Evidence has been offered that the DMF rates among this age group vary with geographic location.

Prevalence Rates in Adults

Driak (1929) examined 1,100 patients in Vienna from ages 10 to 90 years, with 22,672 teeth. Seventy-one per cent of the individuals had dental caries, and 12.9 per cent of all teeth were carious; 4.45 per cent of individuals were edentulous; 53 per cent of all those with carious teeth were in the 20- to 40-year age group.

Cramer (1929) conducted a study of 1,000 adult males in Milwaukee, Wisconsin, from 20 to over 60 years of age. The group includes representatives of different economic levels, being divided into the following categories: 341 executives, 247 skilled workers, 183 semi-skilled workers, 224 unskilled workers and 5 unclassified. It was not a representative population group, however, in that all were clinical patients. The results by age groups are reported in table 14. These individuals averaged 15.06 DMF teeth per person.

Seiler (1931) examined 179 adults in 15 towns in Germany, 101 of whom were from 20 to 40 years of age, while 78 were over 40. Among 4,951 teeth remaining in these persons, 5.8 per cent were carious, 2.2 per cent indicated extraction, and 2.2 per cent were filled, with an average of 2.82 carious and filled teeth per person. There were 777 teeth unaccounted for and presumed missing.

Meyer (1938) compared the caries prevalence among 2,000 Polish land workers with a similar age group in Hamburg reported by Röse. These Polish workers were men and women between 20 and 24 years of age. A few were reported to be younger and still fewer were older than the 20- to 24-year age group. Röse reported that 98.75 per cent of the individuals examined by him in Hamburg had caries, while only 78 per cent

TABLE 14

Average dental experience per patient among 1,000 adult males in Milwaukee, Wisconsin

(Cramer 1929)

Age	Patients no.	Fillings no.	Caries no.	Extractions no.	Total DMF
20–25	129	8.16	5.2	2.29	15.65
25–30	225	7.38	4.53	2.83	14.74
30–35	183	7.7	3.7	3.53	14.93
35–40	167	7.55	3.49	4.1	15.14
40–45	99	8.1	2.57	4.86	15.53
45–50	84	6.3	2.25	6.8	15.35
50–55	48	5.4	1.41	7.2	14.01
55–60	25	6.2	3.12	7.6	16.92
over 60	30	2.9	2.0	16.9	20.8
	1000	7.11	3.6	4.35	15.06

TABLE 15

Caries incidence as measured by affected teeth per person and affected surfaces per person

(Hollander and Dunning 1939)

Age	DMF per person	DMF surfaces per person
17–19	12.66	23.48
20–24	15.82	31.52
25–29	18.28	40.42
30–34	20.22	48.05
35–39	20.49	50.98
40–44	21.61	55.90
45–49	22.13	58.71
50–54	22.36	59.98
55–59	23.03	65.89
60–64	23.37	62.78
65 & over	24.88	70.09

among the Polish land workers had caries; 22 out of every 100 Polish workers were caries-free. While only 13.7 per cent of the teeth of the Polish workers were decayed, Röse found among 20-year-old Germans that 51 per cent of the teeth were decayed.

Hollander and Dunning (1939) reported on dental examinations of 12,753 persons from 17 to over 65 years of age (table 15). They found the greatest DMF increase to take place in the 17- to 25-year age group.

In the older age groups, no provision is made for teeth lost through pyorrhea, and these figures may therefore be slightly higher than they would be were they able to eliminate teeth lost for reasons other than caries. The caries rates are slightly higher in the older age groups than those found by Cramer (1929). Perhaps this reflects the different caries rates of the localities or the inclusion by Hollander and Dunning of extracted teeth due to pyorrhea. The figures of Hollander and Dunning (1939) include both sexes, while Cramer's (1929) studies were done on males only, which may also account for the higher rates in the study by Hollander and Dunning.

Brinton and Johnston (1942) examined 2,365 adult male mine and smelter workers in Utah. They ranged in age from 15 through 64 years. The metal miners had a DMF rate of 14.85, the coal miners 14.46 and the smelters 15.42 per individual. Although the coal miners had better teeth in the younger age groups, they were slightly worse than the smelters and about equal to the metal miners in the older age groups.

Angel (1944) examined 60 males from urban and rural areas of Greece and observed that the males of central Greece who were rural and urban had less caries than men in the entirely rural areas of Macedonia and Chalcidise. Of the 932 teeth among the central Greeks, 15.9 per cent were carious, while of the 676 teeth of the entirely rural areas, 21.6 per cent were carious. No information is given as to the number of individuals with missing teeth in each group.

Summary and conclusions: Although it has been found that among adult males the DMF rate does not increase appreciably with age, it has also been shown that among adults of both sexes the numbers of DMF teeth and surfaces increase markedly with age.

Prevalence Rates in Different Civilized Races

There is ample evidence in the literature to indicate a significant difference in dental caries experience among various races. Many racial groups still live in a primitive environment, and a comparison of the caries experience of these primitive peoples with peoples living under our civilization does not necessarily express a true racial difference in caries experience, because of the different environmental factors such as different nutrition or geographic location. The caries rates among primitive peoples have been discussed under that heading. To establish a true racial difference in caries rates, comparisons must be made between races living under the same general environmental conditions. The American whites and Negroes afford, perhaps, the best opportunity to study two large racial groups residing in the same area. A comparison of the caries rates among these two groups indicates that the Negro has less caries experience than does the white person. Although Negroes, according to Suk (1919), are more precocious as to tooth eruption, nevertheless they present mouths with less decay than do white individuals. This difference has been pointed out by many investigators.

McRae (1933) examined 3,188 white and 1,096 Negro children in the first 6 grades of school in Shelby County, Tennessee. Among the white children, 73.7 per cent had experienced decay, while only 41 per cent of the Negroes had a similar experience.

Among the 433 children, aged 13 years,

examined by Day and Sedwick in Rochester, New York, (1935), the Negro portion of the group had the lowest caries index.

Gafafer and Messner (1936) compared the caries experience of 1,499 white and 409 Negro males at the Ohio State Reformatory. Their ages ranged from 16 to 30 years. At ages 16 through 20, 14 per cent of the whites were caries-free, while among the Negroes 22.5 per cent were caries-free. At ages 21 through 25, 6.8 per cent of the whites were caries-free, while among the Negroes 25.9 per cent were caries-free. At ages 26 through 30, 6.3 per cent of the whites were caries-free, while among the Negroes 14.8 per cent were caries-free. For all age groups, 9.4 per cent of the whites and 22.6 per cent of the Negroes were caries-free.

Blackerby (1939) compared the dental conditions among white and Negro children between 6 and 17 years of age from rural and semi-rural areas of the South (table 16). Dental defects averaged 4.16 per child among the whites and 2.29 per child among the Negroes; 12.7 per cent fewer Negro children than white children required dental attention. The percentages needing dental care in the various age groups were as follows:

TABLE 16

Percentages of white and Negro children requiring dental care in various age groups

(Blackerby 1939)

Ages	White	Negro
6–8	84.2	70.3
9–11	79.2	64.0
12–14	74.8	66.4
15–17	80.0	73.9
Average	80.2	67.5

Among the 144 females at Letchworth Village examined clinically and with X-rays, Lathrop (1940–41) found that in the age groups 12 to 15 the American whites had 30.3 per cent carious teeth, while the Negroes had 25.9 per cent.

Brucker (1943) found that, of 7,447 white and 1,822 Negro children from 6 to 16 years of age examined, 9.9 per cent of the whites and 22.5 per cent of the Negroes were caries-free.

Sebelius (1944) examined 2,928 white and 2,917 Negro children between 3 and 17 years of age in Tennessee (table 17). The white children averaged 4.02 decayed, missing and filled teeth per child, while the Negro children averaged 3.1.

TABLE 17

Average number of decayed, missing and filled teeth among white and Negro children (missing deciduous teeth not counted)

(Sebelius 1944)

Age	White	Negro
3–5	4.67	2.42
6–8	4.87	3.95
9–11	4.01	3.00
12–14	3.15	2.35
15–17	3.59	2.96
Average	4.02	3.1

TABLE 18

Dental caries experience in permanent teeth of Baltimore Negro children

(Knutson 1944)

Age	6	7	8	9	10	11	12
DMF teeth per person	0.26	0.59	0.93	1.38	1.65	1.80	2.24
% of children with DMF teeth	14.6	34.2	48.5	57.9	66.2	68.1	72.9

Knutson (1944) found among 1,272 Baltimore Negro children between the ages of 6 and 12 an average DMF rate for the permanent teeth of 1.26 (table 18). The rates are lower than those determined by Klein, Palmer and Knutson (1938) at Hagerstown or by Knutson (1947) at Nicollet County, Minnesota, for whites of the same age. See tables 7 and 8 for the latter two rates.

Stadt (1947) has summarized the data derived from examinations by Charlotte,

North Carolina, dentists of 5,881 white and 2,413 Negro children between 6 and 16 years of age in the city schools. The white children had an average DMF rate of 4.79 permanent teeth per child and a decayed, indicated for extraction, and filled (def) rate of 3.74 deciduous teeth per child. The Negro children had an average DMF rate of 3.44 teeth and a def rate of 3.26 teeth per child. At age 6, 39.2 per cent of the white children and 32.7 per cent of the Negro children had experienced decay in the permanent dentition. At 12 years of age, 94.5 per cent of the white and 92.3 per cent of the Negro children were thus affected. At 16 years of age, 96.0 per cent of the white and 93.7 per cent of the Negro children had one or more permanent teeth attacked by caries. At all ages where sufficient numbers of children were available the Negroes had a lower percentage of children with DMF permanent teeth.

With reference to other races, Bean (1914) reporting on the decay of teeth states that the temporary teeth of the Americans are worse than those of the Filipinos, which are worse than those of the Germans. The permanent teeth of the Americans are worse than those of the Germans, which are worse than those of the Filipinos. No figures were given to substantiate this statement.

Anderson (1932) reported on the dental caries incidence among 1,000 Chinese, over half of whom were 5 to 15 years of age. There were 20,778 permanent teeth, of which 2.1 per cent were carious. Of the 963 individuals with permanent teeth, 41.9 per cent had caries, with an average of 1.1 carious teeth per person. Of 5,173 deciduous teeth, 16.1 per cent were carious. Of the group having deciduous teeth, 51.0 per cent of the individuals had caries of this dentition, with an average of 3.6 carious teeth per person. Although the Chinese appear to have a lower caries incidence than whites, there are many possible factors aside from a racial resistance, such as fluoride areas, modification in diet, and geographic differences, which may account for the difference in caries prevalence.

Montelius (1933) examined 4,474 Chinese between 5 and 70 years of age. The highest percentage of individuals showing caries was in the 15- to 19-year age group, where 66.5 per cent of the individuals were affected. A large percentage of the population presented evidence of dental fluorosis. The fluorine content of the drinking water unquestionably lowered the prevalence of caries in this group.

Hyde (1944) studied the caries incidence among 3,899 selectees 18 through 20 years of age. These men were from the east coast. In comparing the extent of caries in relation to birthplace of parents, the incidence decreased in the following order: English descent presented the greatest amount of caries, Irish next, then Canadian, Yankee, Italian, Portuguese, Russian (Jewish), Negro and Chinese. Many factors beside racial stock could account for the difference in prevalence rates.

Felizardo, Matius and Carreon (1940) found among 3,880 school children in the Philippine Islands that 71.9 per cent had experienced carries in either dentition. Furthermore, 620 inmates of Welfareville had a lower caries incidence (75.6 per cent) than did 273 factory workers (93.7 per cent).

Chapin and Mills (1942) from studies in the Panama Canal Zone reported that 174 Panama-born Americans in the 12- through 14-year age group had a DMF rate of 7.85, while among 659 natives in the same age group the DMF rate was 7.16. However, Americans living only a short time in the Canal Zone had a lower DMF rate than the natives.

Mellanby (1934) examined the teeth of 154 Arab children between the ages of 5 and 16 years. Fifty per cent of the children had some decayed teeth, and of these there was an average of 3 carious teeth per child.

Neither the type of examination nor the number of children in each age group was reported.

Summary and conclusions: The evidence presented indicates rather definitely that the prevalence of dental caries among Negroes is lower than among whites of comparable age.

The evidence that other modern civilized races have a lower prevalence of dental caries than do the whites is not conclusive and can possibly be attributed to environmental or dietary differences rather than to an innate racial resistance to dental caries. It is extremely difficult to attach a difference in dental caries prevalence to a racial difference when environmental conditions are not similar.

TABLE 19

Percentages of children with caries experience in one or more and five or more deciduous and permanent teeth according to age and sex

(Stoughton and Meaker 1932)

Age	Percentage with DF teeth				Percentage with DMF teeth			
	1 or more		5 or more		1 or more		5 or more	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
6	87.4	87.4	59.9	59.4	22.7	18.2	0.2	0.4
7	91.0	90.6	55.6	59.1	48.4	45.7	0.5	0.5
8	90.0	92.1	53.0	55.2	64.8	61.3	1.6	1.3
9	88.5	90.2	29.5	42.9	71.1	66.0	3.3	3.3
10	65.4	78.6	14.0	21.8	75.8	70.9	7.2	8.2
11	40.6	55.9	5.0	9.9	77.6	74.2	12.2	10.8
12	23.5	35.7	1.4	3.6	84.9	78.5	24.2	17.6
13	11.2	17.8	0.3	1.0	86.1	83.7	34.2	29.4
14	3.8	7.7			88.5	86.7	44.4	41.5
15					92.2	90.5	66.4	53.5
16					93.8	89.2	66.7	58.5
17					94.5	94.4	75.0	70.4
18					98.8	100	82.1	86.1
19					98.5	100	83.1	84.2

Difference in Caries Attack Rate Among Sexes

There is considerable evidence in the literature to indicate a difference in caries experience between the sexes. Both the caries attack rate and the total accumulated caries experience in the permanent teeth have been reported to be greater in females than in males of comparable age. Among the deciduous teeth, males apparently have a greater caries experience. At the ages where both dentitions can be taken into account, males appear to have a greater caries experience as judged from the teeth present at the time of examination. A number of papers dealing with the difference in caries rates among the sexes will be presented to qualify the above statements.

Stoughton and Meaker (1932) examined 12,435 children between 6 and 19 years of age. The percentages of girls and boys with one or more and five or more DF deciduous teeth and DMF permanent teeth are presented in table 19. The caries experience is higher for boys after 7 years of age in the deciduous teeth because of later exfoliation of the deciduous teeth in boys. At these ages, there would be more carious deciduous teeth remaining in the mouths of the boys. Considering the combined dentitions, the caries rates are higher in boys in the younger ages and in girls in the older ages. In the permanent teeth, the DMF rates are higher for girls except in the 18- and 19-year age groups.

TABLE 20

Dental caries prevalence according to sex

(Munblatt 1933)

	Percentage of caries-free children	Percentage with caries in permanent teeth	Percentage with caries in deciduous teeth
Male.....	27.88	15.64	23.72
Female ..	32.35	17.27	19.54

Munblatt (1933) arrived at the same conclusion as Stoughton and Meaker (1932) in a study of institutionalized children between 2 and 12 years of age. Incidence figures from 204 females and 251 males are reported in table 20.

It will be observed that, while the caries experience is greater in females than in

males in the permanent teeth, the incidence of caries is higher in the males for the deciduous teeth.

East and Kaiser (1940) and Kaiser and East (1940) give the mean average number of cavities per 100 children ages 6 to 14 as gathered by Messner *et al.* (1936). By mirror and explorer examination, the permanent teeth of 296,605 white boys were compared with those of 285,103 white girls from rural and semi-rural areas of 358 counties in the United States. It will be observed in table 21 that the females show the greatest number of cavities. The differences are significant. The authors state that in the young children with mixed dentition, the sexes show no difference in caries rate. In the older age groups with permanent teeth, the rate is higher in females.

TABLE 21

Mean number of cavities per 100 children according to age and sex

(East and Kaiser 1940)

(Kaiser and East 1940)

Age	6–8	9–11	12–14
Males	97.3	218.0	348.5
Females	110.0	240.0	384.0

East (1941) has presented evidence to show that the same relationship exists between the sexes among city children as was found among the rural children.

Among 582 freshman students at Oregon State College, Hadjimarkos and Storvick (1948) found females to have the greater caries experience. From ages 17 through 20 years, males averaged 13.60 DMF teeth and 31.63 DMF surfaces per student, while females averaged 14.35 DMF teeth and 34.23 DMF surfaces per student.

East and Pohlen (1942) examined the teeth of 1,005 children between the ages of 5 and 17 years in Bergen County, New Jersey, and found that girls had more caries experience than boys. Although at the age of 6 years, 41 per cent of the boys and 52 per cent of the girls had no DMF teeth, 21 per cent of girls and only 15 per cent of boys had as many as 4 DMF permanent teeth. At 8 years of age, only 4 per cent of the children were DMF-free, while 11 per cent of the girls and only 6 per cent of the boys had 5 or more DMF teeth. The number of children with 4 DMF teeth reached its peak at 8 years of age in girls and 10 years of age in boys. Although the girls showed more caries-free mouths than the boys, the intensity of attack in the affected mouths was greater among the girls.

Sappington (1928) among 2,469 employment examinations found that females showed a lower percentage of caries and a higher percentage of restorations than males. The caries experience rate was evenly divided between the two sexes.

Clements and Kirkpatrick (1938) reported mouth mirror and explorer examinations of 920 preschool and school children in New South Wales; 802 of these children were school children over 5 years of age. The incidence of dental caries in the deciduous teeth was higher in boys and in the permanent teeth higher in girls. Both dentitions were included, and for all children there were 6.13 carious teeth per child, 6.18 per boy and 6.09 per girl. For all teeth, there were more carious surfaces in boys than in girls.

Ainsworth and Young (1925) have tabulated the caries rates of 13,756 upper deciduous teeth among British children and found 43.4 per cent of the cavities among girls and 43.2 per cent among boys. This difference was not significant statistically.

Among the deciduous teeth, Allerbeck (1939) and Roos (1944) found that there was no difference in caries rates between the two sexes, but Oravecz (1937) stated that girls had less caries to a ratio of 3:4.

Jensen (1941) found in 471 Copenhagen children under 6 years of age that 16 per cent of the boys and 21 per cent of the girls had caries-free teeth.

Healey and Cheyne (1943) offered evi-

dence from the examination of 3,234 students at the University of Indiana that females had a higher per cent of carious teeth than did males. In males 35.5 per cent of the teeth were affected, while in females 37.8 per cent were involved.

Brekhus (1931) reported on the dental caries status of 10,445 Minnesota University students examined during the years 1928, 1929 and 1930 (table 22). The 1928 students possessed the highest per cent of caries-free mouths.

TABLE 22

Percentages of University of Minnesota students with no caries experience

(Brekhus 1931 and 1941)

	1928	1929	1930	1939
Males	4.70	2.55	1.58	1.34
Females	1.80	0.81	1.28	0.90

5 to over 70 found that 56.5 per cent of the males and 69.6 per cent of the females had caries. There was evidence of dental fluorosis among this group. The high percentage of caries-free mouths in both sexes was due to the fluoride content of the drinking water. These figures offer some evidence, however, that in areas where fluorosis is endemic the caries rate among males is lower than among females.

Foster (1942) in presenting caries rates among 274 Indians and 189 white children between the ages of 6 and 16 found the DMF rates per 100 children to be 271 in the white males and 195 in the Indian males; 266 in the white females and 243 in the Indian females. The samples of both groups were small and the differences not significant statistically.

Steggerda and Hill (1936) in a study of Maya and Navajo Indians, Jamaicans and

TABLE 23

DMF rates per person for 12,753 adults according to age group and sex

(Hollander and Dunning, 1939)

Age	17–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65 & over
Male	11.95	15.72	17.70	20.08	19.53	20.64	21.25	21.18	23.25	23.59	24.46
Female	12.77	15.84	18.59	20.34	21.60	22.61	23.72	24.65	25.62	22.69	27.50

During the 3-year period, the males had the greater percentage of caries-free mouths and 10 years later this was still true (Brekhus, 1941).

Hollander and Dunning (1939) have tabulated figures on the caries incidence of 12,753 persons between the ages of 17 and 65 years of age. Their figures show that generally speaking, even in the older age groups, the numbers of DMF teeth and DMF surfaces are higher for females than males. The older age groups include teeth lost through pyorrhea, which might slightly alter the figures reported in table 23. There is a total caries experience of 21.45 DMF teeth among females and 19.94 among males from ages 17 to over 65.

Montelius (1933) in a study of 3,680 Chinese males and 794 females from ages

Michigan Dutch state that females have a higher percentage of caries than males, except in the Navajos.

Driak (1929) in reporting on the examination of 22,672 teeth in 1,100 patients between 10 and 90 years of age reports that caries is less frequent among men than among women. In 781 patients with caries examined, 43 per cent were men and 57 per cent were women. Of 270 caries-free persons, 52 per cent were males and 48 per cent females. Of the 49 edentulous persons, 24 per cent were males and 76 per cent females. Of the entire number of carious teeth, 41 per cent were in males and 59 per cent in females. Of the completely destroyed teeth, 45 per cent were in males and 55 per cent in females.

Berk (1943) reporting a study of 198

children age 5 years found the caries rate to be 9.66 for males and 9.70 for females.

Brucker (1943) in a mouth mirror and explorer study conducted among 9,269 white and Negro children between the ages of 6 and 16 years in Newark schools found that among the whites 10.5 per cent of the boys and 9.2 per cent of the girls were free from caries. Among the Negroes, 19.6 per cent of the boys and 25.6 per cent of the girls were free from caries.

Magitot (1867) states from his studies that caries is more common among girls to the ratio of approximately 3:2. Among 1,000 cavities in adults, 583 were in females and 417 in males.

Suk (1919) quotes figures on the percentage of bad permanent teeth among 16-year age groups as follows: American - girls 7, boys 5; German - girls 4.3, boys 3.7; Filipino - girls 2.7, boys 2.2.

TABLE 24

Per cent of the entire dentition attacked annually in boys and girls of school age

(Sloman 1941)

Age, years	All pupils	Boys	Girls
6–12	1.98	1.92	2.05
12–18	2.37	2.10	2.68
6–18	2.17	2.01	2.37

Klein, Palmer and Knutson (1938) conducted dental examinations on 4,416 elementary school children aged 6 through 15 years in Hagerstown, Maryland and report the percentage of girls with one or more DMF permanent teeth to be higher than that of boys (table 7). The higher DMF rate among girls is attributed to the greater posteruptive tooth age. Palmer, Klein and Kramer (1938) point out that at age 14.5 years, boys have accumulated 137.15 years of exposure while girls at the same age have accumulated 148.26 years. When comparing caries rates with the posteruptive tooth ages, Klein and Palmer (1938) state that girls have no greater susceptibility than boys to caries.

On the other hand, Sloman (1941) states that the earlier eruption of the teeth of females cannot account entirely for the higher caries rates in females. As an example, he states that the cumulative caries experience for girls of 18 years of age is 28.4. If the eruption age of boys is brought up to that of girls by adding .414 years to the chronological age, calculating the percentage of the fraction of the annual increment rate (2.01) and adding this figure to the caries experience, the boys will then have a theoretical cumulative caries experience of 24.9, or fully 3.5 per cent less than girls of the same age. If caries rates were equal, there would have to be an eruption-age difference of 2½ years between the two sexes.

Sloman (1941) in reviewing Sloman and Sharp's (1940) findings among the 18-year age group found one carious tooth is added every 18.67 months for boys and only every 15.84 months for girls. The average percentage of the entire dentition attacked annually is indicated in table 24.

Sloman's figures for percentage of children with one or more DMF teeth for children from 12 to 18 years of age closely compare with those of Stoughton and Meaker (1932) (see table 8).

Sloman and Sharp (1940) examined a total of 6,923 males and females from 12 to 18 years of age. This represented about 20 per cent of the total public high school population of San Francisco in this age range. A resumé of the pertinent findings in regard to the sexes is presented in table 25.

Day and Sedwick (1935) found that, on the basis of the number of teeth present in the mouth, caries was less frequent in females than males, although teeth erupted earlier in females. In 433 children age 13, there were 5,467 erupted teeth in males and 5,850 in females. The total defects were 4,661 in the males and 4,762 in the females, giving a caries index of 0.85 in the males and 0.81 in females.

Cohen (1936) observed in a group of 1,372 males and 1,182 females between the ages of 2 and 15 years in Minneapolis that

for the deciduous teeth up to about age 6 there are slightly more perfect teeth among the females, but after age 6 the teeth of the males appear slightly better than those of the females. The number of missing teeth of the females is slightly lower up to the age of 6, and after 6 there is an increase in loss of deciduous teeth over that of the males. In the permanent teeth, females have slightly better teeth than the males up to the age of 13 years. After 13, the males have slightly better permanent teeth than the females. The males have more filled teeth and missing teeth, the females more cavities. After 13, the teeth are about the same for both sexes, with a slight tendency for more missing teeth among the females.

Kloser (1913) reported the similarity of caries attack rate for 33,098 boys and 32,699 girls of school age in Germany. His figures indicate very little difference in the condition of the teeth in the two sexes.

Summary and conclusions: Evidence indicates that caries in the permanent dentition is more prevalent in females than in males (Stoughton and Meaker 1932), (Munblatt 1933), (East and Kaiser 1940), (East 1941), (East and Pohlen 1942), (Clements and Kirkpatrick 1938), (Healey and Cheyne 1943), (Brekhus 1931, 1941), (Hollander and Dunning 1939), (Montelius 1933), (Foster 1942), (Steggerda and Hill 1936), (Driak 1929), (Brucker 1943), (Magitot 1867), (Suk 1919), (Klein, Palmer, and Knutson 1938), (Sloman and Sharp 1940), (Sloman 1941), (Cohen 1936).

Only a few of the investigators have found no difference in caries prevalence between the two sexes when permanent teeth only are considered; among these are Sappington (1928) and Kloser (1913).

The paper by Day and Sedwick (1935) was the only one reviewed in this section to report a higher caries rate among the permanent teeth of males than of females. Their findings were based on the number of erupted teeth in the mouth.

In considering the deciduous teeth only, the males appear to have the higher caries rates based on the number of carious and filled teeth present (Munblatt 1933), (Stoughton and Meaker 1932), (Clements and Kirkpatrick 1938), (Jensen 1941), (Oravecz 1937), (Cohen 1936).

TABLE 25

The caries experience of children 12 through 18 years of age according to sex

(Sloman and Sharp, 1940)

Sex	Age	No. of pupils examined	Percentage of pupils without caries, fillings or extractions	Percentage of teeth unattacked by caries
Male	12	263	13.3	88.5
Female		309	11.3	87.7
Total		572	12.2	88.1
Male	13	390	10.3	85.4
Female		418	7.9	84.9
Total		808	9.0	85.1
Male	14	609	9.2	83.0
Female		610	7.9	81.9
Total		1,219	8.5	82.5
Male	15	822	5.4	80.1
Female		810	4.2	79.0
Total		1,632	4.8	79.6
Male	16	702	4.3	78.5
Female		678	3.7	76.6
Total		1,380	4.0	77.5
Male	17	420	4.5	78.0
Female		496	2.2	74.2
Total		916	3.3	76.0
Male	18	213	3.8	75.9
Female		183	1.6	71.6
Total		396	2.8	73.9
Male	All	3,419	6.8	81.0
Female	ages	3,504	5.4	79.4
Total		6,923	6.1	80.2

Berk (1943), Allerbeck (1939), Roos (1944) and Ainsworth and Young (1925) could find no appreciable difference in the caries rates in the deciduous teeth between the sexes.

When both dentitions are considered, even though females have a higher permanent tooth morbidity, males have a

higher caries experience after about 7 years of age because of the longer retention of carious deciduous teeth, which are exfoliated later in males.

The difference in caries experience in the sexes may be due entirely to the difference in eruption age and consequent length of exposure of the teeth to the oral environment or may be due in part to some heretofore unexplained difference.

If one considers all teeth and all age groups, one can state that the caries experience appears to be higher in males in the younger ages and higher in females in the older age groups.

Comparison of Caries Rates Between Jaw Quadrants

In studying population groups, dental caries in both the deciduous and the permanent teeth has been shown to occur bilaterally. Hyatt (1928), Brekhus (1931), Dwyer (1932), McCall (1938), Cheyne and Drain (1940), Leigh (1940), Bertram and Brown (1943), Roos (1944) among others have observed this similarity in caries prevalence between the left and right quadrants of both the upper and lower jaws. Cheyne and Drain (1940) and Knutson and Klein (1938) have pointed out the bilateral nature of tooth mortality.

Dental caries in specific teeth or individual mouths frequently occurs unilaterally. However, unilateral caries does not always occur in the same direction, and in sufficient numbers of individuals the occurrences of unilateral caries tend to cancel each other, leaving teeth as well as quadrants on the right and left sides of the mouth attacked with equal frequency.

Although the occurrence of caries is bilateral, studies may show, because of the occurrence of unilateral caries, a slight variation in frequency between sides. This difference is not statistically significant and not always in the same direction but is due to chance alone.

Healey and Cheyne (1943) point out the similarity of caries experience between the right and left sides of the mouth. In 4,348 Minnesota University students, 38.9 per cent of the teeth on the right side and 38.8 per cent of the teeth on the left side in boys were affected, and in girls, 40.8 per cent of the teeth on the right side and 41.1 per cent of the teeth on the left side were affected. In 3,234 Indiana University students, 32.3 per cent of the teeth on the right side and 35.6 per cent of the teeth on the left side in boys were affected, while in girls 37.3 per cent of the teeth on the right side and 38.3 per cent of the teeth on the left side were affected.

McCall (1938) has stated from his studies that among the deciduous teeth the difference between the right and left sides of the mouth was insignificant.

American Child Health Association Monograph (1930) reported that the horizontal relationship between carious conditions was closer than either vertical or diagonal correlations.

Driak (1929) found among 1,100 patients 10 to 90 years of age a slightly higher incidence on the right side of the maxillary teeth, but the reverse was true of the lowers.

Suk (1919) in studying 694 school children in Prague found a total of 1,764 decayed teeth. Of this number, 546 in 152 individuals were single decayed teeth, while 1,218 teeth were decayed in couples in 364 individuals. Of the 1,764 DMF teeth, there were:

256 single decayed teeth on the right side
290 single decayed teeth on the left side
400 decayed teeth in 200 individuals showing single bilateral caries
460 decayed teeth in 115 individuals showing double bilateral caries
198 decayed teeth in 33 individuals showing triple bilateral caries
64 decayed teeth in 8 individuals showing quadruple bilateral caries
40 decayed teeth in 4 individuals showing quintuple bilateral caries

24 decayed teeth in 2 individuals showing sextuple bilateral caries
32 decayed teeth in 2 individuals showing octuple bilateral caries

Scott (1944) employed X-ray bitewing films to study the bilateral incidence of carious lesions. In the posterior teeth, 73.1 per cent of the DMF lesions occurred bilaterally, and 15 per cent of these were unilaterally carious in another surface. Only 26.9 per cent were affected unilaterally. In studying individuals, bilateral caries was found in 95.3 per cent of those examined, while only 2 per cent showed unilateral caries only. Less than 3 per cent were caries-free. In bilateral caries, the mandibular first molar was most frequently carious and the mandibular first premolar least carious. In unilateral caries, the mandibular second premolar was most frequently attacked. According to Scott (1944), dental caries *per se* may begin in any quadrant.

Losee (1947) studied 580 bitewing roentgenograms on Marine recruits. There were 11,513 pairs of bilateral surfaces; 6,411 pairs had one or both surfaces involved. Of these surfaces, 61.9 per cent were involved on both surfaces. Involved tooth surfaces occurred 42.1 per cent more frequently in bilaterally corresponding surfaces.

Brekhus (1928) found the loss of teeth only slightly higher on the left side, averaging about 0.33 per cent greater.

Knutson and Klein (1938) observed a bilateral similarity in lower first molar mortality in 4,416 children aged 6 through 15 years. In boys, they found 260 lower first molars missing on the left side and 260 on the right side. In girls, they found 321 lower first molars missing on the left side and 317 on the right side.

Cheyne and Drain (1940) computed the significance of first molar extractions between right and left sides of the mouth as well as for the upper and lower jaws in 8,677 school children. They found no significant difference in first molar tooth mortality between opposite halves of either jaw. There were 342 first molar extractions in the lower jaws as compared to only 120 in the upper jaws, a difference which was significant.

It has been reported by Cheyne and Drain (1940), Hyatt and Lotka (1929), Day and Sedwick (1935), Driak (1929) and Cohen (1936) and many others that dental caries is more prevalent in the upper jaw than in the lower jaw despite the extreme susceptibility of the lower first molars. The freedom from dental caries of the lower anteriors accounts in part for the higher prevalence in the maxillary arch.

Healey and Cheyne (1943) in comparing caries activity showed that in 4,348 Minnesota University students 44.4 per cent and 47.5 per cent of the upper teeth of both sexes were affected, while only 33.1 per cent and 34.4 per cent of the lower teeth were similarly involved. In 3,234 Indiana University students, 40 per cent and 42.9 per cent of the upper teeth were involved as against 31.0 per cent and 32.9 per cent of the lower teeth.

Lathrop (1940–41) among 144 females, aged 12 through 15 years, found caries more prevalent in the maxillary than in the mandibular teeth and more prevalent in the two right quadrants than in the two left quadrants.

Day and Sedwick (1935) among 433 13-year-old Rochester children noted that 60 per cent of all caries occurred in the upper jaw.

Hyatt and Lotka (1929) reported from their studies of 2,943 males and females under 25 years of age that there were 85.71 cavities per 100 upper teeth as compared with 59.32 per 100 lower teeth.

Summary and conclusions: The weight of evidence indicates that dental caries occurs bilaterally in both the deciduous and permanent dentitions. Evidence indicates also that tooth mortality, a sequel to neglected dental caries, occurs bilaterally. Although dental caries does occur bilaterally in the majority of teeth, cavities occur unilaterally

in individual mouths. For this reason, the caries rates in both sides of the mouth among groups of individuals, although closely approaching each other in caries frequency, may vary to a slight extent. The small observed differences are not consistently in one direction or the other and are not considered significant.

In comparing the maxillary and mandibular arches, the evidence is in general agreement that the upper teeth present a higher caries prevalence than do the lower teeth.

TABLE 26

Order of susceptibility of deciduous teeth to caries

Author	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Upper								
Centrals	3	3	8	5		5	8	7
Laterals	5		7	7	3	7	7	
Cuspids	4	3	5	6	4	6	5	5
1st molars	2	1	2	4	2	4	4	4
2nd molars	1	2	1	1	1	3	1	2
Lower								
Centrals			10	10		9	10	8
Laterals			9	9		10	9	
Cuspids			6	8		8	6	6
1st molars			4	3		2	3	3
2nd molars			3	2		1	2	1

(1) Parker (1874); (2) Babini (1913–14); (3) Franci (1915); (4) Techow (1916); (5) Ainsworth and Young (1925); (6) Colditz (1931); (7) Dwyer (1932); (8) Mellanby (1934).

The great freedom from dental caries of the lower anterior teeth more than makes up for the greater susceptibility to dental caries of the lower first molars.

Susceptibility of Individual Teeth in the Mouth

Generally speaking, each tooth in the mouth has a distinct caries susceptibility. As the caries attack rate for a specific mouth increases, teeth become carious in a definite order. If the attack rate is decreased because of various intrinsic or extrinsic factors, certain specific teeth which would otherwise be carious will remain selectively caries-free. This observation can be illustrated in areas of fluorosis, where Dean (1946) found a much greater reduction of dental caries incidence in the four upper anterior incisors than in the other teeth of 12- to 14-year-old children. The susceptibility of the various morphological types of teeth has been discussed by many investigators.

The order of susceptibility of deciduous teeth to caries as reported by various observers is presented in table 27.

Dwyer (1932) gave the per cent susceptibility of remaining defective teeth among the 6- to 11-year age group in over 2,000 girls. Fillings and extractions were not considered and, since these ages represent a period when the teeth were being exfoliated, the data may not represent a true picture of the attack rate of the different deciduous teeth. The author found the deciduous first molars less prone to decay than the second deciduous molars because of their morphology. These teeth are more dome shaped and lack the deep pits and fissures found in the second molars.

Franci (1915) examined Italian children from the first through the sixth grade and observed 4,631 carious teeth out of 6,739 deciduous teeth present. Data of Ainsworth and Young (1925) were on the caries susceptibility of 4,000 to 5,000 British school children from 2 to 15 years of age. The majority, however, were from 5 to 13 years of age. Only the upper deciduous teeth were listed. Colditz (1931) presented the specific order of attack of 651 6- and 7-year old children in Germany. Mellanby (1934) reported the percentage of caries in the deciduous teeth of British children. The order of susceptibility differs from that of Dwyer (1932) in the second molars only. Mellanby (1934) found the lower second deciduous molars the most frequently carious teeth, followed by the upper second deciduous molars.

Cohen (1936) presented the deciduous tooth attack rate for both sexes from 2 through 12 years of age. Even as early as 2 years of age, the first and second molars were found to be the most frequently in-

volved. This high attack rate persisted throughout all age groups.

McCall (1938) in a study of 4,596 preschool children found the upper second molars most affected, as did Dwyer (1932), but the least susceptible were the lower incisors and cuspids. The upper cuspids were less susceptible than the upper incisors but showed some caries.

Roos (1944) observed that the caries attack rate for the various morphological types of teeth varied with age. While at 3 years of age only 2.9 per cent of the molars were carious, 3.9 per cent of the upper incisors were attacked. At 5 years of age, the percentage of carious molars had risen to 17.1 per cent, while that of the upper incisors had increased to only 6.3 per cent. This same ratio was also found in extractions.

Parker (1874), examined individuals from under 8 years to over 65 years of age. Fenchel (1893) listed the susceptibility of the various permanent teeth for 335 school children. Of these children, 323 had 2,471 carious teeth.

The findings of Greth (1939) in 212 skulls of the lower Rhine Franks (400–900 AD) whose average length of life was 35 years are presented in table 28 for comparison of ancient and modern data. There were 2,871 teeth, of which 179 or 6.2 per cent

TABLE 27

Order of susceptibility of permanent teeth to caries

Author	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Upper											
Centrals	5	10	8	11	8	8	4	10		6	14
Laterals	4	11	7	13	6	9	7	11	5	9	13
Cuspids	8	9	12	10	11	11	9	13	6	12	11
1st bicuspids	6	5	5	4	5	7	8	7	3	8	4
2nd bicuspids	3	3	6	9	4	5	5	8		5	7
1st molars	1	2	2	2	2	2	1	2	2	2	6
2nd molars	2	7	4	5	9	4	6	3	2	4	9
3rd molars	7		11	6			15	6			8
Lower											
Centrals		13	16	14	13	13	14	14		11	16
Laterals		14	15	15	12	12	13	15	7	13	15
Cuspids		12	14	16	13	14	12	16	8	14	12
1st bicuspids		6	13	12	10	10	11	12	4	10	10
2nd bicuspids		4	9	8	7	6	10	9		7	3
1st molars		1	1	1	1	1	2	1	1	1	1
2nd molars		8	3	3	3	3	3	4	3	3	2
3rd molars			10	7			16	5			5

(1) Parker (1874); (2) Fenchel (1893); (3) Kloser (1913); (4) Franci (1915); (5) Suk (1919); (6) Ainsworth and Young (1925); (7) Driak (1929); (8) Hyatt & Lotka (1929); (9) Day & Sedwick (1935); (10) Cohen (1936); (11) Greth (1939).

TABLE 28

Percentages of upper teeth attacked by caries in several races

(Steggerda and Hill, 1936)

Race	Teeth						
	Centrals	Laterals	Cuspids	1st premolar	2nd premolar	1st molar	2nd molar
Navajo Indian	0.6	0.6	0.0	7.0	12.7	62.8	15.9
Maya Indian	17.6	9.6	2.4	9.6	13.6	45.6	1.6
Jamaica White	14.8	15.1	2.6	15.3	15.9	27.3	9.4
Jamaica Mulatto	20.0	18.6	4.9	12.5	12.7	23.0	7.8
Jamaica Negro	19.3	19.6	6.7	14.7	9.5	18.2	12.3
Dutch (Mich.)	4.9	6.0	0.9	8.1	8.8	64.2	6.7

were carious; 35.3 per cent of the skulls contained carious teeth.

The order of susceptibility of permanent teeth to caries as reported by various observers is presented in table 27.

Steggerda and Hill (1936) reported the percentage distribution for caries in individual permanent teeth of the upper jaw among the different races studied by them. These are presented in table 26. They state that the resistance to dental caries may be racial and the location of the cavities related to the kind of diet of the group.

Brekhus (1931) listed the percentage susceptibility of the different teeth of 3,711 students averaging 18 years of age at the University of Minnesota as follows:

Upper and lower first molars	95%
Upper and lower second molars	75%
Upper second bicuspids	45%
Upper first bicuspids and lower second bicuspids	35%
Upper central and lateral incisors	30%
Upper cuspids and lower first bicuspids	10%
Lower central and lateral incisors and lower cuspids	3%

Brekhus (1941a) in a later paper breaks down the caries rates for the 1929 and 1939 students according to the percentage of perfect teeth for the various individual teeth. With the exception of the upper lateral incisors and the lower central incisors, all teeth showed a greater percentage of carious teeth in 1939 than in 1929.

In the study at Hagerstown, Maryland, conducted by Klein, Palmer and Knutson (1938), 4,416 school children aged 6 through 15 years were examined, using mouth mirror and explorer. Nearly 91 per cent of all the DMF surfaces in the permanent teeth were found in the upper and lower first molars, lower second molars and upper lateral and central incisors. The first molars contributed 64 per cent of the total caries experience in the upper jaw and 87 per cent in the lower jaw.

Klein and Palmer (1941) in discussing the various morphological types of teeth divided the 28 permanent teeth (3rd molars excluded) into 5 classes of caries susceptibility as follows:

Class I Mandibular 1st and 2nd molars
II Maxillary 1st and 2nd molars
III Mandibular 2nd premolars, maxillary 1st and 2nd premolars and maxillary central and lateral incisors
IV Maxillary canines and mandibular 1st premolars
V Mandibular central and lateral incisors and mandibular cuspids

They indicate that the teeth farthest back in the mouth are the most carious and the lower anterior teeth least carious. The high susceptibility of the teeth placed far back in the mouth may be due to their location in the arch or may be an expression of their morphology. The authors point out that, although these teeth are farthest back in the mouth, they are also the teeth with pits and fissures and with the broadest contact points, and suggest that the DMF rates could be expressed as the number of particular types of teeth attacked by caries per year of exposure in the mouth.

Foster (1942), reports the first molars to represent 69.54 per cent of all carious teeth in children of ages 6 to 16. Healey and Cheyne (1943) state that the lower first molars are the most frequently affected and the lower cuspids the least attacked. On this point there is general agreement in the literature. In comparing the specific susceptibility of particular teeth in the mouth, however, the extremely important factor of years of posteruptive exposure to the oral environment must be considered.

Although in the school child the upper central and lateral incisors are most frequently affected after the first molars, in a group of college students examined by Healey and Cheyne (1943), the second molars were most frequently affected after

the first molars. Healey and Cheyne (1943) have plotted the posteruptive tooth age against caries attack rate and conclude that, although the posteruptive tooth age does play an important part, other factors such as the morphological similarity, structure, development influence or position in the mouth must play more important parts in the carious process and overshadow individual differences due to posteruptive age.

Brucker (1944a), in a study of 17,466 first molars, stated that the lower left first molar ranks first in susceptibility, followed by the lower right first molar.

Summary and conclusions: There is sufficient evidence in the literature to substantiate the belief that the caries attack rate among the various teeth follows a regular pattern. In reviewing papers covering this subject, it becomes obvious that the posterior teeth are more subject to decay than the anterior teeth. Whether the location and position in the mouth *per se* are factors in caries susceptibility has not been conclusively demonstrated, although crowding of the teeth and malpositioning could influence caries susceptibility, as has been shown by Saltzman (1939) and Brucker (1944).

Among the deciduous teeth, Franci (1915), Techow (1916), Dwyer (1932) and McCall (1938) indicate that the upper second molars are the most frequently attacked teeth. Colditz (1931) and Mellanby (1934) found the lower second molars most frequently attacked. However, there is almost general agreement that, of the deciduous teeth, the second molars are most frequently attacked, followed by the first molars.

There is almost universal agreement that, among the permanent teeth, the lower first molars are the most frequently attacked teeth, followed by the upper first molars (Fenchel 1893), (Kloser 1913), (Franci 1915), (Suk 1919), (Ainsworth and Young 1925), (Hyatt and Lotka 1929), (Day and Sedwick 1935), (Cohen 1936), (Klein, Palmer and Knutson 1938).

The susceptibility of the other teeth depends on the age of the persons and the exposure age of the teeth. The lower anterior teeth are the least susceptible in all age groups. Both the upper and lower cuspids are relatively free of dental caries.

Susceptibility of the Various Surfaces

As has been pointed out in many papers, the predisposition to caries of specific surfaces of teeth varies with the type of tooth, location and length of exposure to the oral environment.

In assessing the value of studies on the prevalence of caries on specific surfaces, it must be considered that X-rays are indispensable for this type of study. The exposed surfaces attacked can be detected more readily by explorer and mirror examination than can the proximal surfaces. Mouth mirror and explorer examinations, therefore, may not give a true attack relationship among the different surfaces. As an illustration, McCall (1938) compared the caries prevalence in the various surfaces as found by a group of practicing dentists who did not use X-rays with those of a group of clinic patients at Guggenheim Dental Clinic where X-rays were employed. The ratio of approximal cavities compared to occlusal cavities is much greater in the X-rayed group.

McCall (1938) among 4,596 preschool children found 4.2 times as many proximal as labio-lingual cavities in the deciduous teeth. In the deciduous cuspids, 1.7 times as many proximal cavities as labio-lingual cavities were recorded. In the first molars, the difference in the number of cavities occurring on the occlusal and on the proximal surfaces was not significant. However, the proximal cavities may involve two surfaces (mesial and distal), while the occlusal cavities involve only one. The bucco-lingual surfaces were 7.1 times less involved than the occlusal. In the second deciduous molars, the occlusal surfaces were attacked most frequently, being involved 2.7 times

more frequently than the proximals and 5.6 times more frequently than the buccal and lingual aspects. In the deciduous second molars, 64.9 per cent of the cavities were on the occlusal, 23.6 per cent on the proximal and 11.5 per cent on the bucco-lingual surfaces.

Dwyer (1932) reported the percentages of defects on the upper and lower second deciduous molars in over 300 cases of 5- to 7-year-old children. His figures closely tally with McCall's (1938) figures on the preschool children, indicating that the ratio of surfaces involved does not change appreciably in the deciduous teeth as the children leave the preschool age. A comparison of surfaces involved in the two dentitions is stated by Dwyer (1932) to show a greater

TABLE 29

Caries-involved surfaces per 100 teeth

(Hyatt and Lotka, 1929)

	Lingual	Buccal	Mesial	Distal	Occlusal
Maxillary teeth	7.33	3.46	16.17	13.09	45.66
Mandibular teeth	0.73	11.18	4.96	4.47	37.98
All teeth	4.21	6.93	10.82	8.98	41.65

proportion of mesial defects in deciduous molars and of buccal and lingual defects in permanent molars. Caries was found to be very low on the distal aspect of the deciduous second molars. The surfaces which Dwyer (1932) found to be involved in the first permanent molars in individuals from 16 to 20 years of age indicate the wide disparity between occlusal and other surface cavities, the occlusal surfaces accounting for approximately 75 per cent of all involved surfaces.

Morse, Losch, Godfrey and Castaneda (1935) state that pit and fissure caries has its highest percentage incidence (3.02) in the 6- through 7-year age for the deciduous teeth. Smooth surface cavities reach their highest percentage incidence (15.37) at the 8- through 9-year age group.

Klein, Palmer and Knutson (1938) in their study of 23,753 surfaces in elementary school children found that less than half the affected surfaces were occlusal. The percentages of involved surfaces were as follows: occlusal, 43; mesial, 17; distal, 14; buccal, 13; and lingual, 13.

Dunning (1941) in a study of the buccal and proximal surfaces of premolar teeth of 12,645 employees between the ages of 16 and 59 found that the number of proximal cavities on premolar teeth rises to a high point between 30 and 34 years of age and then levels off, while buccal cavities rise in number throughout the entire period of study, although less steeply after 50. Proximal cavities on the premolars occur almost entirely before 30 years and are more than twice as common as buccal cavities.

In a study conducted by Moore (1936) among 80 persons examined 3 or 4 times yearly from 6 to 21 years of age, the greatest increment of caries was found at 17 years of age. This excluded occlusal caries, which arises earlier in life, leaving the increment of proximal caries which come later in life. Her study, omitting the occlusal surfaces, showed only 20.6 per cent of decay occurring between 6 and 13 years of age, while 79.5 per cent occurred between ages 14 and 21.

Brucker (1944b) found no relationship between the presence of fissures and the incidence of caries on the first permanent molars.

Hyatt (1920) without using X-rays reported the percentage of frequency of occurrence of cavities on the different surfaces of the first permanent molars in 2,101 girls 12 to 20 years of age in Brooklyn. His figures were as follows: buccal, 21; lingual, 3; mesial, 9; distal, 8; occlusal, 59 per cent.

Hyatt (1928) reported the number of buccal and lingual cavities per 100 first molars in each jaw quadrant for 2,943 persons as follows: *Buccal:* UR 2.0, LR 25.3, UL 3.0, LL 30.7; *Lingual:* UR 16.3, LR 1.3, UL 16.3, LL 1.8. From these figures it will be observed that the lower molars have

more buccal caries and the upper molars more lingual caries.

In reporting on the surfaces involved for all teeth in 2,943 persons under 25 years of age, Hyatt and Lotka (1929) report that occlusal cavities make up over 57 per cent of the total surfaces affected. Their findings are presented in table 29.

Day and Sedwick (1935) employing both explorer and X-ray, listed the surfaces involved among 433 13-year-old children. Approximately 57 per cent of the involved surfaces were occlusal, 16 per cent mesial, 10 per cent distal, 9 per cent lingual and 7 per cent buccal. Less than 1 per cent were found in labial, incisal and cervical surfaces.

The authors state that the anatomical structure of the tooth is a factor in location of lesions, i.e., the occlusal and lingual cavities in the upper jaw and buccal and occlusal cavities in the lower jaw.

Summary and conclusions: Among the deciduous teeth, the evidence points to a greater number of occlusal cavities than of any other type. The greatest number of fissure caries occur from the sixth through the seventh year of age, while the smooth surface cavities reach their peak from 8 through 9 years of age.

There is general agreement that occlusal caries is the most prevalent among the permanent teeth. Figures offered indicate from 43 to 75 per cent of all lesions are occlusal. Occlusal caries starts earlier in life, while the maximum number of proximal cavities occurs later. In the anterior teeth, approximal cavities account for the greatest number of lesions. In the entire dentition there are more mesial cavities than distal, and in the lower molars there are more buccal cavities than lingual, while in the upper molars the ratio is reversed. In judging the surfaces involved in caries, great caution must be observed in evaluating the findings, for without an X-ray study of the total number of lesions the relative importance of proximal lesions and all others cannot be properly assessed.

Continuity of Caries Attack Rate in Specific Teeth

Dental disease is a continuous and progressive disease. As age increases, the number of cavities in the mouth is observed to increase and the number of caries-free mouths decreases. The onset and rate of progress of caries in specific teeth at various age levels has been considered in a number of papers.

Ainsworth and Young (1925) in a monograph prepared for the Medical Research Council of Great Britain discuss the progress of dental caries in specific teeth. For the permanent teeth, their findings were as follows:

Upper first molars—At age 7, (about 1½ years after the age at which 50 per cent are erupted) 17 per cent show caries, at age 9, 33 per cent are carious and at age 13, 52 per cent are carious.

Lower first molars—At age 7, (about 1½ years after the age at which 50 per cent are erupted) 28.5 per cent are carious, at age 9 years, 45 per cent and at age 13 years, 61.5 per cent are carious.

Upper second molars—At age 13, (about 1½ years after 50 per cent of these teeth are erupted) 16.5 per cent are carious; at age 14, 23.5 per cent are carious.

Lower second molars—At age 12, (1 year after 50 per cent of these teeth are erupted) 28 per cent are carious; at age 14, 44 per cent are involved.

Upper central incisors—At age 10, (3 years after 50 per cent of these teeth are erupted) 3 per cent are carious; at age 12, 5.5 per cent and at age 13, 9 per cent are carious.

Upper lateral incisors—At age 11, (3 years after 50 per cent of these teeth are erupted) 3 per cent are carious; at 13 years of age, over 7 per cent are carious.

Upper first premolars—At age 11, (1½

years after 50 per cent of these teeth are erupted) 4 per cent are carious; at 13 years, 10.4 are involved.

Upper second premolars—At age 11, (1½ years after 50 per cent of these teeth are erupted) 5.6 per cent are carious; at 13 years of age, over 13 per cent are carious.

Cohen (1937) in a study of 2,554 Minneapolis school and preschool children found that at 3 years of age 70 per cent of the lower second deciduous molars and 60 per cent of the upper second deciduous molars were defective. At age 5, 80 per cent of the lower deciduous molars were defective and 15 per cent had already been extracted. At age 7, 40 per cent of the deciduous second molars had been extracted. Among the permanent teeth at age 8, 75 per cent of the lower first permanent molars had experienced decay. At age 10, 80 per cent of the lower first molars and 70 per cent of the upper first molars were defective. At age 15 years only 12 per cent of the children had no decay experience in the lower first molars; 28 per cent of the lower first molars had been extracted.

Summary and conclusions: Among the various teeth, dental caries becomes more prevalent with increased years of exposure to the oral environment. The caries attack rate for the different morphological types of teeth, however, is not consistently of the same magnitude.

Caries in Adjacent Teeth

Bodecker and Ewen (1937) studied full mouth X-ray films from 516 patients selected at random; 103 of these patients presented 179 lesions in which the surface in juxtaposition was not affected.

Van Huysen, Moon and Davidson (1939) examined 105 patients for caries originating on the adjacent surfaces of 1,802 teeth. The average age of the patients was 46 years (27–74). Of the 2,566 adjacent surfaces studied, 42 per cent contained caries. The coefficient of association was greater for caries on opposite sides of the jaw than for caries on adjacent surfaces.

Losee (1947) conducted explorer and X-ray examinations of 580 Marine Corps recruits having a median age of 21.2 years. These men had received no previous dental treatment. The author found that 50 per cent of the abutting paired surfaces among the proximal and the occlusal surfaces from the distal of the cuspid posteriorly had cavities; of these carious pairs, 75.4 per cent had both surfaces involved while 24.6 per cent had only one surface involved. With respect to the bilateral nature of the occlusal caries, 55.7 per cent of the matched surfaces showed cavities. Of these bilateral pairs, 61.9 per cent were involved on both matched surfaces while 38.1 per cent were carious on only one surface.

Summary and conclusions: The evidence indicates that caries can occur on only one of two adjoining surfaces but suggests that caries on both adjacent surfaces is more common than on only one surface.

Yearly Increment of Dental Caries

The attack of teeth by dental caries is a continuous process throughout life. The yearly increment varies directly with the accumulated dental caries prevalence in any given group and with the age and sex of the individuals in the group. Generally speaking, the difference between the accumulated dental caries at the beginning and end of any specific span of time for any specific group would be the caries increment for that period of time. Because of the wide deviations in caries rates, the yearly increments are likely to vary widely when the difference in total caries experience among groups is compared.

Klein, Palmer and Knutson (1938) found a yearly increment of 1.32 carious permanent surfaces among 4,416 Hagerstown elementary school children 6 to 15 years of age.

Sloman (1941) computed the average attack rate of 53,198 children in San Fran-

cisco between 6 and 18 years of age to be about 2.17 per cent of the entire dentition annually. The author lists the average per cent of the entire dentition attacked by caries during the 6- through 12-year age period as 1.98 and during the 12- through 18-year age period as 2.37 per cent. Sloman states that one carious tooth is added every 18.67 months in boys and every 15.84 months in girls.

Klein and Palmer (1941a) in their study of 6,257 white children aged 6 through 19 years in Hagerstown and environs observed an attack rate of 0.7 of a permanent tooth per year. There was an accumulation of carious surfaces at the rate of 1.4 per year.

Thompson (1943) in his study of boys at Boystown between the ages of 12 and 17 years found an average yearly increment of 1.4 new cavities per boy.

Among Hagerstown high school children 13 to 19 years of age, Klein and Palmer (1940) observed that the incidence of new caries was approximately 0.6 affected permanent tooth and two affected permanent tooth surfaces per child per year.

Hollander and Dunning (1939) found that in 12,753 persons, from 17 to over 65 years of age the number of DMF teeth and surfaces rose rapidly, corresponding to a rate of about 1.75 surfaces per person per year, until about 34 years of age when it started slowing down. After 34, the average rate of caries was about 0.5 of a surface per year of age, or a gross reduction of about 70 per cent. After about 25 years of age, there was a definite but gradual reduction in formation of new lesions which could not be associated with the passing of adolescence.

Schlack (1940) found among 1,047 U.S. Navy personnel that the yearly increment of new and recurrent carious defects at 17 to 19 years of age was 1.15 tooth areas per year; at 20 to 24 years, it was 1.19; at 25 to 29 years, it was 1.30; at 30 to 34 years, it was 1.66; at 35 to 39 years of age, it was 0.68; and at 40 to 51 years, it was 1.27. However, in a later study Schlack (1941), with the employment of X-rays on a group of 209 Navy personnel, found that the yearly increment was not as great as appeared by visual inspection. Apparently, incipient lesions were detected by X-ray which did not develop during the year. Since there were very few individuals over 40 years of age, it is questionable whether the figures in the older age groups are representative of these ages.

A more exact method of determining caries increments is the employment of groups of individuals in a longitudinal study. Since the caries pattern varies among individuals, an accurate assessment of the rate of caries increase can best be secured by following the same individual over an extended period of time. From this type of study, one can determine: (1) whether there are periods when caries may be expected to advance more rapidly than in others; (2) whether a tooth non-carious for several years after eruption is less likely to be attacked than a tooth newly erupting into the mouth; (3) whether the caries advancement is a steady or an intermittent process. Boyd and Cheyne (1946) made such a study. The records of 60 boys and 41 girls from 2 to 11 years of age from the Iowa College of Dentistry files were tabulated for a period averaging 67½ months for each child. Their results showed an average caries increment in the mixed dentition of 4 newly affected surfaces per year.

Boyd and Cheyne (1946a) compared the data derived from their study with that of Klein, Palmer and Knutson (1938) at Hagerstown. Thirty-five per cent of the teeth of the Iowa City children were carious as contrasted with 15 per cent at Hagerstown. The average number of DMF surfaces per DMF tooth was 1.85 in Iowa City as compared with 1.93 at Hagerstown. The average number of DMF surfaces in Iowa City children was 6.9 as compared with 5.5 at Hagerstown. The average annual in-

crement of DMF surfaces was 2.7 among Iowa City children and 1.3 among Hagerstown children. There are several reasons that might account for this difference: (1) the Iowa City children were Clinic patients while the Hagerstown group were school children; (2) Iowa City children had more dental corrections, the fillings perhaps occupying more surfaces than would the original lesions; (3) cleaning and drying of the teeth as well as X-rays were used in the Iowa City study, while the Hagerstown study was done with explorer alone.

Summary and conclusions: Evidence indicates that there is an attack rate among school children of slightly less than one permanent tooth per child per year, with roughly two carious surfaces for every carious tooth. The caries attack rate begins to decrease after about 35 years of age. This reduction is perhaps a reflection of the reduced number of teeth and the reduced number of available surfaces for attack.

Mortality Rate of Teeth

Correlated with the progress of dental caries is the loss of teeth. Although the loss of teeth does not necessarily follow the caries attack rate, it closely parallels it unless corrective treatment has been instigated. In a sense, therefore, mortality rates reflect the effectiveness of a dental health program. Knutson and Klein (1938) illustrate this point by showing that among students without fillings boys have 58 per cent and girls 33 per cent more missing permanent teeth than those with fillings. Knutson and Klein among 4,416 Hagerstown school children between 6 and 15 years of age observed the tooth mortality rates expressed as missing teeth per 100 children ranging from 2.5 at 7 years to 119.0 at 15 years. At equivalent ages girls had a higher tooth mortality than boys. As to the kinds of teeth missing, Knutson and Klein report that among 742 teeth missing in the 2,232 boys studied there were 3 canines, 3 second molars, 5 second bicuspids, 8 first bicuspids, 8 lateral incisors, 18 central incisors, and 697 first molars. The teeth lost among grade school children are primarily the first molars, which in no age or sex group account for less than 90 per cent of all tooth mortality, and in all school age groups they account for 93.9 per cent of the tooth mortality in boys and 96.2 per cent in girls. The lower first molars alone contribute 70 per cent of all tooth mortality in boys and 80 per cent in girls. Tooth mortality, like dental caries, is bilateral in occurrence. Therefore, as pointed out by the authors, the adequacy of dental care in a given community can be determined by determining the mortality rate of the lower first molar of either half of the jaw.

Cheyne and Drain (1940) arrived at the same conclusion in their study of 8,677 school children age 7 to 14 years, where the first molars represented 88 per cent of the total tooth loss. The lower first molars alone accounted for 65.8 per cent of the mortality. Although there was no significant difference in the frequency of missing first molars in the right and left quadrants, there was a statistically significant difference between the loss of the upper and lower first molars. Although females had a greater tooth mortality in the maxillary molars, the total tooth loss was not significantly different between the two sexes. Of the 520 permanent teeth lost, 462 were first molars, 36 premolars, and 22 other teeth. This is a greater loss of premolars than reported by Knutson and Klein (1938).

McCall (1938) reported the following percentage losses of first and second deciduous molars in 4,386 children in New York City: 7.4 at age 3, 9.7 at age 4, 9.5 at age 5, and 12 at age 6. There was a greater loss among the second deciduous molars than the first, although the difference was not great.

Hagan and Cook (1949) in an examination of the teeth of 7,500 children observed the following permanent tooth mortality: At age 7, a loss of 0.04 permanent teeth; at

age 9, 0.23 teeth; at age 11, 0.51 teeth; at age 14, 0.96 teeth; and at age 16, 1.11 teeth. The tooth mortality was lower in those mouths having fillings.

Knutson (1942) in a study of 700 children between the ages of 10 and 14 years observed a tooth mortality among first molars of 74.82 per 100 boys and 77.66 per 100 girls.

Among the 13- to 19-year age group numbering 1,841 in Hagerstown, Klein and Palmer (1940) found a permanent tooth mortality of 125.8 per 100 boys and 118.8 per 100 girls. The boys in the high school age group had the higher tooth loss. Apparently the girls as they grow older have greater concern for their appearance and hence take better care of their teeth.

Arnett and Ennis (1933) examined 883 students at Drexel Institute, averaging 18.75 years of age. Of these students, 419 had 896 missing teeth for an average of 1.01 missing tooth per student examined. This is about half the number of missing teeth reported by Klein and Palmer (1940) among 18-year-olds, indicating that this group probably received better than average dental care.

Welty (1937) reported on 26 students at California Dental College. Freshmen averaged 1.308 missing teeth, sophomores 1.616, juniors 1.692 and seniors 2.038 missing teeth per student.

Hyatt (1920) examined 2,101 Brooklyn girls from ages 12 to 20 years. There were 1,141 first molars missing, or 13.5 per cent of the entire number that should be present. These were so divided that 6.7 per cent were upper first molars and 20.3 per cent were lower first molars. The loss of second premolars totalled 213 or 2.5 per cent. There were 1.7 per cent of the upper second premolars missing and 3.3 per cent of the lower second premolars.

Miller and Seidler (1940) studied 1,003 persons between the ages of 11 and 60 years. They found that females had more missing teeth than males. The females had 7.4 per person as compared with 6.8 for the males.

Dunning (1944) reported on 1,208 midshipmen averaging 21.6 years of age. These men had apparently been under good dental care, for there was only 0.68 of a missing tooth per person.

Brekhus (1929) reported on the loss of teeth in 1,202 male patients and 1,521 female patients from 10 to 80 years of age. The percentage of teeth extracted because of caries among females was slightly greater than among males. Of all teeth lost, the percentage of loss due to caries or its sequellae only is presented in table 30. When dental caries and pulp involvement due to dental caries are considered, his figures indicate that caries is the major cause of loss of teeth before 50 years of age. After 50, periodontoclasia becomes the major cause of loss of teeth.

TABLE 30

Teeth lost due to caries only as per cent of total loss of teeth

(Brekhus, 1929)

Age group..	10–20	20–30	30–40	40–50	50–60	60–70	70–80
Male.....	51.0	33.8	23.9	22.5	16.6	18.0	23.9
Female..	51.2	35.5	31.8	22.2	17.5	18.4	24.9

Brekhus (1928) stated that among 2,723 patients at age 10 years, 10 per cent of the boys and 15 per cent of the girls had lost lower first permanent molars. At age 15, there was a 30 per cent loss in boys and a 30 per cent loss in girls. At age 20, there was a 40 per cent loss in boys and a 47 per cent loss in girls. At age 40, there was a 63 per cent loss in men and a 75 per cent loss in women. There was a 0.33 per cent greater loss on the left side than on the right side.

Cramer (1929) in an examination of 1,000 male factory employees from executives to unskilled laborers in Milwaukee, found there was an average of 4.35 extracted teeth per person, ranging from 2.29 lost teeth at the 20- to 25-year age level to 16.9 teeth over

the 60-year age level; 341 of the 1,000 employees examined were executives.

Klein (1943) reported the tooth mortality rates for the age groups from 15 to over 65 years. There is an unbroken rise in tooth mortality rates.

Age group	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65+
Lost teeth	2.3	3.6	5.4	7.9	10.6	13.7	17.4	18.5	21.0	22.8	23.8

Allen (1943) made a statistical study of the primary causes of extraction in persons 6 to 81 years of age. In the younger age groups, caries was the primary reason for extractions; above the 31- to 40-year age group, other causes assumed greater importance. The author reported the following percentages of extraction for caries: at 1–10, 85.7; 11–20, 63.1; 21–30, 72; 31–40, 43.9; 41–50, 39.6; 51–60, 25.6; 61–70, 40.2; over 70, 48.8.

Wisan (1938) has shown the lost permanent tooth index to be greater in communities with inadequate dental programs. He also found it to vary with the size of the community and with the economic status of the individuals.

Klein (1943) found more teeth lost in the lower income groups. Beyond 18 years of age, there was an average loss of one tooth for every 2½ years. It was also noted that at age 18 years, the average white person has 29.8 serviceable teeth remaining in his mouth.

Summary and conclusions: The loss of teeth is inversely correlated with the amount of dental treatment received. The evidence indicates that girls have a higher tooth mortality than boys. This is a reflection of the higher caries rates among the permanent teeth in girls. Tooth mortality among school children primarily involves the first permanent molars and the second deciduous molars. In the younger age groups, caries is the major cause of loss of teeth. After 30 to 40 years of age, periodontoclasia becomes the major cause.

Dental Needs Today

There is no doubt that there is a great disparity between dental needs and dental correction today. As individuals grow older, the gap between dental needs and correction becomes wider. The United States Public Health Service has covered this field rather thoroughly.

Klein, Palmer and Knutson (1938) have analyzed the records of 4,416 Hagerstown children for remedial defects and observed that 59.8 per cent of the boys and 58.7 per cent of the girls, age 6 through 15 years, had one or more teeth needing fillings. In the deciduous teeth, more boys had filled teeth, while in the permanent teeth, more girls had filled teeth.

Klein and Palmer (1940) computed the dental status of 1,841 high school students in this same community. Among the 13- to 19-year age group, there were 7.1 DMF teeth and 14.5 DMF surfaces per child. Only 3.3 teeth and 4.7 surfaces had been filled, and 1.22 teeth had been removed or were merely remaining roots. There were 2.86 untreated carious teeth per child. There were, therefore, less than half of the DMF teeth filled and these teeth originally presented small cavities without too great surface involvement. In these age groups, an average of over one tooth had already been lost through neglect.

Klein and Palmer (1941a) discuss the findings on 6,257 white children 6 through 19 years of age in Hagerstown and environs. They state that the DMF surfaces develop at a rate nearly 5 times faster than they are being filled. Teeth were filled at the rate of 0.25 of a tooth per year and more than 0.2 of a tooth was extracted or indicated for extraction. There is, therefore, a residuum of 0.2 of a carious tooth per child to accumulate per year. There is a disparity between

the prevalence of caries attack and the amount of correction, so that the number of untreated cavities increases as age advances. Of every 100 tooth surfaces attacked, approximately 20 are filled, nearly 80 remain unfilled. Of the 80 surfaces remaining unfilled, 60 are eventually extracted or so indicated. More than three-fourths of the tooth surfaces that become carious per year remain without fillings; and of the latter, two-thirds extend to a point where they are extracted or so indicated. According to the authors, the average school child, 6 through 19 years of age, shows a present need for fillings of about 3 permanent tooth surfaces, with 3 surfaces lost and a little more than 2 surfaces filled.

Sandler and Strusser (1949) report on the dental treatment requirements of 3,282 children aged 2 to 16 years in New York City. These children were reporting for the first time to the New York City Department of Health Clinics. The average number of teeth requiring treatment (fillings and extractions) increases from 3.5 at 2 years of age to 6.8 teeth at 7 years of age. The average number of permanent teeth requiring treatment increases from 0.2 teeth at 4 years of age to 6.7 at 14 years of age. The deciduous teeth increase from 3.5 at 2 years of age to 4.4 at 3 years of age and then gradually decline.

Klein (1941) in comparing the dental status of men acceptable and rejectable for draft status among 642 West Virginia and Maryland men aged 21 through 35 years reported the following findings:

	DMF rate	Carious	Extracted	Filled
Acceptables..	11.	5.5	3.3	2.4
Rejectables...	22.5	5.4	15.4	2.1

It will be observed that 87.2 per cent of the DMF teeth in the acceptable group have had to be extracted or remain without fillings. In the rejectable group, 68.4 per cent of the DMF teeth have been extracted or require it. Although the rejectables had over twice the DMF rate of the acceptables, they had less filled teeth, indicating persistent neglect.

Walls, Lewis and Dollar (1941) found that in ages 15 to over 65 only 3.2 per cent of the males and females needed no dental attention. The need for fillings was greatest in the 15- through 19-year age group. For males at this age group, there was need for 6.8 fillings per person and for females 6.5 fillings per person. The need for fillings decreased (because of extractions) by approximately 0.6 of a filling with each 5-year increase in age. The average need for all persons over 15 was 4.3 fillings for males and 4.4 for females. With age, there was an increase in size of the cavities, with a greater preponderance of two- and three-surface cavities. The need for extractions also increased with age. In the 15- through 19-year age group, there was about 0.6 of an extraction per person needed. Beyond 55 years of age, it leveled off to about 4 extractions for males and 3 for females. At 50 years of age, approximately 30 per cent of all patients needed full upper dentures. The need for lower full dentures followed the same pattern, but, because of the resistance to caries of the lower anterior teeth, at a lower level. At 40 years of age, 23 per cent of the females and 20 per cent of the males needed partial dentures. The need for fixed bridges was greatest between 30 and 35 years of age, when approximately 22 per cent needed such restorations.

Rypkins (1922a) reported that nearly two-thirds of all children entering school needed dental attention.

The Philadelphia Hospital and Health Survey (1929) stated that among 8,500 children from kindergarten through the eighth grade, 1.8 per cent were caries-free; 74 per cent had cavities but received no attention; 24 per cent had cavities but received only partial attention; and only 0.2 per cent received complete attention.

The amount of neglect among the popula-

tion as a whole may be expressed in another way as the amount of time necessary to complete the rehabilitation of these mouths. According to Brandhorst (1939), the average time required in hours to render the needed dental services for one year was as follows: kindergarten child, 5.23; grade child, 6.35; teachers' college student, 7.7; rural grade child, 7.8; high school student, 8.8; vocational pupil, 10.6; professional person, 12.1; college student, 12.2; white collar person, 15.7; urban adult, 17.2; merchant, 18.7; unclassified person, 18.8; housewife, 18.9; rural adult, 19.2; housemaid, 20.8; farmer, 21.4; trade and factory worker, 23.5.

Summary and conclusions: There is an appalling disparity between dental needs and dental corrections today. This difference becomes greater with age, although among preschool children the need is also very marked. The literature indicates that among school children less than half of the teeth needing correction receive it. The high mortality rates among this group are a result of this neglect. Not only is the accumulated problem not being met, but the yearly increment of new cavities is not being adequately treated. The time required to rehabilitate the mouth increases with age and with the ability to secure adequate dental attention.

GENERAL SUMMARY

1. There is imperative need for standardization of examining procedures in dental caries prevalence studies in order that differences now extant in the literature can be minimized or avoided in the future.

2. There are a number of methods of recording data that have much to recommend their continued usage, although none is altogether satisfactory.

3. Ancient man was relatively free of dental caries. When caries was present, it was generally the result of wearing away of the tooth surfaces due to attrition. Ancient man possessed well formed and developed jaws.

4. Modern primitive man resembles ancient man to some extent in the prevalence of dental caries. This relative freedom from dental caries disappears, however, on contact with civilization.

5. There are marked geographic differences in dental caries prevalence, as well as changes among the same group of individuals from time to time.

6. Dental caries is rampant. It attacks the preschool child soon after eruption of the deciduous teeth. It becomes exceedingly prevalent among the school population and young adults and persists into old age.

7. There are racial differences in caries prevalence. Negroes have less caries experience than do whites. Because of environmental differences, a comparison with other civilized races is difficult to make.

8. In the deciduous dentition, caries appears to be more prevalent in males. In the permanent dentition, it is more prevalent in females of comparable age. In the mixed dentition, males appear to have the greater caries experience because of later exfoliation of the deciduous teeth in males.

9. In both the deciduous and permanent dentitions, dental caries and tooth mortality occur bilaterally. Dental caries experience is more prevalent in the upper than in the lower jaw because of the extreme freedom from attack of the lower anterior teeth.

10. Among individual deciduous teeth, the caries experience is greatest among the upper and lower second molars, closely followed by the first molars. Among the individual permanent teeth, the lower first molars are the most frequently involved, followed by the upper first molars. The order of involvement of the other teeth in the mouth depends a great deal on the age of the individual.

11. Occlusal cavities are the most common in both the deciduous and permanent dentitions. Occlusal cavities in the permanent teeth occur earlier than do the smooth-surface cavities. Buccal cavities are more common on the lower molars and lingual cavities on the upper molars.

12. Dental caries in individual teeth increases in both the number and size of the lesions proportionately to the number of years of exposure of these teeth to the oral environment. There is a difference in attack rate for the various morphological types of teeth.

13. Dental caries is a continuous process with yearly increments of new lesions. The yearly increment of new lesions tends to taper off during middle age.

14. Loss of teeth is inversely correlated with dental treatment. The first molars account for the greatest per cent of tooth mortality. Caries is the greatest cause of loss of teeth in the younger age groups. Periodontoclasia becomes a major cause in later life.

15. There is considerable disparity between dental needs and dental corrections. Not only is the accumulated problem not being met but dental corrections are not keeping pace with the yearly increment of new lesions.

PATHOLOGY OF DENTAL CARIES

A Biological Approach

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Contents

PATHOLOGY OF DENTAL CARIES

This review emphasizes the "vitalistic" aspect of dental caries. It does not represent the view that is presently popular, and it will not be surprising if such attention as it may receive in the literature will be critical and adverse.

The experimental biological approach to the problem of dental caries is comparatively recent, although the hypothesis is old. Evidence that teeth are in biologic connection with the body by way of a tissue fluid is accumulating, and further experimentation and investigation in this direction may well conclusively determine that tooth resistance to external attack is related to this biologic connection.

An effort has been made in this report to review the work of all investigators who approach the problem from a vitalistic viewpoint. They are few compared with the great number of those who regard the teeth as inert mineral masses. The reviewer, who is in this small group, has for a long time concentrated on the biologic phase and has written extensively on the results of his investigations. Consequently, this review must refer to many of his own reports with seeming immodesty.

The hypotheses stated are working hypotheses, advanced for the purpose of mapping out future investigations. Some may be refuted, others modified or extended, but the fundamental concept is believed to be sound. The popularly accepted concept of dental caries, namely, that acid formation on the surface of the tooth is the complete cause of its destruction, has been followed for more than half a century. Nevertheless, it has not led to a complete comprehension of the dental caries mechanism as evidenced by paradoxical clinical observations. Nor has it led to the prevention of the disease.

Dental caries has long been considered the result of environmental conditions. Many investigators working on this phase of the problem have contributed many data which define the mechanism of the exciting cause of tooth destruction. As a result of their findings, the conclusion has been reached that only by mouth cleanliness can caries be prevented. This concept has given rise to the slogan "A clean tooth does not decay" and to untold numbers of dentifrices supposed to keep the teeth clean. However, clinical observations show that, in spite of the conscientious effort of the public to fight dental caries with the tooth brush and dentifrice, tooth destruction is as active as ever.

Some persons, for example primitive tribes living on natural diets, have a low incidence of dental caries, even though they use neither tooth brush nor dentifrice (Waugh, 1930, and others). This fact seems to strengthen the hypothesis of the small group of investigators who consider that dental caries is not due solely to external destructive factors, but that it is a disease related to body resistance or, more precisely, to tooth resistance. The hypothesis, which requires further substantiation, has been set up that the factors causing dental caries are twofold as in the case of infectious diseases, namely, the exciting and the predisposing. The presence of exciting factors active around the teeth (food retention, bacteria, saliva) has been amply demonstrated, but the activity of predisposing factors still remains somewhat obscure. Should the predisposing factor be established as one of the causes of dental caries, then the often puzzling immunity to dental caries among certain individuals would be explained. Many persons are exposed to infectious diseases but do not necessarily contract them unless a predisposing factor, such as lowered body resistance, also appears.

With this approach the dental caries prob-

lem may be more amenable to solution. The fundamental evidence which must be presented before this concept can be accepted is the definition of the vital relationship of the teeth with the body. In other words, does a tissue fluid permeate the calcified dental tissues and thus link the teeth with general metabolic processes?

ANATOMY OF THE TEETH

Dental caries affects the five surfaces of the teeth to a varying degree due to differences in their form and location and their relation to other teeth, as well as to differences in their gross and microscopic structure. The physiologic reaction of the teeth to caries also seems important in the consideration of this problem, since evidence is accumulating that systemic disturbances such as nutritional deficiencies are related to caries activity. A brief description of the anatomy and histology of the teeth is taken from cited textbooks, the selections being principally those data which may have some bearing on dental caries (Bodecker 1944; Fish 1932; Gottlieb 1921, 1947; Kronfeld 1933; Meyer 1935; Noyes 1938; Orban 1944).

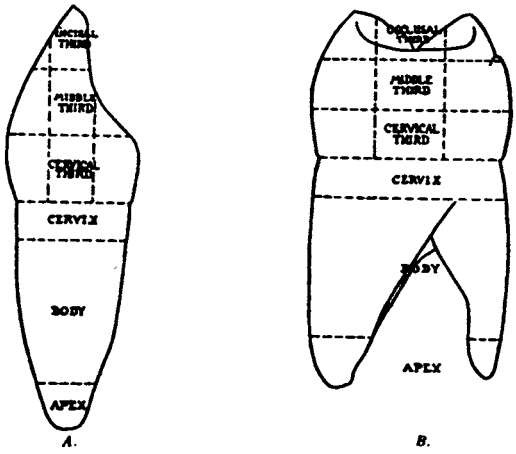


FIG. 1

A. Anatomical divisions of crown and root of anterior tooth; *B*. anatomical divisions of crown and roots of posterior tooth. (Courtesy of M. Diamond).

Teeth of man are of the omnivorous type. They function both as food cutting and as crushing tools. The incisive edges of the 12 anterior maxillary and mandibular teeth serve to cut food into small morsels which are then crushed on the occlusal surfaces of the 20 posterior teeth. The thorough and energetic mastication of detergent foods has a mechanical cleansing effect on some, but not all, tooth surfaces which thus receive a certain protection against dental caries (Wallace 1934). Forceful chewing also has the beneficial effect of stimulating the flow of blood to the pulp, which is the formative and nutritive organ of the tooth. The study of the problem of dental caries requires at least a superficial knowledge of the anatomy of the teeth, as their varying forms make some surfaces more susceptible than others to the disease.

Tooth surfaces: Teeth are divided anatomically into two parts, the crown and the root (fig. 1). The crown is subdivided into three parts, an incisal third in the anterior teeth and an occlusal third in the posterior teeth, a middle third and a cervical third. The root also has three subdivisions, the cervix, the body and the apex. Dental caries may affect the entire crown, the cervix of the root and, rarely, the body of the root. The three segments of the crown, as well as the tooth cervix, are further subdivided into four areas, related to the position of the teeth in the jaws. The proximal areas—those located between the teeth—have two designations, namely, the mesial surfaces, directed toward the median line of the face, and those directly opposite, the distal surfaces. The tongue, lips and cheeks, coming in contact with the remaining surfaces of the teeth, supply designations of lingual, labial and buccal surfaces.

Enamel grooves, pits and fissures: Grooves are found normally on the occlusal surfaces of molars and premolars. These are located on the surface of the enamel and are often so deep as to be regarded as faults. They are then designated as fissures and are re-

garded as exceedingly prone to dental caries. Enamel pits likewise may be faults and susceptible to caries. In addition to being found on the occlusal surfaces of molars and premolars, they also occur on the lingual surfaces of the maxillary or upper incisors and molars and on the buccal or cheek surfaces of the mandibular or lower molars. The reason these areas are so prone to caries is discussed in the section on dental caries.

HISTOLOGY OF THE TEETH

Teeth are composed of three highly calcified and two noncalcified tissues. The calcified areas are depicted in figure 2: enamel (E); dentin (D); cementum (C). Figure 3 shows the non-calcified tissues: pulp (P), the so-called nerve of the tooth; and the periodontal membrane (PdM).

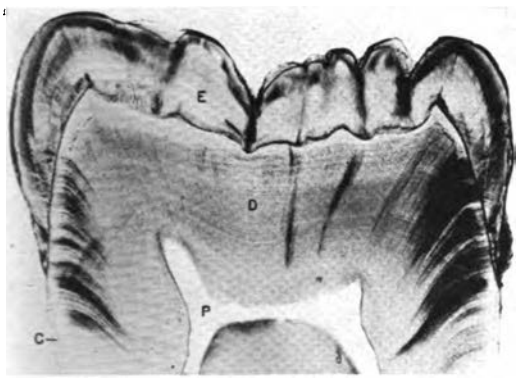


FIG. 2
Ground (undecalcified) section of crown of molar tooth, showing enamel (E); dentin (D); pulp chamber (P); cementum (C).

Attention will be focused in this report entirely on the enamel, dentin and dental pulp, as these tissues are affected most deeply by dental caries. Cementum (fig. 2, C), normally covered by the gingiva or gum tissue (fig. 3, G), may become carious when exposed to the fluids of the mouth by recession of the gingiva. The periodontal membrane (PdM), alveolar bone (AB) and to some extent the cementum become diseased as a result of periodontoclasia or pyorrhea alveolaris. This results in a loosening and frequent loss of teeth, a subject outside the scope of this discussion.

Enamel

A fairly detailed knowledge of the structure of the enamel is essential in any discussion of the pathology of dental caries, since it is the first barrier of defense against this disease. Much is obscure concerning the varying resistance of enamel to destruction, which may account for the fact that the problem of dental caries still defies complete solution.

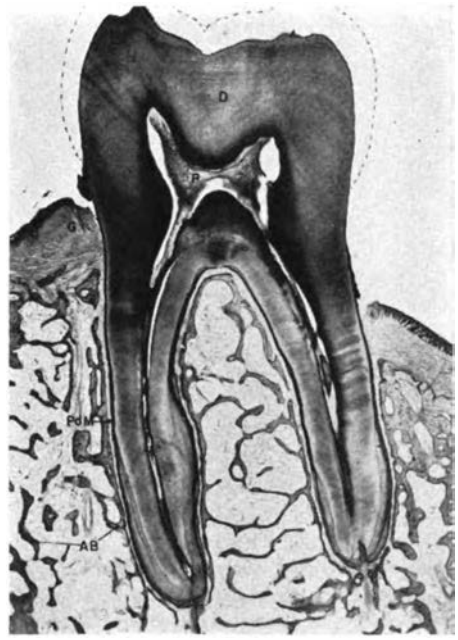


FIG. 3
Decalcified section of lower molar in alveolar bone. Dentin (D); pulp (P); gingiva or gum tissue (G); periodontal membrane (PdM); alveolar bone (AB). Enamel (dotted line) has been destroyed by the acid used in preparation of the specimen.

The enamel is the hardest and most highly calcified structure in the body, containing, according to Hodge (1944), 96 per cent ash, 1.7 per cent organic matter and 2.3 per cent water. Enamel covers the crown of the tooth in varying thickness and, because of its extreme hardness, enables it to withstand the

stress of mastication. The principal structural elements of the enamel are the rods, rod sheaths, inter-rod or cement substance, lamellae and tufts.

The enamel rods, having a diameter of about 4 microns, run in a more or less wavy course from the underlying dentin to the surface of the tooth (figs. 4 and 5). When the direction followed by the rods is definitely wavy, as in figure 5, it is termed "gnarled enamel". Their course is important in the clinical study of dental caries, inasmuch as the destructive process penetrates much more easily along the enamel lamellae and the rods than across their axes.

Normal enamel appears translucent when viewed under the microscope, showing only faint demarcations of the enamel rod sheaths under high magnification (fig. 6). Frequent brownish bands, the incremental lines or striae of Retzius, are distributed with a fair degree of regularity through the enamel (fig. 4, R). These areas of increased organic or protein material are more marked when nutritional disturbances occur during tooth formation.

Enamel cuticle: A thin, dense membrane, the enamel cuticle, covers the entire crown of the tooth. This membrane macroscopically and microscopically is invisible unless the tooth is subjected to acid. Figure 7 is a microphotograph of a ground section of a tooth decalcified with 5 per cent nitric acid under the coverglass. The mineral elements of the enamel are completely destroyed, leaving the enamel cuticle (C). Figure 8 shows a similarly treated section of dentin in which is seen the enamel cuticle (C) with the imprints of the enamel rods ending on the surface of the tooth. Originally the enamel cuticle was called Nasmyth's membrane, the remnant of the enamel-forming organ. However, Gottlieb (1921) and Gottlieb and Orban (1938) observed that it is a double membrane and applied the terms primary and secondary cuticles, the primary

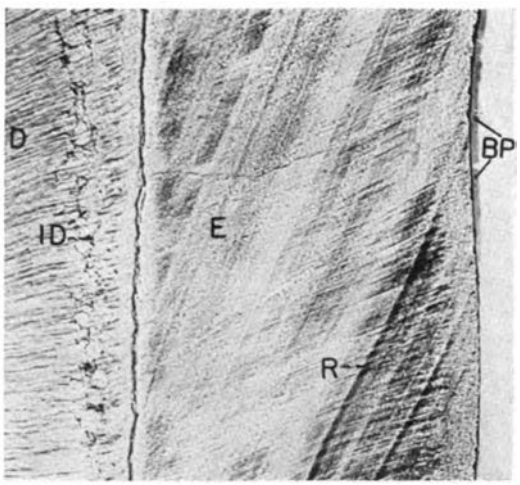


FIG. 4
Ground section of enamel (E) showing straight rods, under low magnification passing from dentin (D) to surface of tooth; striae of Retzius (R) or incremental lines; interglobular dentin (ID) areas of insufficient calcification due to nutritional disturbances during tooth formation; on the surface of tooth, bacterial plaques (BP) which when acid-forming decalcify the enamel.

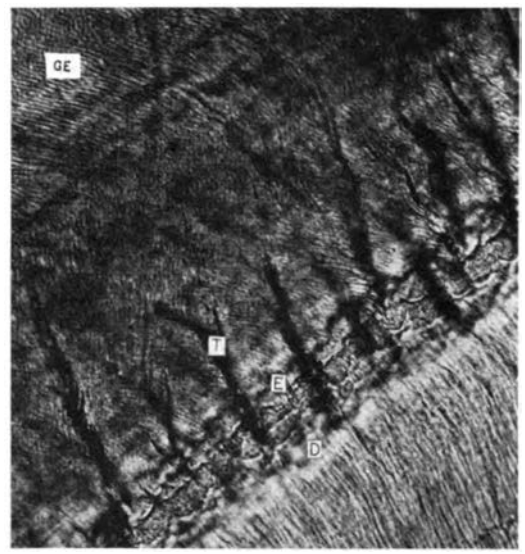


FIG. 5
Ground section of dentin (D) and enamel (E) showing the twisted course of the enamel rods, gnarled enamel (GE); tufts (T).

originating from the enamel organ and the secondary from the oral epithelium. Gottlieb (personal communication) gives further details as follows: "The primary cuticle is the final product of the ameloblasts after the enamel matrix is completely formed. The secondary cuticle originates from the enamel organ, consisting of reduced enamel epithelium, upon merging with the oral epithelium." These cuticles, composed of keratin, are remarkable for their resistance to acids. Their relation to dental caries will be discussed under the heading Enamel Resistance to Caries.

Organic constituents of enamel: The question as to whether enamel contains organic material has been a highly controversial

FIG. 6
Section of enamel under high magnification, etched, showing rods in longitudinal and oblique section in area of gnarled enamel.

one for more than 100 years. The importance of arriving at a decision may be seen from the fact that organic structures may affect the enamel, not only by forming channels for the passage of tissue fluid from within the tooth, but also by offering paths of entry for microorganisms and their products from the exterior. Therefore, because of the role these structures may play in enamel caries, this subject will be considered in some detail.

Sognnaes (1948) lists 19 investigators active in the study of the organic enamel matrix. He states that in 1843 Linderer observed "Bueschel" in ground sections of enamel which later, on isolation and sectioning, proved to be organic structures. On isolation in 1906 (Bodecker) they were named "tufts" and later "whorls" (Williams 1923), each time without knowledge of Linderer's previous description.

Abbott (1887) described a delicate reticulum of organic matter in the enamel, while C. F. W. Bodecker (1878–1879) noted fine organic fibers lodged between the enamel rods, which were probably parts of prism sheaths. Miller (1902) decalcified thin ground sections of enamel and dentin under the coverglass and observed occasionally that

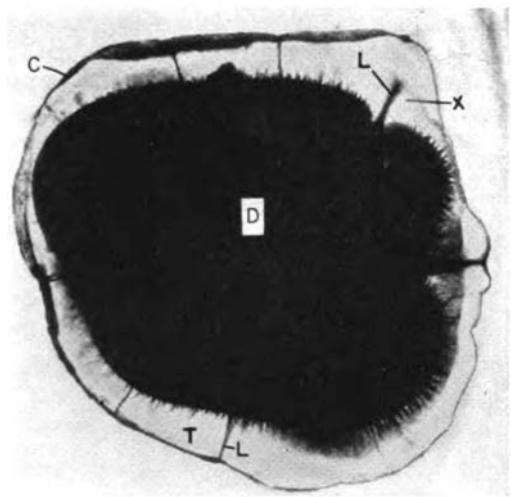


FIG. 7
Transverse section of a molar crown, decalcified under coverglass and photographed with ultra-violet light shows remaining organic structures; enamel cuticle (C); enamel lamellae (L); enamel tufts (T); dentin (D); enamel rod sheaths (X).

"Fibrillenbuendel" (bundles of fibers) remained, connecting the enamel cuticle with the dentin. He also observed intertwining fibers attached to the surface of the dentin, which were interpreted as being composed of uncalcified enamel rods. Without a knowledge of this work, the former structures were named enamel lamellae and the latter, enamel tufts (Bodecker 1906).

Most of the above observations were discredited in 1896 by Williams (1896) and later by Hopewell-Smith (1926) on the ground that only sketches, with no photo-

graphic evidence, were presented by the older investigators. Williams was so adamant in his conviction concerning the completely mineral character of the enamel that he

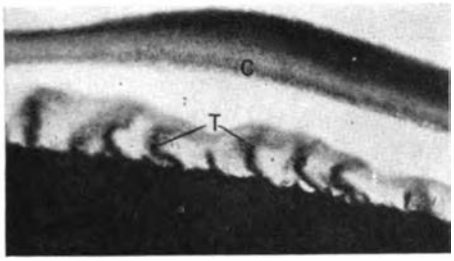


FIG. 8

Section treated in same manner as that in figure 7, shows surface of enamel cuticle with imprints of enamel rods at "C"; tufts (T).

FIG. 9

Enamel matrix, isolated by celloidin decalcifying method; organic matrix appears more profuse at the dentino-enamel junction (D-EJ) and surface of tooth, than in the middle third; dentin had been removed for better decalcification of specimen.

compared it to countless glass rods held together by glass of a lower fusing point. This concept influenced the approach to dental caries research for many years. In 1906 (Bodecker) a special method of decalcification was devised by which it was possible to procure exceedingly thin serial sections of the entire thickness of the enamel matrix, (fig. 9). Three structures were found: the enamel lamellae (previously observed by Miller (1902)), enamel tufts ("Bueschel" of Linderer) and enamel rod sheaths (Bodecker 1906, 1928). Malleson (1924) confirmed these observations in 1924.

Enamel lamellae: Serial sections of the organic matrix showed these structures as "sheet-like processes of organic matter,

FIG. 10

Enamel lamellae (L) and tufts (T) seen in an undecalcified ground section of dentin (D) and enamel (E).

originating at the dentino-enamel junction and passing to the outer boundary of the enamel." Owing to the remarkable similarity of lamellae to cracks in the enamel (Bodecker 1924–1926, 1928; Miller 1902) (fig. 10, L), they had usually been regarded as such. Evidence that these structures are not all cracks was presented by decalcifying ground sections of enamel under the coverglass (Miller 1902). Later (Bodecker 1906) such sections were photographed with ultraviolet light. Figure 11 shows a specimen prepared in this manner with a lamella (L) and the remnant of the organic enamel

matrix (EM) attached to the dentin. This method, however, merely demonstrates the presence of the lamella; it does not permit a study of its structure. The celloidin decalcifying method, which simultaneously embeds and decalcifies the enamel, permits exam-

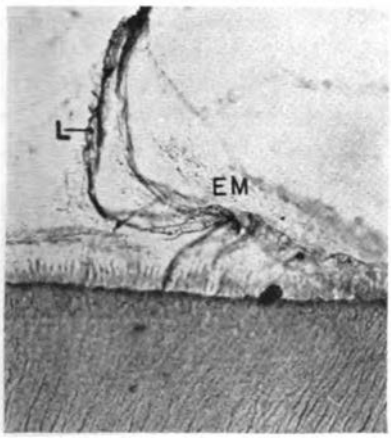


FIG. 11

'Ground section decalcified under coverglass showing lamellae (L) removing all mineral matter from the enamel, leaving the unstained enamel lamella (L); photographed with ultra-violet light; remnant of enamel matrix (EM).

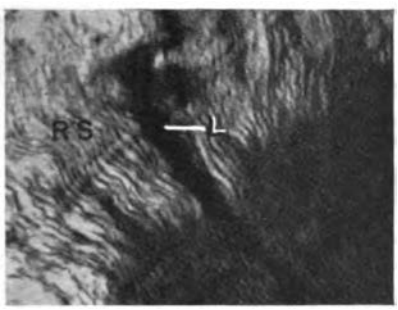


FIG. 12

Organic enamel matrix prepared by celloidin decalcifying method showing organic lamella (L) and rod sheaths (RS) in longitudinal section.

ination and study of the organic enamel structures and the details of enamel lamellae, the enamel rod sheaths and enamel tufts (fig. 12).

According to Orban (1928) there are two kinds of lamellae, one arising from the dentino-enamel border and the other originating from within the dentin.

Enamel rod sheaths: These structures are described as delicate envelopes enclosing the enamel rods (Bodecker 1906). Figure 13 shows them in transverse section under high magnification. The enamel rod sheaths are not always in close apposition with each other; in such cases, a cement substance joins these structures (fig. 13, C).

Enamel tufts: Bodecker (1906) observed a modification of the lamellae, which he named "tuft-like processes" because they appeared in ground sections like tufts of beach grass springing from the margin of the dentin (fig. 14). Beust (1934c) showed the incorrectness of Bodecker's conception of the enamel tufts, in that they do not rise from

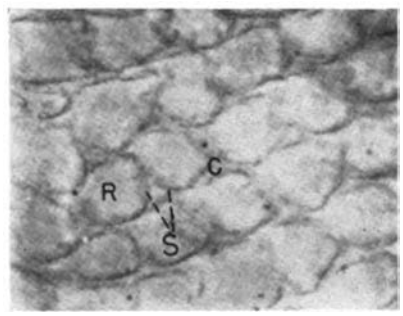


FIG. 13

Organic enamel rod sheaths (S); rods (R) and cement substance (C).

a single point on the dentin but are ribbon-like structures, similar to but smaller than lamellae.

Endings of dentinal tubules in enamel: Figure 15 shows dentinal tubules of various forms passing into the enamel as points, loops and knobs located in the enamel rod sheaths. The enamel spindles, another form of dentinal tubule ending, run obliquely across the course of the enamel rods (fig. 16). They are distributed principally on the cusps of the dentino-enamel junction. Their function is as yet debatable, although some consider them as sensory end organs.

Dentin

Dentin is an elastic, highly calcified connective tissue, fibrous in nature, mesodermic in origin, forming the bulk of the crown and

root of the tooth and giving to each its characteristic form. The tusks of elephants, known to the layman as ivory, are composed principally of dentin. Dentin is made up of a collagenous matrix, permeated by innumerable delicate tubules about 2 microns in diameter, running from the pulp to the surface of the dentin. These are more discernible in ground sections (fig. 2) than in decalcified ones (fig. 3). The number of tubules passing through one square millimeter of dentin varies from 15,000 to 75,000, depending upon their location (Meyer 1935, p. 49). These channels and their state of permeability appear to be important factors in the varying activity of the rate of penetration of dental caries. Further details concerning the microscopic structure of the dentin will be discussed subsequently.

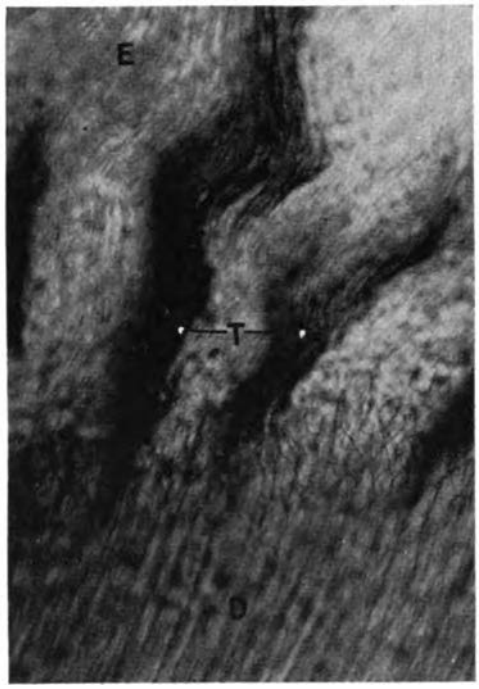


FIG. 14

Ground (undecalcified) section of dentin (D) and enamel (E); enamel tufts (T) arising from dentin and passing into enamel. Branched endings of dentinal tubules at dentino-enamel junction.

Dentin is divided into two types, known as primary and secondary dentin according to the time of its formation and to its structural difference. The distinction in structure of these two types is important in its effect on the rate of carious penetration.

Primary dentin: The tubules in primary dentin run with a great degree of regularity from the central cavity—pulp chamber and pulp canal—to the surface of the dentin (fig. 2). Primary dentin is designated empirically as being formed up to the time of the completion of the young tooth, i.e., of the root end, which occurs about 3 years after tooth eruption, and is usually referred to simply as dentin. It is only when secondary dentin is also under discussion that the word "primary" is added to differentiate the one from the other.

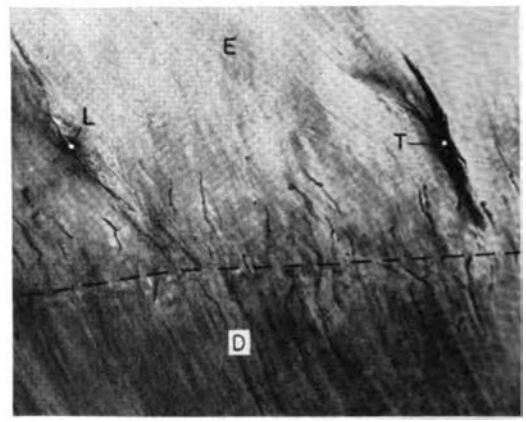


FIG. 15

Dentin (D) and enamel (E) show the passage of many dentinal tubules into the enamel across the dentino-enamel junction (broken line); tufts (T); lamella (L).

Secondary dentin: Secondary dentin is formed intermittently after the completion of the root end, the rapidity of its formation depending greatly upon the chemical and thermal irritation reaching the dental pulp by way of the dentinal fibers lodged in the tubules. The manner of its formation is described under Dentinogenesis. The reduction in the number of dentinal tubules in secondary dentin is of considerable benefit to the

tooth, as this retards the penetration of dental caries. The teeth of some persons do not form secondary dentin as readily as those of others, making such persons more susceptible to the disease. The reason for this difference has not been determined.

A description of the varying structure of secondary dentin is necessary for the comprehension of the protection against caries which this tissue lends to teeth. Hopewell-Smith (1924) recognized various structural differences in secondary dentin and classified them as "physiologic" and "adventitious". The latter type he subdivided into six variations: "alveolar, cellular, fibrillar, hyaline, laminar, tubular, singly or combined". He stated that all adventitious types were subject to the penetration of infection.

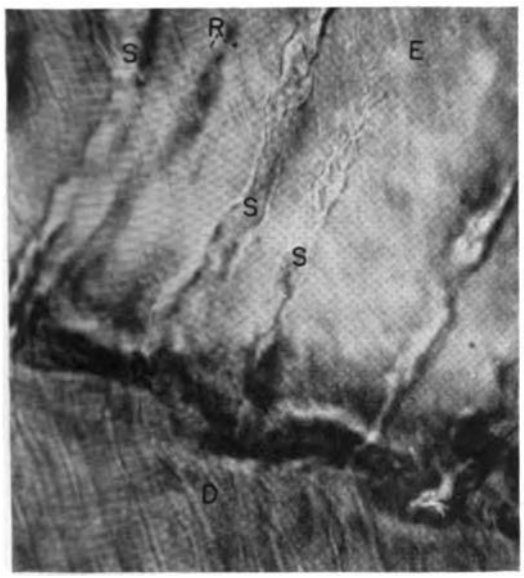


FIG. 16
Enamel spindles (S) arising from the dentin (D) pass obliquely across the enamel rods (R).

Bodecker (1944) simplified this classification, basing it on the structure and physiologic significance of secondary dentin as: "1. Physiologic; 2. protective; and 3. pathologic."

Physiologic secondary dentin: The pulp chamber and pulp canal or canals of a young tooth are large. As the tooth ages, their size, particularly that of the pulp chamber, is gradually decreased by the formation of secondary dentin. (fig. 17, Phy. D). This tissue has been termed physiologic secondary dentin. It is formed more slowly and usually is separated from primary dentin by an incremental line caused by a deviation in the course of the dentinal tubules (fig. 17).

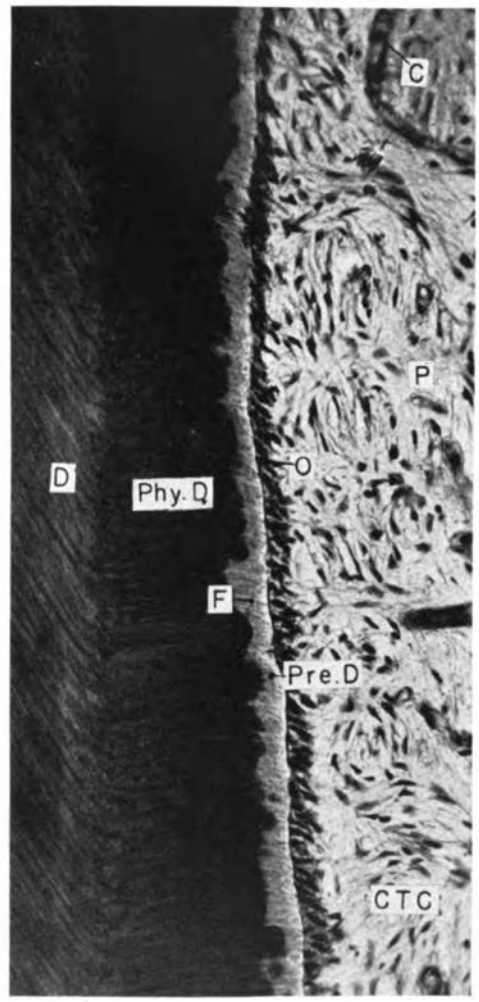


FIG. 17
Physiologic secondary dentin (PhyD) shows a change in the direction of the dentinal tubules from those in the primary dentin (D); predentin (PreD); odontoblasts (O); dentinal fibers (F); pulp (P); capillaries (C); connective tissue cells (CTC).

Protective secondary dentin: Protective secondary dentin may be developed after a certain amount of the physiologic type has been laid down, or it may form contiguous to the primary dentin. Protective secondary dentin reduces the degree of irritation reaching the pulp from the interior, as it has fewer and more irregular tubules (figs. 18, 19). The cause of its formation is external pulpal irritation by way of the dentinal fibers in the primary dentin, such as attrition or caries. Figure 18 presents examples of protective secondary dentin formation resulting from attrition (A) and caries. The incisal edge is markedly worn, so that the original pulp chamber is filled with protective secondary dentin (Pro. D). Cervical caries (C) on the lingual aspect resulted also in the formation of the like type. The labial surface carries a filling (F) which, together with the irritation of previous caries, caused a large amount of secondary dentin formation (Pro. D) which has a somewhat greater number of tubules than that in the incisal area.

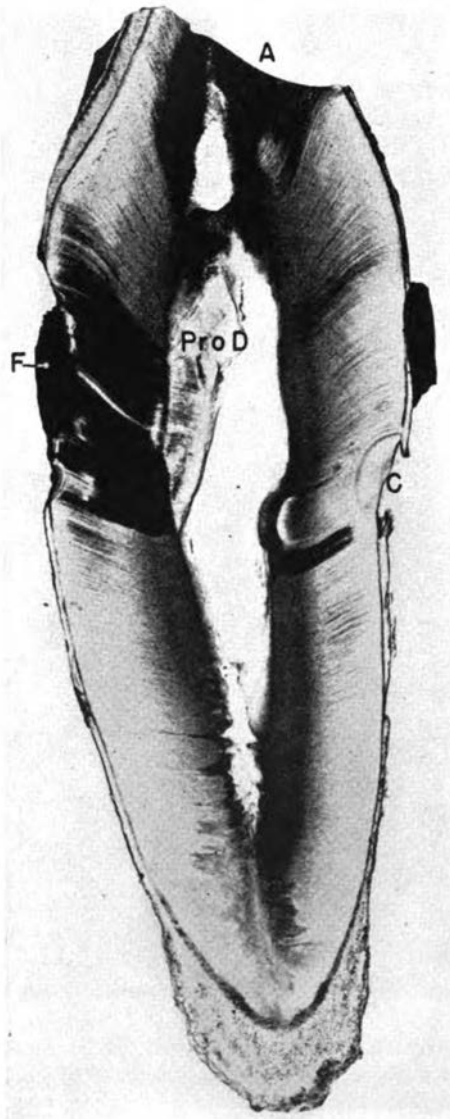


FIG. 18
Protective secondary dentin (ProD) formed as result of attrition (A); caries (C); filling (F).

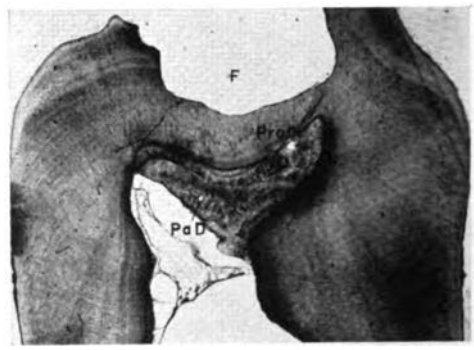


FIG. 19
Deciduous molar with protective secondary dentin (ProD) and pathologic secondary dentin (PaD) filling most of the pulp chamber. F, a large cavity previously filled.

Figure 19 shows a large cavity which previously contained a filling (F). The irritation originating from caries, as well as from the cavity preparation and the filling, resulted in the formation of two types of secondary dentin: protective (Pro. D) and pathologic (Pa. D). The structure of the former shows a reduction in its number of tubules; of the latter, channels of larger calibers, which are discussed below.

Pathologic secondary dentin: This type of secondary dentin likewise has few and very irregular tubules and, in addition, contains

large channels which enclose strands of pulp tissue which, as will be shown, are a menace to the tooth (fig. 20, Pa. D). Subsequently-formed tissue was laid down in this tooth as so many pulp remnants as to appear like osteoid tissue (fig. 21, Pa. D).

The varying degrees of permeability of secondary dentin are important in dental

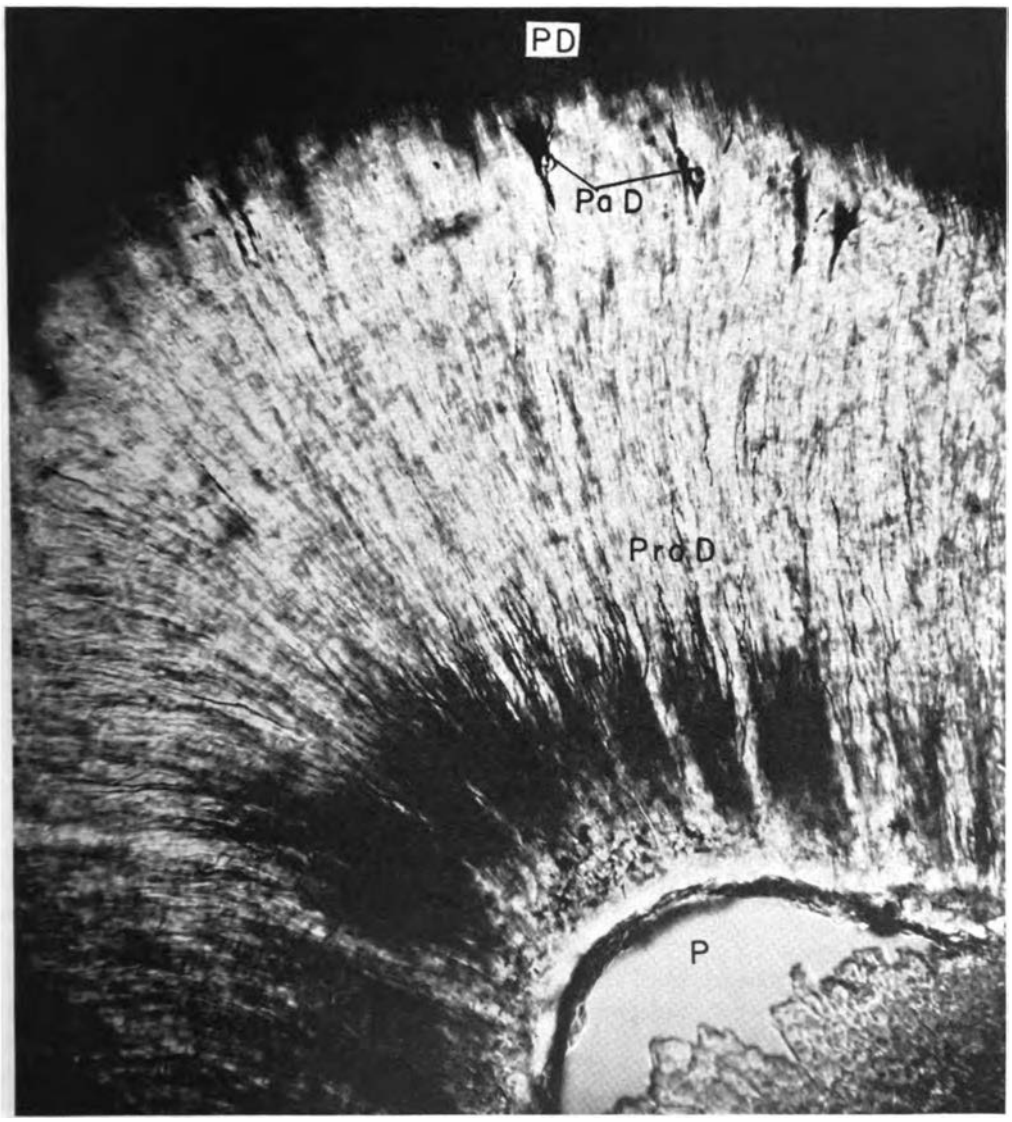


FIG. 20

Both protective secondary dentin (ProD) and pathologic secondary dentin (PaD) under higher magnification; primary dentin (PD); pulp chamber (P).

the protective type (Pro D). The primary dentin (PD) is so opaque as to prevent completely the passage of light. Pathologic secondary dentin may on occasion contain caries activity, as will be discussed subsequently. The physiologic type has almost the same degree of permeability as primary dentin; the protective type has a greatly

reduced permeability; the pathologic type has great permeability due to the inclusion of strands of pulp tissue. Pathologic secondary dentin furnishes little or no protection to the pulp because, when caries or attrition exposes this tissue, microorganisms penetrate its grosser permeable channels and thus cause the death of the pulp.

Gottlieb (personal communication) makes this addition: "Secondary dentin, during its formation, may be disturbed structurally as a result of chemical, thermal, mechanical or bacterial irritants. Under these conditions, the structure is more rapidly formed and, therefore, imperfectly formed. The latter is what is intended to be characterized in the report as protective secondary dentin. With the proper connotations, I believe the classification of physiologic and protective secondary dentin is adequate. The third classification of pathologic secondary dentin is unnecessary. What is described under the heading is a matter of degree of disturbance resulting during formation in a greater irregularity of structure".

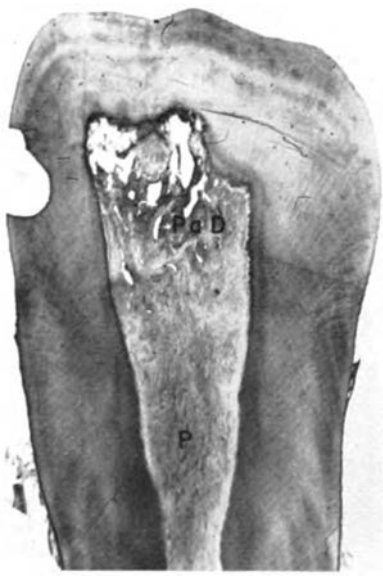


FIG. 21
Pathologic secondary dentin (PaD) which includes strands of pulp tissue; pulp (P).

The purpose of the third classification of pathologic secondary dentin is to differentiate the harmful effect of this tissue on the welfare of the pulp from that of the "protective" type.

A suggestion is also made by Gottlieb (personal communication) to distinguish between "young" and "old" primary and secondary dentin. This subject is discussed under Protective Metamorphosis of Dentin.

The most common types of secondary dentin are the physiologic and the protective, while the pathologic is rare. Neither of the two classifications (Bodecker 1944; Hopewell-Smith 1924) is in general use. The latter appeared only in a textbook, but a more detailed paper is in preparation. Some further details concerning the dentinal tubules and their varying permeability, necessary for the comprehension of the physiologic activity of dentin and its reaction to caries, will be discussed subsequently.

The Dental Pulp

The dental pulp, popularly called "nerve" on account of its acute sensitiveness, occupies a central cavity or pulp chamber and canal or canals of the tooth (figs. 3, 17, 22). It is composed of embryonic connective tissue, rich in blood vessels and nerves. Its vital function is the formation of primary and secondary dentin, the furnishing of the necessary products for the continued calcification of this tissue and the young enamel in the newly erupted teeth. The sensory function of the pulp is to respond to chemical, thermal and traumatic irritation.

A layer of highly differentiated and specialized connective tissue cells, the odontoblasts (fig. 22, O), columnar in form, with an oval nucleus, cover the surface of the dental pulp. Each cell sends a cytoplasmic process into a dentinal tubule, known as the dentinal fiber or Tomes' fiber. The function

of the odontoblasts will be discussed in the section on the physiology of the dentin and enamel.

The dental pulp is described by Noyes, Schour and Noyes (1938, pp. 146, 147) as "an extremely vascular tissue, and the arrangement of the vessels, the structure of their walls, and the nature of the intercellular substance through which they run render the tissue especially susceptible to pathological conditions which are associated

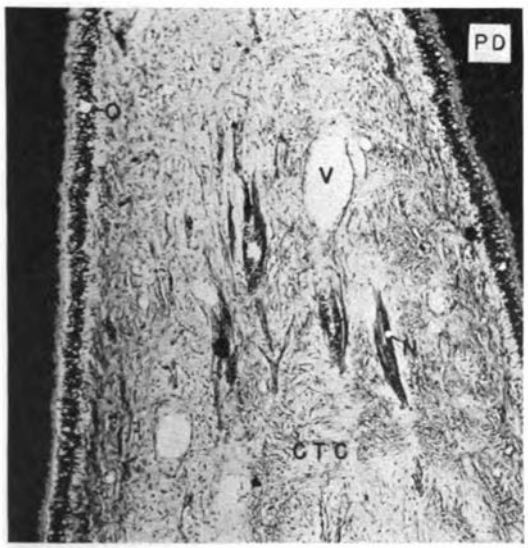


FIG. 22
Dental pulp, odontoblasts (O); primary dentin (PD); blood vessels (V); nerves (N); connective tissue cells (CTC).

with alterations in the circulation. . . . The delicacy of the walls of the blood vessels is one of the most striking histological characteristics of the dental pulp. This peculiarity renders the tissue unusually susceptible to hyperemia and inflammation." The intense pain of odontalgia (toothache) during pulpal inflammation is caused by the heightened blood pressure on the nerve fibers of the dental pulp confined within the unyielding pulp chamber.

Nerves of the dental pulp: According to Noyes, Schour and Noyes (1938, p. 149), "few subjects in connection with dental histology have received more attention than the distribution of the nerves in the dental pulp, especially in relation to the

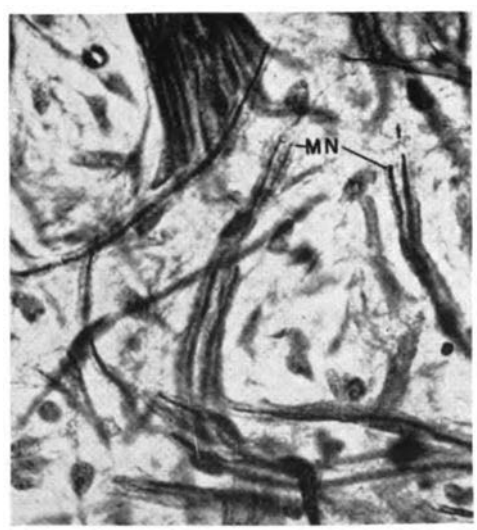


FIG. 23 (above)
Nerve trunk (N) in dental pulp.
FIG. 24
Medullated nerve (MN) in dental pulp.

sensitiveness of the dentin [p. 151]. . . . Usually three or four nerve trunks enter the dental pulp through the foramina which contain eight to forty medulated nerve

fibers". Bodecker (report in preparation) shows that well stained specimens of the pulp contain a profuse supply of nerves (figs. 23, 24). The reason for the abundance of nerves is puzzling, because this organ has no contact with the exterior except under pathological conditions, when exposed by dental caries. The purpose of these abundant nerves after the completion of the tooth is not apparent unless their presence is necessary for some as yet obscure function related to the physiologic activity of the pulp. Further work on this subject seems indicated.

The innervation of the dentin is still controversial. Some workers (Dependorf, Toyoda, Mummery, Morgenstern, Ambrose and others) show structures resembling nerve fibers in the dentinal tubules, which, if present, would explain the intense pain occasionally caused by dental operations; others regard these as artifacts. In the absence of evidence that can be duplicated by others, showing nerve fibers in the dentin, the dentinal fibers are regarded by many histologists as the structure which transmits sensation. This view is weakened somewhat by the fact that nowhere else in the body do connective tissue fibers (mesodermic) take on the function of nerves which are of ectodermic origin.

PHYSIOLOGY OF THE DENTIN AND ENAMEL

Investigations during the last decade indicate that teeth are affected by nutritional disturbances and by fluorides, as well as by other factors. These investigations, discussed in other sections of this monograph, hold out some hope that at some future date dental caries may be prevented on a large scale by other than reparative means. The only preventive treatment instituted generally for the protection of the teeth against destruction is oral cleanliness and impregnation of the enamel with a fluoride for the purpose of making this structure less soluble. The benefit of fluoride in drinking water used during tooth formation is discussed in a separate section. The fluorine content of the enamel, however, does not seem to be the complete reason for caries immunity. Clinical observations show that certain persons may have a caries immunity for 10 or more years and then suffer a relapse. If this long immunity to caries is due to the fluoride content of the enamel, then it follows that this chemically bound fluoride must have been removed. This does not appear likely. Hence, even though fluoride may give a certain protection, it does not appear as the complete explanation of caries inmunity.

Systemic conditions are believed to have no effect on the enamel because of the fact that its formative organ is destroyed when the teeth erupt. For this reason only symptomatic treatment, repairing the damages caused by dental caries, is used in combating this disease. This view is commonly held by the majority of the profession as well as by many investigators. However, the fallacy of the conception will be discussed under Enamel Calcification. Evidence is accumulating that dentin and enamel receive some products necessary for their maturation from the pulp after tooth eruption; therefore a disturbance of systemic conditions may affect the teeth from within. Too little is known of the histopatholgy of the dental tissues and still less of the physiologic activity of the pulp, dentin and enamel, as well as of the possible bearing this physiological activity may have on dental caries. Further knowledge must be obtained through investigations in dental physiology, a new, vast field for future research.

There is no evidence of any specific disease being constantly linked with dental caries. Many observations of the beneficial effects of optimal diets on dental health are counterbalanced by those of Day (1944c), Schour and Massler (1947) and others, who show a reduction in dental caries in malnourished people. These contrary observa-

tions indicate the existing confusion and show that much work remains to be done before reaching a solution of the problem.

The manner in which teeth are developed must be considered in order properly to evaluate the erroneous belief that enamel is segregated from the dental pulp after tooth eruption. Enamel calcification of the first formed teeth, the deciduous set, begins at the 16th week of intra-uterine life and progresses outwardly, locating the enamel organ on the surface of the tooth (fig. 25, EO). After completion of the enamel, its formative organ undergoes atrophic degeneration before the tooth crown pierces the gingival or gum tissue to become a functioning unit in the oral cavity (fig. 25–3). Its remnant forms a part of the enamel cuticle, which, being cut off from all blood supply, can no longer have a vital function. Thus it seems apparent that the enamel is an inert structure. It is for this reason that a large group of investigators believe that systemic disturbances cannot affect the enamel after tooth eruption and that, therefore, enamel of fully formed teeth can be influenced only from the exterior, i.e., by food retention, by bacteria present in the oral cavity and by the saliva. This concept has long been a barrier to the investigations of the possible predisposing factors of dental caries which may affect the enamel from within.

The question, therefore, of whether or not fully formed dental enamel supports slight metabolic activity which may affect tooth resistance seems highly important. If metabolic activity is absent, then the sole possibility of preventing dental caries is oral cleanliness, improvement in the quality of the saliva, or the more recently advocated impregnation of the enamel with fluoride. On the other hand, if certain phases of metabolism are present, future investigations will be focused more intensely on possible systemic factors which may affect the teeth and thus predispose them to caries. The effort is made in this report to present detailed evidence concerning changes in enamel

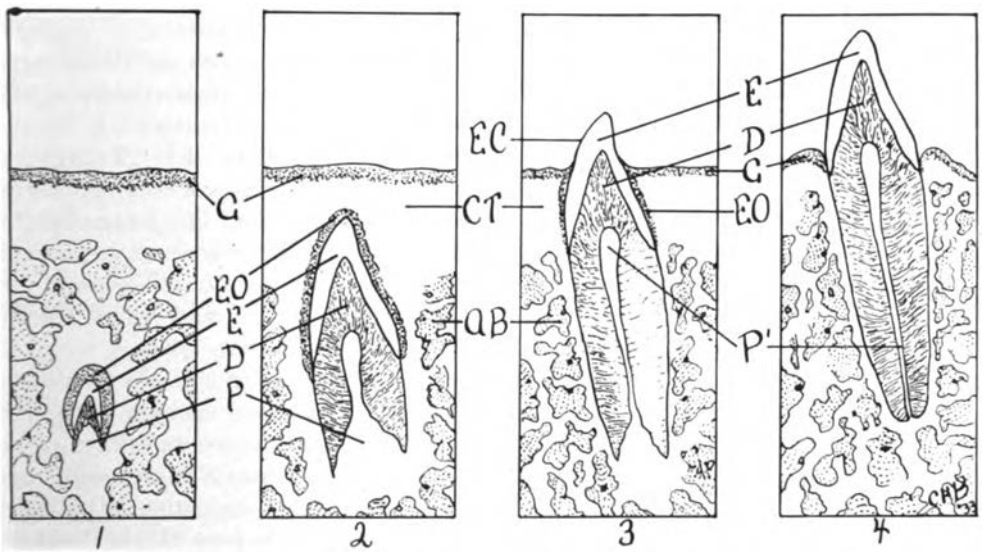


FIG. 25

Diagram showing destruction of enamel organ (EO) during tooth eruption; papilla (P) which later forms the pulp (P'); dentin (D); enamel (E); gum or gingival tissue (G) covering the alveolar bone (AB). (Courtesy: Nelson New Loose-Leaf Medicine, Chapt. I-A, pp. 15–20G, Fall, 1935.)

and dentin which appear to be of a metabolic nature, as well as to define the paths by which a tissue fluid permeates dentin and enamel. The observations of those holding a contrary opinion will be mentioned, although, since they are well known, they will be considered briefly.

Consideration of Metabolic Activity

The first step in presenting the physiology of the dental tissues is the definition of the term "metabolism" as related to this subject. Halliburton (1913) defines metabolism as the sum total of chemical changes that occur in living tissues. This definition would not apply to changes occurring in the enamel, because it does not appear as a living tissue. Churchill (Meyer 1935, p. 42) discusses metabolism in considerable detail as follows: "We recognize vitality in the body cells by their performances of functions we conventionally regard as characteristics of life. However, we find it difficult to accept the view that these cells possess a monopoly in regard to such a vital phenomenon.

"We might add: Is metabolism limited to the cellular elements of the body? It is important to point out that, to some extent everywhere in our tissues, chemical changes occur which in a broader sense may be called metabolic. The body fluids, such as the lymph, carry the products of cell activities to and from the intracellular substance, adding and subtracting according to osmotic and utilitarian conditions. This fact, together with the interdependence of cells and ground substance, broadens our concept of vitality. In this wider concept, anabolism, which is a synthetic process, and catabolism, which is analytic in nature, are factors which, in addition to intercellular processes, are components of metabolism. . .

"Karlstroem's researches incline us to believe that changes in human enamel do occur. Fish found occasionally a small degree of penetration of methylene dye into the enamel from within. Whatever tissue fluid he observed in the enamel is always confined to the prism sheaths. Sprawson has shown the presence of what he terms 'vital fluids' in the enamel. . . .

"Although certain facts point to the probability that in a broad sense enamel partakes of metabolic changes, such changes have in our estimation not yet been incontrovertibly proved. Whatever processes in the broadest physiologic sense do occur must be extremely slow in this densest of human structures. Experimental evidence points to changes brought about from within rather than from without. . . .

"Summarizing, we may say that the question of vitality of the hard structures of the teeth is decided by definitions.

"Those who believe that vitality is dependent on the presence of phenomena characteristic of cell life as metabolism, respiration, growth, regeneration, reproduction, irritability, contractility and conductivity, naturally would regard the hard tooth structures as non-vital.

"However, those who would consider the physical and chemical changes in the intercellular substance as representative of vital activities in a broader sense, will regard enamel, dentin and cementum as participating in the vital process of the body. We tend towards the latter point of view."

The controversial question of metabolic activity in the calcified dental tissues can also be approached from the clinical angle. It may be judged by determining the effect on dentin and enamel of removing the dental pulp from young teeth. If this operation has an effect, then dentin and enamel may be regarded as being supplied with a tissue fluid necessary for their maintenance; if no effect is noted, then these structures are inert and cannot be influenced after the eruption of the teeth.

Examination of the following evidence of the presence of a tissue fluid should give the answer to the question whether or not dentin and enamel support a low degree of metabolism, judged on the basis of the definition set up by Churchill.

Two methods have been used to determine the possible connection of dentin and enamel with the blood stream: (1) isotopes and (2) vital staining. These methods complement each other in the specific use to which they are put in this problem. Isotopes (Hevesy 1939; McCauley 1935) introduced into the blood stream are subsequently defined in pulverized samples of dentin and enamel, demonstrating that these structures receive some products from the blood. Vital staining, the far older method, has the advantage of defining the exact areas in the dentin and enamel and the precise paths through which the tissue fluid passes. This in itself gives more valuable histo-physiologic data. The use of isotopes, on the other hand, is simpler and has corroborated observations made by the vital staining technic.

Amelogenesis and Calcification of Enamel

A brief analysis of the mode of enamel formation is essential for the comprehension of the physiology of this structure.

Enamel formation is a complex phenomenon which has recently been investigated again by Diamond and Weinmann (1940), Gottlieb (1943), Saunders, Nuckolls and Frisbie (1942), Orban (1928) and Orban, Sicher and Weinmann (1943). Details need to be given on only the one phase of the subject which has a bearing on the physiology of the enamel as related to the problem of dental caries. It concerns the source of the material necessary for the calcification of the enamel and the information as to whether or not the supply persists after tooth eruption.

The enamel organ is regarded as the formative organ of this structure, which functions by building the organic matrix and furnishing the elements necessary for its calcification. As already stated, the atrophy of the enamel organ during the eruption of the teeth makes impossible any later anatomical change in the enamel. The fact must be emphasized that no additional enamel can be formed after the degeneration of its formative organ. However, the post-eruptive calcification or maturation of the enamel, observed by hardness test and clinical observations, gives evidence that physical changes do occur in enamel after its anatomical completion. Karlstroem (1931) using the Herbert pendulum hardness tester, showed that the enamel of fully matured teeth is markedly harder in the *inner zones* than that of newly erupted teeth. It is significant that the surface hardness of the mature and immature enamel is nearly the same, suggesting that the source of the mineral salts necessary for the post-eruptive calcification is in the pulp and not in the saliva.

Head (1910), Pickerill (1912, p. 113), Bunting and Rickert (1918) and Fish (1931), all consider that the inorganic material which causes the continued hardening of the enamel after tooth eruption originates from the saliva. Bunting and Rickert, discussing the permeability of enamel, conclude: "Enamel and dentin are porous . . . and admit the passage of salts and solvents of saliva to the pulp, and blood to the exterior." Fish (1932), in staining vital teeth of a dog, used, among other reagents, an aqueous solution of methylene blue under crowns. He observed that the stain persisted on the surface of the enamel for several days and deduced that the enamel was permeable from without inward. His report, however, does not include the important examination of sections of these experimental teeth showing actual penetration of the dye.

On the other hand, Leigh (1925), Turner (1929), J. L. Williams (1928) and Lefkowitz and Bodecker (1938) oppose the theory that saliva penetrates the enamel. Williams questioned the premise that the physiological function alleged to take place in a living tooth is identical with a chemical phenomenon occurring in a dead tooth outside the mouth. Turner, in disagreeing with the theory of porosity in enamel, states: "Since horny epithelium is in all other parts of the body a protective layer, we may conclude that, at least, it was not nature's original

plan to place any dependence on post-eruptive hardening of the enamel by means of lime salts derived from the saliva."

Whether or not enamel is permeable from without is important in defining the physiologic activity of this structure. Evidence will be presented below that it is permeable only from within. It would seem rather absurd that saliva or any of its products could penetrate normal enamel and reach the pulp.

Enamel, a calcified epithelial structure, has the same embryonic analogy as the skin. A consideration of this similiarity presents an interesting analogy. Discussing the permeability of skin, Starling (1936) states: "It may be regarded as established that the uninjured skin is impermeable to watery solutions of salts and other substance. On the other hand, it is possible to produce a certain amount of absorption of substances dissolved in fatty vehicles." Sollman (1936) reports that the absorption of fatty substance occurs only through the hair follicles; therefore, we may consider that the epidermis is impermeable from without, unless hair follicles are present.

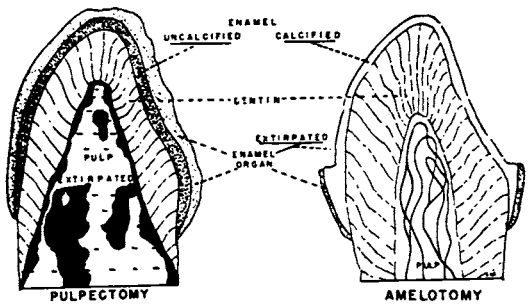


FIG. 26

Diagram showing the result of pulpectomy and amelotomy on unerupted, partially formed teeth.

Lefkowitz and Bodecker (1938) state: "The enamel, resting upon the dentin of mesodermic origin and receiving from it the dental lymph (tissue fluid) is comparable to the stratum corneum of the skin, being supplied by an interstitial fluid from the underlying mesodermic tissue. Both enamel and the stratum corneum are permeable from within but not permeable from without, classifying both tissues as uni-directional membranes (semi-permeable)." Evidence of this statement is presented below.

The permeability of the enamel from the exterior was tested experimentally on dogs and man (Lefkowitz and Bodecker 1938). The saliva of dogs was continuously stained for a period of 3 weeks. This was made possible by placing compressed potassium permanganate tablets in perforated receptacles, attaching them to gold crowns, and cementing them to the posterior teeth. In this manner the saliva was stained a purple brown color for the entire duration of the experiment. The teeth in the immediate vicinity of the dye-bearing receptacles were deeply stained, but sectioning showed that the stain was limited to the surface of the enamel. Experiments on teeth of man gave like results; no stain penetrated the enamel unless previous surface decalcification abnormally established permeability. Since these observations indicate that the saliva is not active in affecting the continued calcification of enamel, the following experiments (pulpotomy and amelotomy) were carried out to determine whether or not the pulp is responsible for this change.

Pulpotomy: Lefkowitz, Bodecker and Shapiro (1944) eliminated the pulps from partially formed, unerupted teeth, so as to ascertain the possible function of this organ in relation to enamel calcification (fig. 26). For the first experiments, young cats were selected in which the enamel was still in the matrix state (Chase 1935), i.e., calcified only about 33 per cent instead of 96–99.5 per cent, as it is in enamel of matured teeth. The two animals were sacrificed 3½ and 7 months after the operation. Histological sections showed that the enamel had remained in the matrix state, i.e., had not continued to calcify, as had that in the control teeth on the other side of the mouth. Because of the fact that the operation of removing the pulp might have disturbed the function of the enamel organ and thus pre-

vented the continued calcification of the enamel, the experiment was reversed, i.e., amelotomy was performed instead of pulpotomy.

Amelotomy: This operation (Lefkowitz, Shapiro and Bodecker 1947) consists in partially removing the enamel organ from the incisal half of the crown of incompletely formed, unerupted teeth of dogs. Figure 26 shows the effects of both pulpotomy and amelotomy. The success of the amelotomy operations was demonstrated by sacrificing one experimental animal immediately after the operation. No enamel epithelium was present, demonstrating that the complete enamel organ had been removed from the operated area. The possibility of trauma, disturbing the physiologic function of the tissues, also was determined by sacrifficing one of the animals 2 weeks after its operation. Examination of the sections showed that all tissues had returned to normal, proving that the results observed in the enamel of the experimental teeth were due to the removal of the enamel organ.

Twenty-four weeks after amelotomy, ground sections were made of two experimental teeth for histologic study. Those areas of the teeth from which a part of the enamel organ had been removed showed hypoplastic but calcified enamel. Preparation of the soft tissue sections of the other operated animals is not yet complete. They may throw further light on details of enamel calcification.

Even though the number of animals in these experiments is small, the fact that enamel calcification occurred after the removal of the enamel organ suggests that the enamel is dependent on the pulp for its completion.

One reviewer (Diamond, personal communication) states that the "pulpotomy and amelotomy experiments regrettably draw conclusions based upon erroneous basic knowledge". He points out that enamel development occurs in two states—formation and calcification—and further that the enamel matrix in the first stage contains approximately only one-third of the total mineral elements. He states that this "calcification stage has its own path, separate and distinct from the path of incremental formation."

It was with this basic knowledge that the pulpotomy and amelotomy experiments were set up for the particular purpose of determining the source and path of the mineral salts for the completion of enamel calcification. These have been shown to be a product of the dental pulp passing through the dentinal tubules into the enamel matrix.

Diamond (personal communication) disputes this observation and states, "Assuming that in the pulpotomy experiments, calcification (secondary) of the enamel did not occur, the conclusion drawn that therefore the pulp is responsible for the calcifying materials of the maturation of the enamel is not *ipso facto* and is unwarranted. The source of calcifying materials for the maturation stage of enamel is still one of the important but unanswered questions. Diamond and Weinmann have suggested that the high vascularity immediately surrounding the enamel matrix after the reduction of the enamel epithelium may be the direct source. Numerous factors in the evidence so far accumulated would mitigate against the pulp playing the role."

The question is: why then did the enamel matrix fail to calcify after pulp removal if the pulp is not a factor in the second stage of calcification?

The results of these amelotomy and pulpotomy experiments throw light on the clinical observation that the removal of the pulp from recently erupted teeth causes brittleness of the enamel and a darkening of the teeth. They also suggest that pulp removal cuts off prematurely the tissue fluid supply necessary for the maturation of both dentin and enamel. It is a common clinical observation that pulpless teeth, particularly those in which the pulps have been removed early in life, are most prone to fracture.

Dentinogenesis

A discussion of dentin formation is essential to the subject of metabolic activity in this tissue. The odontoblasts of the dental pulp, as their name implies, were considered as dentin-forming cells. Von Korff (1928), however, showed that the dentin matrix is derived, not from odontoblasts, but from other cells of the pulp, either from fibroblasts or amorphous intercellular substance (Orban, Sicher, and Weinmann 1944, p. 120). Hence it seems evident that the odontoblasts are not concerned directly with the formation of the dentin matrix although their presence seems necessary to initiate matrix formation. Orban (1929) has established this phase of dentinogenesis.

Gottlieb (1946) recently explained that external irritation does not stimulate the odontoblasts of the pulp in the formation of secondary dentin but, on the contrary, tends to cause their destruction and thus allow the underlying connective tissue cells to deposit dentin matrix more readily. This observation further suggests that the odontoblasts are not concerned with dentin formation.

The precise manner in which dentin is calcified is not as yet completely clear. The work of Bevelander and Johnson (1946a) throws light on this subject. They examined dental pulps to determine the presence of alkaline phosphatase, which is related to the calcification of bone. They state: "Within the past decade, methods have been developed which now permit the histochemical localization of the phosphatases (1946b). Since these methods have been developed, considerable literature has appeared which deals with histochemical localization of these enzymes in various tissues and organs.

"In regard to the localization of phosphatase in teeth, Robinson and Somes (1924) reported considerable amounts of this enzyme in the rapidly growing teeth of young rodents. Kabat and Furth (1941) illustrated sections of the enamel organ of the rat, in which phosphatase also appears to be present in several of the components. Horowitz (1942) contended that alkaline phosphatase is present in both ameloblasts and odontoblasts of young teeth. Gomori (1943) likewise remarked on the presence of this enzyme in the various components of the developing tooth. Finally, Bevelander and Johnson (1946b) described the histochemical localization of alkaline phosphatase in the developing tooth of the pig and showed among other things that this enzyme is present in odontoblasts before and during early calcification of dentin."

They conclude (1946a):

"1. During the differentiation of the odontoblast, alkaline phosphatase comes to be localized within the nucleus, the cytoplasm, and the dentinal fibril just before and during the early calcification of dentin.

"2. In older dentin, this same situation holds true and, in addition, Tomes' dentinal fibril is also strongly phosphatase positive.

"3. On the basis of the well-known function of phosphatase in connection with normal calcification, we can now state with a fair degree of certainty that the function of the odontoblast and Tomes' fibril is to a great extent biochemical—to produce an immediate phosphatase transfer which in turn leads to the splitting of the phosphatase in the very region in which we know it is deposited."

Permeability of Dentin

The varying permeability of dentin and enamel to fluid penetration from the interior as well as the exterior is important in comprehending both the exogenous and endogenous factors of dental caries activity. The permeability of the dentin will be discussed before that of the enamel, as dentin is the path through which the tissue fluid reaches the enamel. The dentin matrix, or basic substance, contains innumerable dentinal tubules and a profusion of lateral branches (fig. 27). In these are lodged the dentinal

fibers, the vital projection of the odontoblasts of the dental pulp. One of the first proofs of the permeability of dentinal tubules during life was presented (Bodecker 1894) by the observation of an oxide of an amalgam filling diffusing into the adjacent dentin, which outlined these channels precisely (fig. 28).

The degree of physiologic activity in dentin is affected greatly by the character of the odontoblastic process enclosed in the dentinal tubule, i.e., whether it is a fiber or a tube.

Orban *et al.* (1944, p. 103) state: "The odontoblast processes are solid protoplasmic extensions of the cells, with a denser and

FIG. 27

Dentinal tubules (T) with a profusion of lateral branches (LB) stained vitally with silver nitrate, shows channels for distribution of tissue fluid (dental lymph).

slightly deeper-staining outer layer. The structural difference between the outer layer and the core has led to the interpretation of the odontoblast processes as tubes. Agreement on this point has not been reached as yet." The view that the dentinal fibers are solid is not shared by Hanazava (1917), Bodecker (1914, 1922) and Wolf (1931). According to Bodecker (1922) their commonly observed appearance as fibers is due to the coagulation of the fluid content within the processes by the fixative used as the preservative of the specimens. He shows that when fresh dentin fragments are washed with pyridin *in vacuo* before fixing, the fibers appear as delicate tubes (fig. 29). Wolf (1931) describes the dentinal fibers as hollow, filled with a fluid or semi-fluid protoplasmic content. Bodecker (1922) and Fish (1932, p. 30) describe a capillary space between the fiber and dentinal tubule and

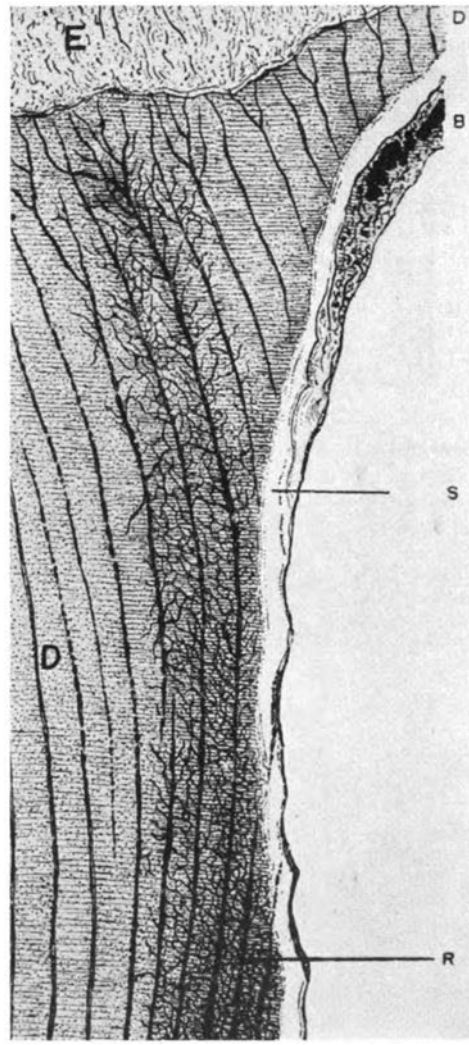


FIG. 28

Silver oxide of an amalgam filling in a vital tooth diffusing through neighboring dentinal tubules and lateral branches (R); dentin (D); enamel (E).

suggest it carries lymph, but other authors consider this space to be due to shrinkage. Fish (1927) further observed that the dentinal tubules are channels through which flows a stream of lymph of sufficient volume

to carry solid pigment particles such as India ink one-third of the distance into the dentin from the pulp.

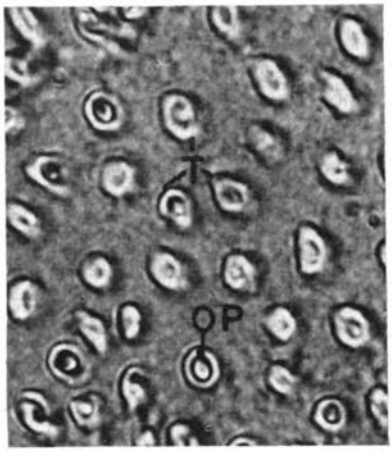


FIG. 29

Dentin treated with pyridine in vacuum and photographed with ultra-violet light, shows tubular character of odontoblastic process (OP); dentinal tubules (T).

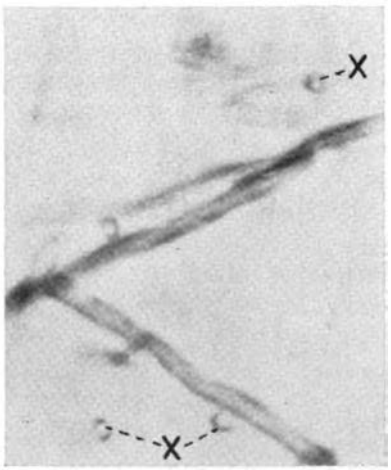


FIG. 30

Odontoblastic processes (X) shown in transverse section isolated from dentin by acid and previous heat treatment.

More recently, Bodecker (1947) isolated processes of odontoblasts, which had the appearance of tubes. These specimens were prepared by an incineration-decalcification method, an odd and apparently illogical technic. Even so, it shows that the periphery of the odontoblastic process is composed of a substance highly resistant both to acid and to heat, which may be of interest in relation to tooth resistance against caries. With the purpose of demonstrating the apparent indestructibility of the dentinal fibers, a brief description of the technic follows: Ground sections of dentin were incinerated (1100 degrees F.) for 2 minutes, which was sufficient to volatalize only the easily destructible organic elements; then the specimens were subjected to a very small quantity of 25 per cent nitric acid under the coverglass, which destroyed the greater part, but not all, of the mineral elements of the dentin. However, decalcification was incomplete, leaving a residue of brittle, highly resistant dentinal fibers or odontoblastic processes which appear tubular in character (fig. 30).

The tubular character of the odontoblastic process is not generally accepted. In spite of this, the available evidence on the subject has been presented because of its possible bearing on the physiologic activity of the dentin, which, in turn, may be related to the resistance of the teeth to caries.

Fish (1932, p. 49) summarizes: "There are reasonably consistent figures in the analyses of human premolars to indicate that there is a physiological addition of calcium to the preformed dentine after eruption of the tooth of the order of 4.3 per cent of the amount present at eruption. This is sufficient to indicate the occurrence of some degree of metabolic change in the dentine.

"The calcium content of the unaffected dentine of teeth which have become carious is normal.

"It has not been found possible to modify the calcium content of the dentine either by parathyroidectomy, calcium-deficient diet, pregnancy, or feeding on therapeutic or excessive doses of vitamin D; nor did two dogs with extreme natural softening of the bones show any loss of calcium from the dentine."

Permeability of Enamel

The determination of enamel permeability from within is important in establishing the entrance of body fluids as well as the possibility of physiologic reactions to external irritation. There is no doubt of the permeability of enamel in the teeth of the oldest existing marsupials; here all dentinal tubules pass into the enamel and terminate near its surface. Figure 31, a photomicrograph of a ground section of a tooth of an opossum, shows the enamel (E) containing as many tubules as does the dentin. The fact that there is an obvious vital connection between dentin and enamel in this, a lower form of mammal, is evidence that in these animals the enamel continues to receive some products from the blood after tooth eruption. By defining an oxide of an amalgam filling in vital teeth, Applebaum (1929) showed, similarly, that dental enamel is permeable from within. Furthermore, Howe (1926) observed enamel permeability during life by demonstrating the presence of lead in the enamel tufts in the teeth of a person who died of lead poisoning. It is clear, from an histological aspect, that the lead was deposited in this tooth from body fluids and not by the saliva, as it appeared in the enamel near the dentino-enamel junction and not near the surface of the tooth. Pickerill (1912, p. 93) states that the permeability of enamel is not entirely developmental but is largely acquired after tooth eruption. Inasmuch as amelotomy experiments, previously described, show that the enamel continues to calcify after tooth eruption, it seems evident that the dental pulp is concerned with the changes in permeability of enamel of fully developed teeth.

Function of the Organic Enamel Matrix

The organic matrix serves as a framework for the mineral elements of the enamel during its formative stages. On completion of the enamel and the eruption of the tooth, enamel permeability is slowly reduced as shown below. Evidence will be presented of the presence of a tissue fluid originating from the dentin and permeating the enamel by means of the organic matrix. Beust (1912) first stained enamel *in vitro*. Bunting and Rickert (1918), Klein and Amberson (1929) and Applebaum (1929) demonstrated the permeability of enamel *in vitro* by electo-endosmotic means. Vitally stained enamel was demonstrated by Fish (1925), Prinz (1928), Bertram (1934), Bodecker and Lefkowitz (1937, 1938, 1941, 1946) and Lefkowitz (1943). Beust (1912) on the basis of a special *in vitro* staining technic, long considered that an increase in mineral matter occurs in the teeth after eruption. This seems to be corroborated by Karlstroem (1931) who offered evidence derived from hardness tests which indicated that enamel continues to calcify after the eruption of the tooth.

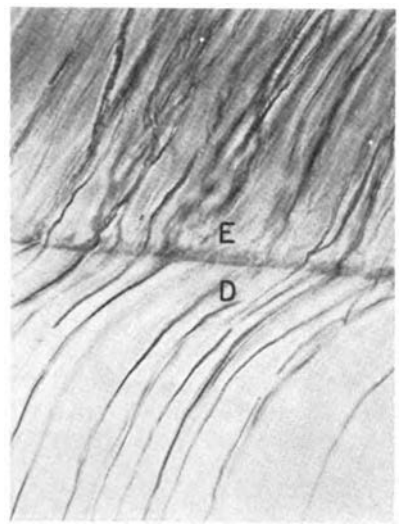


FIG. 31

Dentinal tubules pass from dentin (D) through enamel (E) in teeth of opossum.

Structural changes, which occur in both dentin and enamel as a result of caries in vital teeth, are absent in pulpless ones (Kronfeld 1933, p. 94). This observation demonstrates that the pulp is instrumental

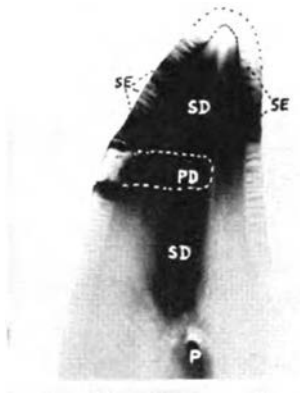


FIG. 32

Potassium permanganate placed in experimental cavity (PD) in tooth of dog for seven days, was dissolved by tissue fluid, staining dentin (SD), enamel (SE) and penetrated afferently to the pulp (P) and efferently to the surface of the tooth.

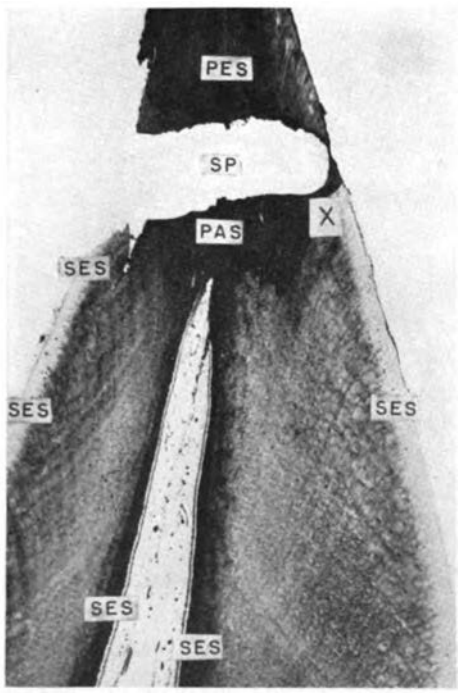


FIG. 33

Powdered potassium permanganate placed in a stain pocket (SP) of a lateral incisor of a 17-year-old person; dye dissolved by tissue fluid, passed inward to the pulp (primary afferent stain PAS). This organ then distributed the dye (secondary efferent stain SES) to the surface of the tooth.

in effecting these changes, a subject further detailed in the section on dental caries.

Tissue Fluid Movement in Dentin and Enamel

The two-directional *in vivo* movement of fluid in dentin and enamel was demonstrated by Fish (1927), Bodecker and Lefkowitz (1937), and Lefkowitz (1943). The technic used by these investigators was fundamentally the same, i.e., an experimental cavity was drilled into the enamel and dentin of sound, vital teeth and a dye sealed in with dental cement (fig. 32). Bodecker and Lefkowitz used dry dyes instead of solutions, for a duration of from 6 minutes to a number of weeks, and have reported findings (1946) on 224 vitally stained human and animal teeth. The condition in pulpless teeth was compared with that in vital ones, showing a striking difference in stain distribution. This they consider an indication that pulp vitality affects both dentin and enamel, a subject discussed under pulpless teeth.

Ground and decalcified sections of the vitally stained teeth of dogs and man were examined and showed that dry dyes placed in experimental cavities are dissolved by a tissue fluid in the dentin and transported to the pulp and the surface of the enamel (1937). Figure 32 is a photomicrograph of a ground section of a dog's tooth, stained *in vitro* with potassium permanganate for 7 days. The dissolved dye penetrated to the surface of the enamel as well as through the dentin to the pulp. For comprehension of the physiologic phenomenon of caries resistance of teeth, it is necessary to examine in detail the diffusional channels in dentin and enamel. Figures 33–36 are photomicrographs of a tooth section of man, stained vitally with potassium permanganate for 6 hours. The dye placed in the stain pocket (SP) was dissolved and carried to the pulp) primary afferent stain, PAS), then redistributed by the pulp to the surrounding dentin areas (secondary efferent stain, SES), penetrating the enamel (E) to the surface of the tooth. Inasmuch as this tooth had been in the

mouth for approximately 10 years (a lateral incisor of a 17-year-old person), this and similar observations present evidence that the channels in dentin and enamel remain permeable for at least this length of time after tooth eruption, a fact which demonstrates that the enamel and dentin receive products of the blood long after tooth formation.

Vital staining experiments have defined the paths followed by the tissue fluid as passing along the tubules in the dentin and only lightly across their axes. In the enamel, the dyes penetrate its organic structures, i.e., tufts, enamel rod sheaths, and enamel lamellae (figs. 9, 10, 13). The tissue fluid has been noted flowing in two directions, efferently and afferently. However, its passage from the stain pocket to the pulp (afferent) may be open to question. This can be explained by simple diffusion along the cut tubules and not as a flow or current of tissue fluid, as is the case in the secondary efferent staining (fig. 33, SES).

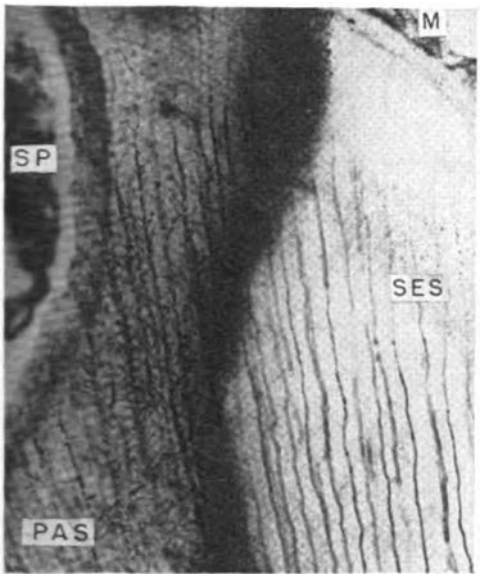


FIG. 34

High magnification of area (X) of figure 33, shows part of stain pocket (SP); the primary afferent stain (PAS) proceeded towards the pulp and the secondary efferent stain (SES) passed outward to the enamel matrix (M) on the surface of the tooth.

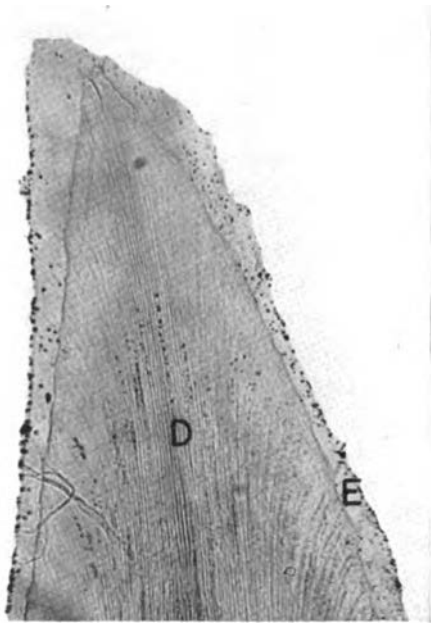


FIG. 35

Dentin (D) and enamel (E) of the tip of the same tooth as figure 33.

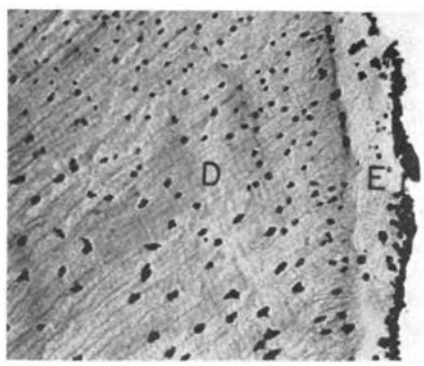


FIG. 36

Higher magnification of part of figure 35 shows the dye located primarily in the tubules of the dentin (D), enamel matrix (E) and at the surface of the tooth.

Origin of Tissue Fluid

Orban *et al* (1944) state: "The function of the odontoblasts is not yet fully known,

but it is believed that they play a part in the nutrition and sensitivity of the dentin." A possible secretory function of the odontoblasts is suggested by three observations:

(1) Bevelander and Johnson (1946a), previously cited observed the presence of alkaline phosphatase in these cells;
(2) Beams and King (1933) noted a change in the polarity of the Goigi apparatus of odontoblasts, an indication of their secretory nature; and
(3) Noyes, Schour and Noyes (1938, p. 143) showed, in their histochemical studies, the presence of calcium, phosphorus and potassium in the cytoplasm of active odontoblasts. These observations suggest that the odontoblasts are concerned with the calcification of the dentin and enamel.

The injection of isotopes into the blood stream has been used extensively for defining metabolic activity in dentin and enamel and corroborates observations previously made by vital staining. This subject is covered well by McCauley (1935), who includes an extensive bibliography.

Recently Bartelstone, Mandel, Oshry and Seidlin (1947) reported on the injection of radioactive iodine which was then identified in the enamel, dentin and cementum. They state: "The results obtained demonstrate the efficacy of the use of radioautography with radioactive iodine as a method for studying the physiology of the fluid in teeth in communication with the systemic circulation."

Fish (1925) observed, as a result of injection of an iron solution into the gingival tissue of dogs, that after 10 minutes the cementum and root dentin became stained. This was later corroborated by McCauley and Gilda (1943) as described below and indicates that nutrition of the roots of the dog's teeth, composed of dentin and cementum, is not cut off completely when these become pulpless. The teeth of man, however, react differently.

Pulpless Teeth

McCauley and Gilda (1943) used radioactive phosphorus to determine differences of metabolic activity in vital and pulpless teeth. For this purpose the "teeth of diametrically opposing quadrants in a young adult dog were rendered pulpless by extirpation and the canals filled with a paste of zinc oxide and eugenol. The animal was then injected intravenously with an aqueous solution of Na_2HPO_4 containing approximately 8,000,000 counts per minute of the radioactive isotope P^{32} and was sacrificed 36 hours later. The teeth were removed and sectioned in longitudinal slabs about 1 mm. thick. These were backed with lead foil and placed with the exposed side downward on no-screen X-ray film, where they remained undisturbed for 46 hours. At the end of this period the sections were removed and the film developed. The resulting radioautographs reveal a striking decrease in the amount of P^{32} deposited in pulpless teeth, as compared with that in vital teeth. The preponderance of the small deposit in pulpless teeth appears contiguous on the cemental covering of the root, indicating a nutritive function of the cementum and peridontal membrane. In normal teeth P^{32} appears in greatest density adjacent to the pulp, its deposit lessening with distance from circulatory elements."

Bodecker and Lefkowitz (1938) observed that the death of the dental pulp has the most marked effect on young teeth, further indicating that this organ is physiologically most active during youth in supplying products to the dentin and enamel. Evidence was presented that the removal of the dental pulp temporarily increases the permeability of the dentin. They state, on the basis of their experiments: "The difference in stain penetration in vital, recently devitalized, and old pulpless teeth of both dogs and man permit the following conclusions:

"1. Vital teeth are less permeable than teeth immediately after pulp extirpation.

"2. The permeability status of pulpless teeth changes. Immediately after pulp extirpation, the permeability of the tooth is highest, reducing slowly thereafter." These observations are a further indication that pulp activity influences dentin and enamel.

The immediate, vast increase of permeability after pulp extirpation appears to be a sign of a cessation of life in dentin and enamel, according to physiologists. Osterhout (1922) states: "It is well known that death is accompanied by an increase in permeability. Thus a slice of red beet kept in water will live for a long time without giving off pigment, but as soon as it is killed, the color begins to escape from the cells. . . . It is a matter of common observation that cells may resist the penetration of certain dyes as long as they are alive, but absorb them readily as soon as they are killed." Thus the augmented dye diffusion through pulpless teeth does not disprove vitality; on the contrary, it appears to be one of the strong indications of vitality of dentin and enamel.

The following conclusions may be drawn from observations on the above-described vital staining experiments (figs. 33–36) (Bertram 1934; Bodecker and Lefkowitz 1937, 1938; Bodecker 1941, 1946; Fish 1925, 1927; Lefkowitz 1943):

(1) The presence of a fluid in dentin is established by the fact that the dye placed in the experimental stain pocket was in powder form (fig. 33, SP);

(2) The dissolved dye passed in two directions, efferently and afferently from the stain pocket.

The purpose of the emphasis placed on "powder form" of the dyes was misunderstood by one reviewer. He states, "I do not believe that dry dyes will diffuse very far". These were selected for the express purpose of determining whether or not a tissue fluid is present. The fact that the tightly sealed dry dyes were distributed answered the question in the affirmative.

Some critics state that this phenomenon might occur by means of diffusion, even if the fluid present in dentin and enamel were stagnant. The concept that the fluid is stagnant is erroneous, as shown by the fact that the pulp, receiving the dissolved dye from the stain pocket, further distributes it to the dentin and enamel, not in direct communication with the stain pocket (fig. 34, secondary efferent stain, SES). Therefore:

(3) the tissue fluid originates from the pulp;

(4) the tissue fluid present in the tooth is not stagnant but flows centrifugally from the pulp.

Physiologic Changes in Dentin

The structural changes of sclerosis, usually noted as transparencies and opacities, occur in dentin as a result of ageing and the irritation of caries, attrition and erosion. These changes are now comprehensible on the basis of the previously described physiologic activity of the odontoblastic processes. Comparatively few investigators have interested themselves in the character of these changes, because most recent histopathologic research on teeth has been done on decalcified sections. These show little or no evidence of the state of calcification of the dentin and none concerning the gas content of the dentinal tubules. It is only by the study of thin, ground, i.e., undecalcified sections, that these data become available.

Dentin sclerosis: Tomes (1859), W. D. Miller (1903) and many observers since their time have noted that ground sections of dentin are not uniform in appearance; they show various degrees of translucency and opacity when viewed by transmitted light under the microscope (fig. 37). These differences have been defined as arising from structural changes of the dentinal fibers within the tubules. The interpretation of translucent and opaque areas is still in a state of some confusion. Orban *et al*, (1944, pp. 117–118) describe the process of sclerosis

as follows: "Calcium salts may be deposited in or around degenerating odontoblast processes and may obliterate the tubules. The refractory indices of dentin in which the tubules are occluded are equalized, and such areas become transparent. Transparent dentin can be observed in old teeth, probably as a final stage in this progressive narrowing of the tubules. On the other hand, zones of transparent dentin develop around the dentinal part of enamel lamellae . . . and under slowly progressing caries. In such cases the blocking of the tubules may be considered as a defensive reaction of the dentin. Roentgen ray absorption tests have shown that such areas are denser than normal dentin (Van Huysen 1933, 1935)."

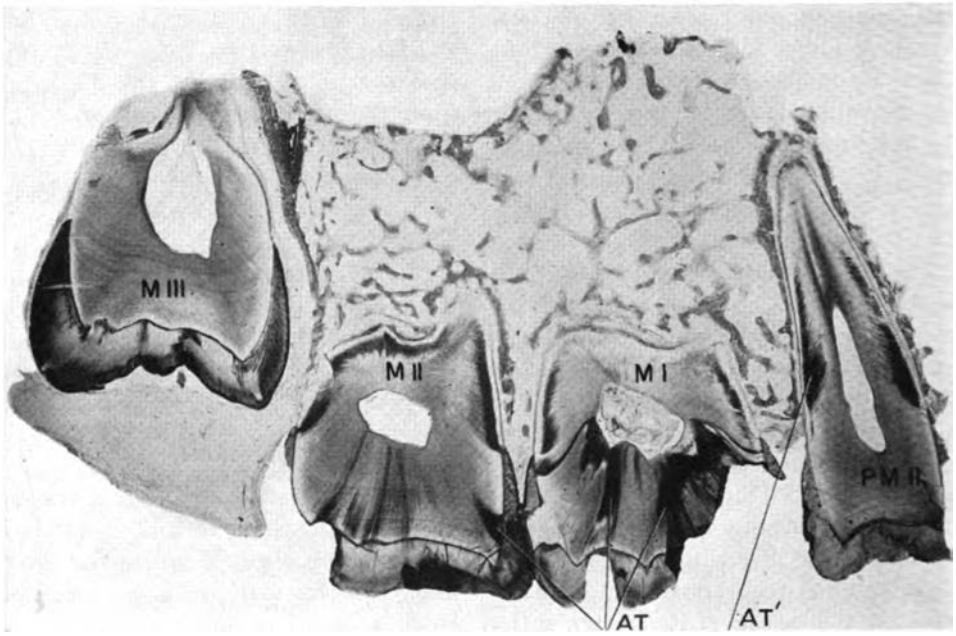


FIG. 37

Photomicrograph of a ground section of four teeth embedded in the maxillary bone. Dark areas in the dentin (AT) are present in the three erupted teeth, i.e. the second premolar (PM II), first and second molar (M I, M II). Heretofore these changes have been attributed to caries, abrasion and attrition. That this cannot be the complete explanation is shown by the similar dark areas in the ROOT dentin (AT') which has not been affected by such external irritants. Another cause of these dark zones is thought to be possible drying during grinding specimen. This, also, does not explain the phenomenon, because the unerupted third molar (M III) which was subjected to like manipulation, nevertheless shows normal dentin.

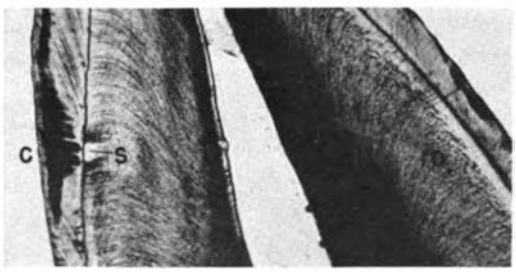


FIG. 38

Incipient enamel caries (C) caused translucent dentin sclerosis (S); interglobular dentin (ID).

Fish (1932) also notes that lamellae, permeable to mouth fluids, are often sealed by a translucent zone in the dentin (fig. 38).

Noyes, Schour and Noyes (1938, p. 139) describe transparent dentin as sclerotic. They state: "This is a vital reaction to the

irritation from caries and may be due to a fatty degeneration of Tomes fiber. An impermeable barrier of lime salts may be observed in the dentin immediately below the carious lesion (translucent zone of Tomes)".

Beust (1934a) states: "Sclerosis is an expression of factors of resistance to decay possessed by a tooth. . . . A tooth's resistance to caries increases in proportion to the amount of its sclerosis."

In the opinion of the above-named investigators, dentin sclerosis seems a sign of dentin response to external irritations and may be regarded as a physiological reaction.

Maturation or protective metamorphosis of dentin: The second type of physiologic change is controversial; it likewise is visible only in ground sections of teeth by transmitted light and causes an opacity, instead of a translucency, in dentin. This change was believed to be due to external irritation, such as caries, attrition, erosion or a desiccation of the section during its preparation for observation under the microscope (Bodecker and Applebaum 1930). Bodecker and Applebaum (1931) contributed to this subject by making ground, instead of decalcified, sections of a large block of tissue composed of four teeth embedded in the maxillary bone (fig. 37). Teeth of different ages, including an unerupted third molar, were present in this tissue block and, what is particularly important, all were subjected to the same technical manipulation of preparing the section. The following conclusions were drawn, based on evidence stated in the legend:

Dentin opacity (1) occurs primarily as a result of age changes in the dental pulp and the formation of secondary dentin; (2) is hastened greatly by external irritation such as caries (fig. 41), abrasion (figs. 82, 94) or erosion; (3) protects the pulp by reducing dentin permeability; (4) is not necessarily the result of desiccation during the preparation of the section; (5) has been termed protective metamorphosis, a condition not necessarily accompanied by sclerosis of the dentinal tubules.

Beust (1912) very active in the early investigations of physiologic changes in dentin, designated it as maturation. He showed that opaque areas take up little or no stain, while most translucent dentin, with the exception of that which is sclerosed, stains readily. On the basis of these observations, he named the former "chromophilic" and the latter "chromophobic" dentin. By means of *in vitro* staining, Beust showed the variation in permeability of opaque and translucent dentin and regarded the former as being sclerosed throughout. The latter part of his statement does not seem correct, according to reports of van Huysen (1935b) and Applebaum *et al.* (1933). The former concludes his observations with the statement: "The staining properties of dentin do not necessarily reflect the degree of infiltration of calcific material." This subject will be considered in greater detail below.

Gottlieb (personal communication) writes, "It was definitely shown (Gottlieb 1947) that opaque hypercalcification is produced by a pouring in of calcium salts into the dentinal tubuli either from the saliva or from neighboring decalcified dentin. The transparent hypercalcification of the dentin develops when the dentin fibers are calcified by the surrounding tissue fluid. Both kinds of hypercalcification are best observed in the neighborhood of caries and both show their hypercalcification in the Grenz-ray, where no differentiation can be made between opaque and transparent hypercalcification. The decalcified dentin also appears translucent and the explanations for the fact that hypercalcified dentin may be opaque or translucent—translucent dentin may be decalcified or hypercalcified—are given in the book (Gottlieb 1947)."

Typical metamorphosis of the dentin crown, making it almost opaque to transmitted light, is noted in figure 39. This change has occurred without external irritation, as no caries is evident. In contradistinc-

tion, figure 40, a section of a young tooth, shows but little dentin opacity or metamorphosis except that stimulated by caries and subsequent fillings on the mesial and distal surfaces.

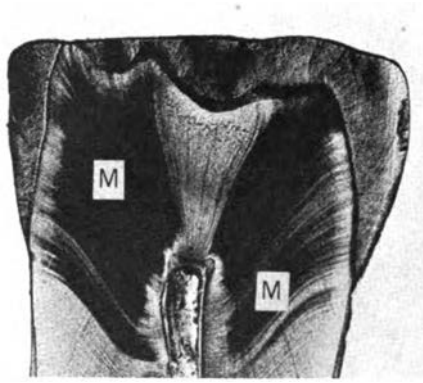


FIG. 39

Extensive protective metamorphosis (M) in crown and root dentin typical in old teeth.

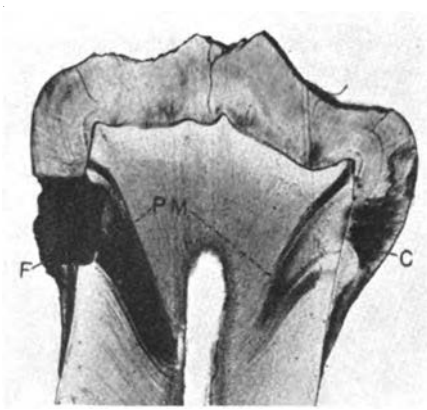


FIG. 40

Protective metamorphosis (PM) is hastened in a young tooth by amalgam and cement filling (F), and by caries (C).

With the purpose of determining whether or not calcification is increased under carious areas and fillings, Bodecker and Applebaum (1930, 1931) introduced the use of X-rays for this purpose. Figure 41 is a photomicrograph of a tooth slab illuminated by reflected light. A dark zone is noted under the filling. A roentgenogram of this specimen reveals a radio-opaque zone which indicates hypermineralization (fig. 42). For a further and more detailed study of variations in calcification in dentin and enamel, Applebaum *et al* (1933) adapted to this purpose the Grenz-ray (soft X-ray), which is a more sensitive tool as shown by the marked variations in radio-opacity. Van Huysen *et al* (1933) continued to use the hard x-rays, while Warren *et al* (1934) adapted an apparatus by which delicate variations of radio-opacity in the X-ray film could be determined precisely.

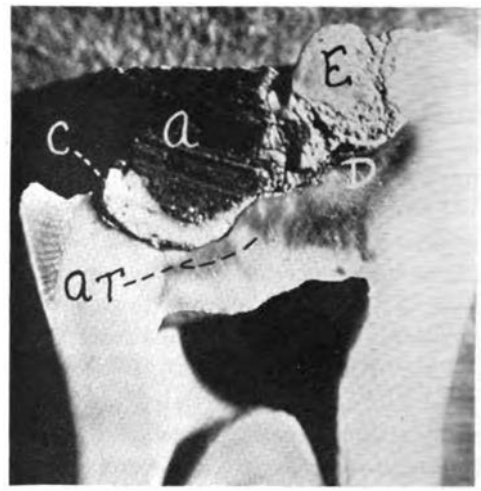


FIG. 41

Tooth slab photographed by reflected light, shows enamel (E), amalgam (A), cement filling (C), a dark zone of affected tubules (AT) and dentin caries (D).

Whether opaque dentin is sclerosed or is made less permeable by other means may be a factor in the mechanism of tooth resistance to caries. Generally, opaque areas are considered as being sclerosed. Applebaum *et al* (1933) and van Huysen (1935a) observed that X-ray photographs of thin, ground sections of dentin show only occasional scleroses. Both translucent and opaque or metamorphosed dentin are seen in a photomicrograph of a tooth section (fig. 43). A Grenz-ray view (fig. 44) of this specimen, enlarged to the same extent as the photo-

micrograph, shows that some of the opaque or metamorphosed dentin areas are sclerosed (S), while others, though equally opaque, show no sclerosis (NS). For this reason, Bodecker and Applebaum (1933) consider

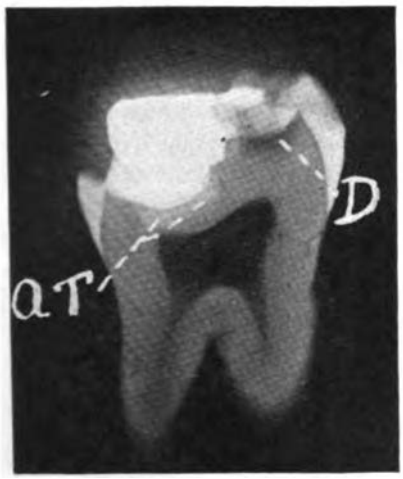


FIG. 42

Roentgenogram of tooth slab (fig. 41) shows dark zone (AT) is radio-opaque, indicating its hypermineralization; dentin caries (D) is radio-lucent.

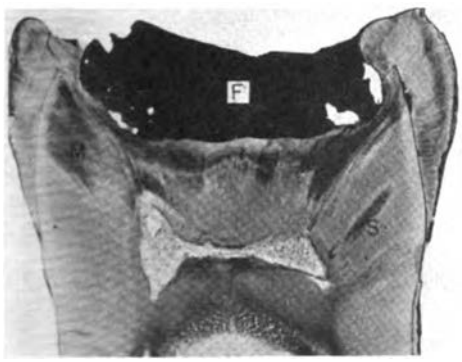


FIG. 43

Photomicrograph of thin ground section of tooth with filling (F) shows various shades of dentin metamorphosis (M, S).

the term "sclerosis" incorrect as applied to opaque dentin. Also, it has been shown that opaque areas often are caused by caries and not always by ageing (fig. 40). Hence the term "maturation" does not always apply. On this basis, therefore, a designation with a less distinctive meaning has been suggested, namely "metamorphosis".

The interpretation of the changes occurring in metamorphosed dentin is, as already mentioned, controversial; however, the following evidence is presented substantiating this hypothesis: The initiation of this con-

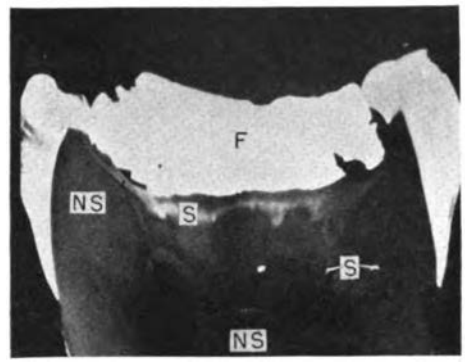


FIG. 44

Grenz-ray photograph of tooth section (fig. 43) shows some metamorphosed areas are not sclerosed (NS) while others are sclerosed (S).

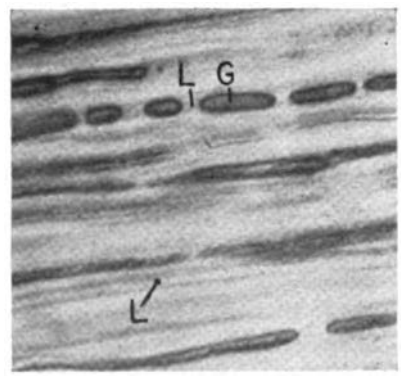


FIG. 45

Ground (undecalcified) section of dentin shows beginning of protective metamorphosis: formation of gas (G) in tubules that contain tissue fluid (dental lymph, L).

dition appears to be the formation of a gas (fig. 45G), which appears like beads comparable to bubbles in a capillary tube filled with air and water (Bodecker and Applebaum 1931). Separating the bubbles of gas in the dentinal tubule is a fluid, the tissue fluid or dental lymph. One proof that a

fluid is present is that, in vital staining experiments, the areas between the gas bubbles are stained deeply by the dye, i.e., a fluid acting as a solvent of the experimental dye. Figure 46 shows the final phase of dentin metamorphosis, i.e., a disappearance of the tissue fluid. This conclusion is reached

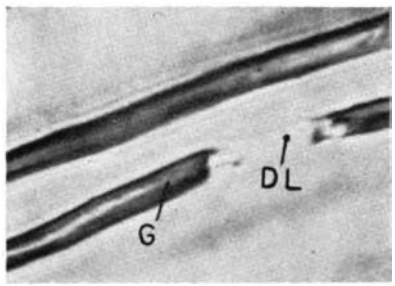


FIG. 46

Completed dentin metamorphosis: tubules filled with gas (G) and dessicated tissue fluid (dental lymph, DL) with a possible trace of fat.

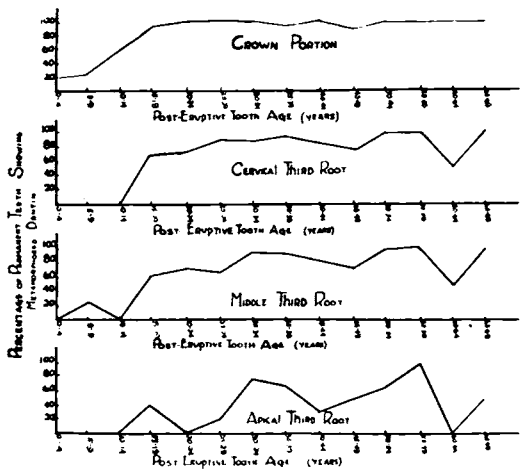


FIG. 47

Graph of age progress of protective metamorphosis in crowns and roots of teeth. (Courtesy of W. Lefkowitz (1942)).

because of the fact that the menisci of the gas bubbles are no longer rounded but jagged, suggesting the absence of fluid. It is clear that dentin permeability, from within and from without, is eliminated in tubules thus blocked by gas and plugs of desiccated lymph.

A critic states: "What tests have been made for the presence of a 'gas'? The microscope certainly cannot answer this question." When a wet dentin section is dessicated, an increased opacity of the dentinal tubules results. Individual tubules in axial section, when dry, show a high degree of refraction of transmitted light under the microscope, a sign of gas inclusion (fig. 46).

Gottlieb (personal communication) writes: "The problem of opaque hypercalcification was solved by the experiment reported in the book (Gottlieb 1947) pages 137 to 139, figs. 121–123, where it was shown that de-

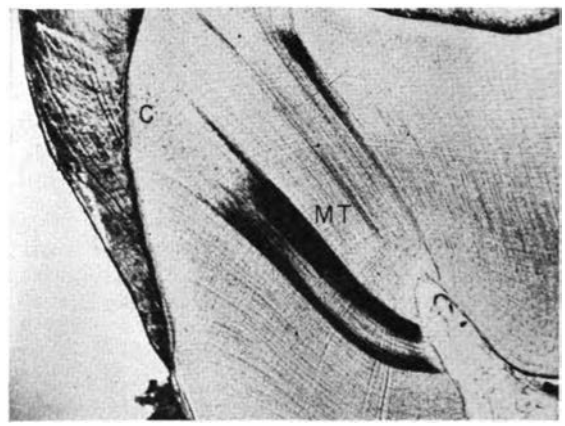


FIG. 48

Photomicrograph of caries (C), not entirely in the plane of section, resulted in metamorphosed tracts (MT) in dentin. (Courtesy of E. Applebaum).

calcification of these with addition of tricalcium phosphate produces the same opaqueness as seen in connection with caries and that such opaqueness may be removed by washing out the calcium salts from the tubuli."

Dentin metamorphosis sets in at a definite time after tooth eruption. Lefkowitz (1942) showed that it usually commences in the crown, 5 to 10 years after the tooth takes its position in the mouth, and progresses apically, increasing with age (fig. 47).

Whether or not hypermineralization of the dentinal tubules occurs as a result of

caries seems dependent upon the state of the dental pulp. Figure 48 is a photomicrograph of a ground tooth section in which the irritation of caries has resulted in the formation of metamorphosed tracts (MT) in the dentin (Applebaum *et al.* 1933). Figure 49, a Grenz-ray view of this section, shows sharply defined radio-opaque tracts (hypermineralized, HT) corresponding to those seen in figure 48, leading from the pulp to the vicinity of the dentino-enamel junction. In this tooth, the pulp appeared very active and deposited a clearly perceptible quantity of mineral salts. It is of interest that hypermineralization of the dentin around carious areas seems dependent on the viability of the dental pulp, because pulpless teeth, and even some vital ones, do not show this deposit.

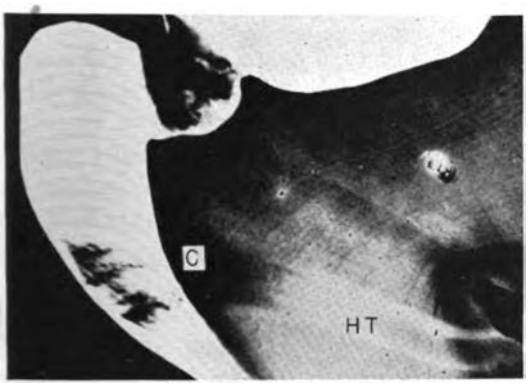


FIG. 49

Grenz-ray photograph of section (fig. 48) shows radio-opaque tracts (hypermineralized, HT) correspond to metamorphosed tracts in figure 49. (Courtesy of E. Applebaum).

Ellison and Halpert (1947) made an interesting contribution to the small store of available knowledge concerning metamorphosed dentin. They showed by means of micro-analyses that the slight increase in mineral matter in these areas is composed of neither calcium nor phosphorus, but of some other, as yet unidentified, radio-opaque substance. Hence, the observations of these two workers suggest that it may be more proper to speak of "hypermineralization" rather than of "hypercalcification" which occasionally is present in metamorphosed dentin.

The reduction of dentin permeability may be important in the comprehension of the mechanism of caries; hence it seems advisable to distinguish precisely between the two processes which cause this effect:

Dentin Sclerosis	Protective Metamorphosis
1. Dentin appears translucent	1. Dentin appears opaque
2. Causes obliteration of dentinal tubules	2. Accentuates visibility of the tubules,
3. Tubules filled homogeneously with mineral salts	3. Tubules filled with a gas and traces of fat and only occasionally with mineral salts
4. A slow process	4. A rapid process
5. Individual tubules impermeable	5. The same
6. Reduces rate of carious penetration	6. The same
7. Increases brittleness of dentin	7. The same

This section has dealt principally with the physiologic reactions occurring in dentin. Some post-eruptive changes also have been shown in the enamel, but whether the latter can be termed physiologic may be debatable. The study of the enamel is even more difficult than that of the dentin, owing to its low organic content and brittleness. These qualities make the preparation of very thin ground sections difficult; also, different methods of examination from those used for soft tissues are necessary. The study of the crystalline structure of enamel is made possible by means of polarized light, on which subject Kitchin (1938) has made a notable contribution. The varying degrees of decalcification in and around incipient enamel lesions can best be determined by Grenz-ray, as developed and reported by Applebaum *et al.* (1933), (figs. 48, 49), while a special procedure of decalcification is essential for the study of the organic enamel matrix

(Bodecker 1906; Frisbie *et al.* 1944; Malleson 1924; Saunders *et al.* 1942). These three approaches in the study of enamel may be fruitful in increasing our comprehension of the manner of its carious destruction and its possible reaction to attack.

Finally, it must be reiterated that the concept of slight metabolic activity in dentin and enamel is shared by only a small minority of investigators. The majority definitely deny the existence of any biologic connection of dentin and enamel with a body fluid originating from the pulp. The active prosecution of research in the physiology of the enamel and dentin, with particular emphasis on the former, may uncover more evidence and aid in the further elucidation of the varying resistance offered by these structures to dental caries.

Conclusions

The possibility of physiologic changes occurring in dentin and to a lesser extent in enamel is a highly controversial subject. However, the following observations, previously detailed, suggest that these occur in teeth for 10 or more years after eruption:

1. Both dentin and enamel are permeated by a tissue fluid originating from the dental pulp (vital staining).

2. Pulp removal from unerupted, partially formed teeth halts calcification of the enamel, suggesting that this structure requires some products from the pulp for its maturation (pulpotomy and amelotomy experiments).

3. Pulp removal from young, erupted teeth subsequently makes dentin and enamel brittle and results in a marked darkening of the teeth (clinical observations).

4. Pulp removal from teeth having a postformative age of 10 or more years also causes brittleness of dentin and enamel but less discoloration, suggesting that maturation of the teeth is more advanced (clinical observations).

5. Dentin undergoes age changes related to a reduction of tissue fluid supply (dental lymph) from the dental pulp; this causes a lowered permeability (histological observations).

6. Pulpless teeth show no hypercalcification of dentin or enamel as a result of external irritation; this is evidence that the dental pulp is effective in causing these changes.

The existence of a tissue fluid originating from the dental pulp offers an explanation of the manner in which the teeth of young persons may be benefited or possibly harmed by systemic conditions. This subject will be discussed in the following section.

DENTAL CARIES

The subject of dental caries has attracted a great many investigators, but the problem is not yet solved. Bunting (1930) gives the following brief but comprehensive outline of the clinical aspects of dental caries:

"1. Dental caries is a destruction of the hard substance of the tooth by a process, the initial stage of which is adecalcification by acids.

"2. The acids active in caries are not generally distributed in the saliva, but are localized and concentrated on certain areas of the tooth surfaces.

"3. Carious lesions occur most frequently in the pits and fissures of the occlusal surfaces and on certain areas of the approximal, buccal and lingual surfaces of the teeth, at which locations there is opportunity for stagnation and the retention of foreign matter. They do not occur on smooth enamel surfaces which are frequently cleansed.

"4. All initial lesions of caries contain acid-forming bacteria capable of producing and living in acids of sufficient potential to decalcify the enamel.

"5. The hardness or softness of the teeth may affect the rate of progress and extent of caries but does not alone determine its occurrence. Caries, as a rule, runs a more rapid and extensive course in poorly formed teeth than in the hard and well-formed varieties, but instances commonly occur in which

the poorest formed teeth are wholly free from the disease."

"6. Malhygiene of the mouth frequently favors the inception of dental caries and increases its activity, but alone does not determine its occurrence. Mouths that are habitually unclean are often wholly free from caries and, conversely, mouths that are scrupulously clean may be seriously affected by the disease."

Bunting states further that the following bodily conditions are perhaps best known as systemic factors which either favor or oppose dental caries:

"(a) Heredity—the tendency towards dental caries or an immunity to the disease may be transmitted from parent to child, according to the laws of familial inheritance. . . .

"(b) Age—after 20 years caries is markedly decreased. . . .

"(c) Health . . . severe onset of dental caries follows attacks of general disease and disturbances of body health. . . .

"(d) Racial Influence—The natives of Africa, South America, and the South Sea Islands, the Esquimaux and many other primitive peoples are notably free from the disease, while those who live in the more civilized communities are extremely susceptible to it. . . . The most constant and important variable between immune and susceptible races appears to be that of diet."

Noyes, Schour and Noyes (1938, pp. 332–244) present a histopathologic study of the progress of dental caries, which they divide into three stages or periods:

"FIRST PERIOD—If a tooth can be examined during this stage, a white spot will be seen. The area appears white because the cementing substance has been removed from between the enamel rods. . . .

"If the section is ground through the white area of such a tooth, the enamel rods will be found to be entirely separated because of the solution of the interprismatic cementing substance, and the cross-striations will be much more apparent because the unevenness in the diameter of the rods has been increased by the action of the acid.

"In the disintegrated area, during the first as well as the second stage, the diameter of the enamel rods is always considerably reduced and the striation rendered more apparent.

"The boundary between the sound and the disintegrated area in enamel is usually marked by a darker zone, the significance of which is not now understood." (See explanation given by Applebaum (1935); Mummery (1926) below). Miller (1890) indicated that such spots are more resistant to the progress of caries than perfect enamel surfaces. At any time during the first period, therefore, the destruction may be arrested by immunity of the patient.

"SECOND PERIOD—This period extends from the time when the action of the acid reaches the dentin-enamel junction until the rods are destroyed or fall out. As soon as the solution of the cementing substance reaches the dentin-enamel junction at the apex of the advancing cone, the solution of the inorganic salts in the dentin matrix begins. . . .

"The action of the acid follows the tubules of the dentin towards the pulp and spreads through their branches laterally near the dentin-enamel junction, so that the form of the disintegrated dentin is always that of a truncated cone with the base at the dentin-enamel junction and the apex towards the pulp chamber.

"It must be remembered that the acid acting upon the dentin is formed by the microorganisms on the surface of the enamel and filters through the spaces between the enamel rods. In this stage no microorganisms have entered the dentin, and the effect upon it is the result of the action of substances formed upon the surface."

The foregoing quoted paragraph is particularly noteworthy. It is incomprehensible why an acid formed on the surface of the tooth should filter between the enamel rods to act on the dentin beneath, disregarding

the mineral salts present in the enamel. This subject will be discussed under Enamel Proteolysis. The quotation (Noyes, Schour and Noyes 1938) continues:

"The decalcification of the dentin may be considerable, while the surface of the enamel is still preserved.... The decalcified dentin matrix shrinks and more or less of a space is formed under the enamel.... The rate of progress of decay in the dentin may often become modified by the presence of a translucent zone of hypercalcified dentin. This zone was first described by Tomes and has been termed sclerosed dentin.

"THIRD PERIOD—This is the period after the enamel rods have begun to fall out and an actual cavity is apparent. As soon as this occurs, the surface of the tooth, at the point where the formation of the colony began, is destroyed and the protected point is lost, and the extension of surface attack ceases. The microorganisms are admitted to the dentin, where they grow through the dentinal tubules, spreading rapidly at the dentin-enamel junction. The dentin is always decalcified in advance of the penetration of the microorganisms. The decalcified dentin matrix becomes food material for the bacteria, and the spaces produced by the destruction of tissue accommodate more decomposing foodstuffs.

"The acid formed within the cavity now attacks the enamel from within outward, producing what has been called backward or *secondary decay of enamel.* The condition may progress until the entire occlusal enamel has been undermined and greatly weakened before the site of original attack is noticeably enlarged."

The two quotations, from Bunting and from Noyes, Schour and Noyes, present a clear and concise description of the clinical and histopathologic activity of dental caries with particular emphasis on the effect of acid. Following is a detailed discussion of various predisposing causes such as permeable enamel lamellae, lack of secondary dentin formation, lack of hypercalcified zones around incipient caries lesion, high permeability of enamel and dentin, variations in composition of the teeth, certain types of hypoplasia, hypocalcification, etc., which may initiate or hasten the caries destruction of the teeth. In addition, there is a discussion of a controversial factor of dental caries which, as well as acid, may be active in tooth destruction, i.e. enamel proteolysis.

The terms "susceptibility", "immunity" and "resistance" in connection with caries are mentioned occasionally in the succeeding pages, as they are used by other investigators such as Bunting (1930), Beust (1934a), Orban *et al.* (1944b, pp. 117–118). These designations are regarded by some workers as inapplicable to teeth, primarily because of the chemical analyses of Black (1908) who observed no differences in the composition of carious and non-carious teeth, a statement occasionally quoted even today. Consideration must be given to the fact that since the analyses of Black, chemical methods have been improved greatly. Even so, this erroneous statement has done much to hinder a biologic concept of the teeth. Crowell, Hodge and Line (1934) completely disproved Black's statement by showing, not only that variations in tooth composition do exist, but that they occur even in teeth taken from the same mouth. Furthermore Deakins (1940) detailed chemical differences between carious and non-carious enamel.

That group which believes that teeth are inert explains the clinically observed caries immunity and susceptibility by stimulating the presence or absence of carbohydrate food debris and *L. acidophilus* as the only factors controlling caries activity. But this concept appears negated by the observation of unilateral caries, previously mentioned and again discussed at the end of this section. Still further evidence of the varying resistance of teeth to dental caries has been presented by Hunt, Hoppert and Irwin (1944), who bred a "caries-susceptible strain and a caries-resistant strain" of rats.

Klein (1946) made an important contribution in this respect by showing that heredity also operates in man, as he observed variations in caries resistance in children whose parents showed similar tendencies. The observations of Hunt *et al.*, made under controlled laboratory conditions, suggest strongly that teeth react differently to caries attack. Generally, the terms "immunity" and "susceptibility" are applied to the body in relation to infectious diseases; hence it seems logical to use them also regarding the reactions of the teeth to caries. Possibly "variable resistance" may be a more acceptable term.

One reason for the failure to obtain a solution of the problem of dental caries may be the fact that teeth have been regarded as inert. The cause of their destruction has therefore been sought, with comparatively few exceptions, solely in variations of oral conditions.

Mellanby (1934, p. 8), in discussing her research, states: "Attention was paid rather to the predisposing than to the exciting causes of dental decay. It was assumed that a tooth is a living organ and must therefore be considered as any other organ in the body in regard to its susceptibility to infective invasion."

Food retention, bacteria and saliva, all important causes of dental caries, may be classed as EXOGENOUS factors of this disease, while the ENDOGENOUS ones are considered as causing changes within the teeth, affecting their resistance to attack. Some data advanced by the supporters of the exogenous factors were mentioned only briefly in the section on physiology of dentin and enamel; the importance of these appears to be emphasized by the following observations on diet.

Relative to the thesis that body health does not affect caries activity, Volker (1947) states, from observations of the National Youth Administration during World War II: "The health picture in New England was one of the best, if not the best in the nation, but the tooth decay of the examinees was the most extensive encountered."

Day (1944) observed that children in those areas of India where famine has been frequent in recent years and who consume rations deficient in calories, essential amino acids, and most of the known vitamins, have excellent teeth. Such a condition prevails even though the children actually show all other symptoms associated with deficient nutrition.

Schour and Massler (1947) report that the caries incidence in four postwar Italian cities, in which 3,905 persons were examined, is approximately two to seven times lower than that observed in the United States.

These observations might suggest that nutrition plays no part in dental caries activity. Contrary evidence is presented in the section on Nutrition and Dental Caries in this monograph, in which the beneficial effect of vitamin D, calcium and an optimal diet is described as reducing the activity of this disease. It is evident from these conflicting observations that some factors in the etiology of dental caries, which might explain these paradoxical observations, must still be lacking.

Some views of the "vitalists" have been mentioned briefly in the last section in relation to the physiology of the dentin and enamel; these will now be considered in relation to the dental caries problem. Supporters of the biologic concept of dentin and enamel believe, not only that exogenous factors do attack the teeth, but that these forces may be weakened or completely annulled by the physiologic protection inherent in the teeth as well as in the saliva.

The endogenous factors may relate to abnormal structural variations, such as permeable enamel lamellae, varying chemical composition of enamel and dentin, chemical differences in the tissue fluid (dental lymph) within the tooth, which in turn are dependent on blood conditions and also important localized pulpal activity. The great majority

of past investigations have been focused on the exogenous factors, while the fields related to the endogenous ones have been studied by only a comparatively small group, viz:—Beust (1931a), Bodecker (1935, 1942), Boyd and his associates (1943), Broderick (1928), Fish (1925), Howe (1926), Karstroem (1931), McBeath *et al.* (1942). The work of those investigators concerned with the effect of diet on dental caries activity (Boyd 1943; McBeath *et al.* 1942; Mellanby 1923, 1930, 1934; and others), is discussed in the section on Nutrition and Dental Caries.

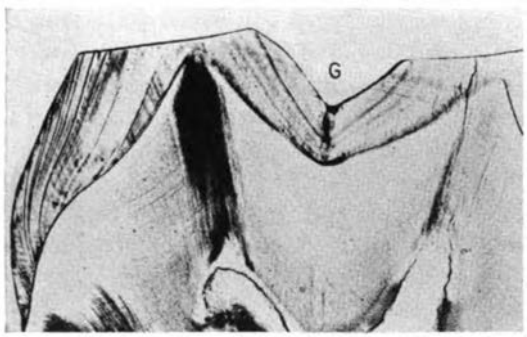


FIG. 50

Section of tooth showing a non-retentive groove (G) on occlusal surface.

Dental Caries as Related to the Macroscopic Anatomy of Teeth

The destruction of the teeth by dental caries begins in the crown portion on the surface of the enamel, or on the cementum if this tissue is denuded of gingival or gum tissue. The form of the carious lesion is dependent on a number of factors, such as the location of the lesion and the age of the tooth. The reason for this will become apparent in considering the various phases of dental caries activity.

The two principal carious lesions on the crown are: (1) pit and fissure lesions and (2) smooth surface lesions. The surfaces of the entire tooth never become carious simultaneously, nor does caries progress with uniform rapidity in the above-mentioned areas. This clinical observation suggests that certain factors are active in reducing or increasing caries activity on particular tooth surfaces.

The most commonly and primarily affected ones of the permanent teeth are on the occlusal surfaces (Hyatt 1930). Next are the proximal surfaces, mesial and distal, and the cervical third. Finally, the least affected are the labial, buccal and, most rarely, the incisal edges of the anterior and the lingual surfaces of the posterior teeth. When the tooth cervices become carious, it is usually a sign of maximum caries activity. The relative condition of the anterior to that of the posterior teeth also indicates the degree of caries activity; the posterior ones always become carious first, but when the anterior teeth are also affected, the carious process may be regarded as somewhat above the average.

The fact that all tooth surfaces do not become carious with equal rapidity is explained partly by their food-retentive qualities. O'Brien (1940) expressed the commonly accepted view that caries susceptibility is primarily dependent on tooth form and arrangement in the arch, which determines the degree of food retention. Hirschfeld (1930) states that a tooth may be tilted in such a manner that one of its proximal surfaces is constantly cleansed by the excursion of food during mastication, whereas the other, not being cleansed in this manner, becomes carious. However, neither position in the arch nor relation to adjoining teeth appears to be the complete answer to the problem, because as shown, (Bodecker and Ewen 1937) occassionally caries may affect only one of the two closely adjoining teeth (figs. 73, 74). If food retention, bacteria and saliva are the only factors in dental caries activity, then both closely contacting tooth surfaces should be attacked equally. This observation suggests not only that dental caries is caused through exogenous means, but that endogenous factors may modify tooth resistance.

Enamel pit and fissure caries: Shallow grooves are normally present on the occlusal surfaces of molars and premolars (fig. 50 G), making the opportunity for food retention minimal in these areas. Very frequently, however, the grooves are so deep and narrow (fig. 51 F) that they are highly food retentive and as such are termed fissures and pits. In the past, the attention of the dental profession has frequently been called to the seriousness of dental caries originating in enamel pits and fissures. Hyatt (1930) states that in 1835 William Robertson was one of the first to point out that "the shape and form of the tooth increases the liability of the tooth to decay". Hyatt mentions more than 15 authors who subsequently emphasized the danger of enamel pit and fissure caries. The early treatment of these areas is now an established procedure. There are two reasons why pit and fissure lesions become carious so readily and deeply.

(1) Enamel lamellae. These are commonly present in pit and fissure areas and, when permeable, are destroyed rapidly through proteolysis (Bodecker 1927; and Bodecker 1929).

(2) Enamel rod direction. Caries progresses more rapidly along the enamel rods than across their axes. The directions of the rods in pit and fissure areas diverge as they pass from the surface of the enamel to the interior (figs. 52 A, 53). Hence, caries entering the tooth from a small opening on its surface involves a much larger area at the dentin-enamel junction (Bodecker 1931). The fact that the orifice of the cavity is small and inconspicuous has two grave sequelae: (a) the patient, and sometimes even the dentist, is not aware of the destruction going on in the depth, because of the minuteness of the opening; (b) saliva cannot readily penetrate the small surface opening and reach the base of the cavity; thus bacterial action is not impeded (Bodecker 1929). For these reasons, carious lesions originating in enamel pits and fissures frequently progress to great size, sometimes

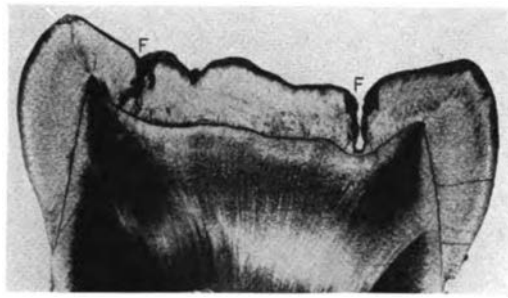


FIG. 51

Deep groove or fissure (F) permitting food retention and therefore a menace to tooth; no caries.

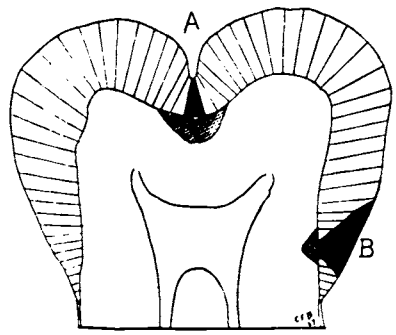


FIG. 52

Diagram of a molar tooth showing differences in enamel rod direction: *A*. rods diverge from surface of tooth to dentino-enamel junction; therefore occlusal cavity has small orifice and enlarges within; *B*. rods almost parallel; cavity has large orifice.

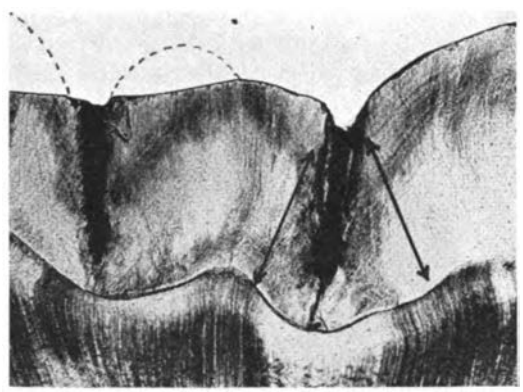


FIG. 53

Ground (undecalcified) section of molar showing directions of enamel rods on occlusal surface (arrows).

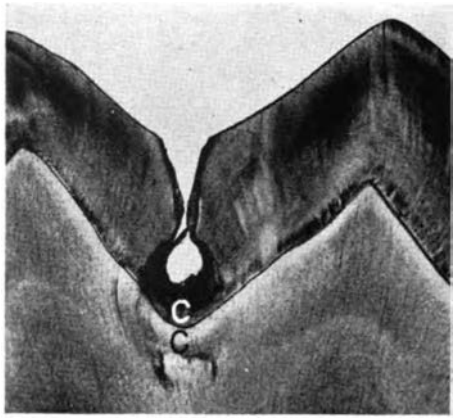


FIG. 54

Deep food retentive fissure shows slight caries (C) of enamel and dentin.

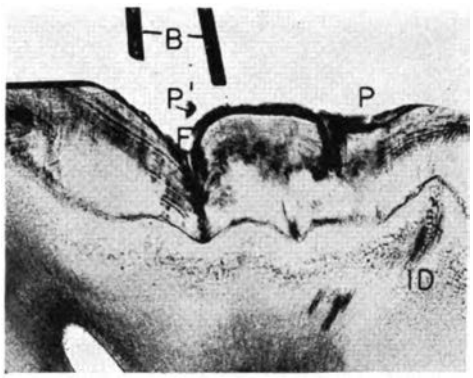


FIG. 55

Ground (undecalcified) section shows deep fissure (F); the large size of tooth brush bristles (B) shows impossibility of keeping some fissures free from food debris; mucin plaque (P); loose ragment (P'); interglobular dentin (ID).

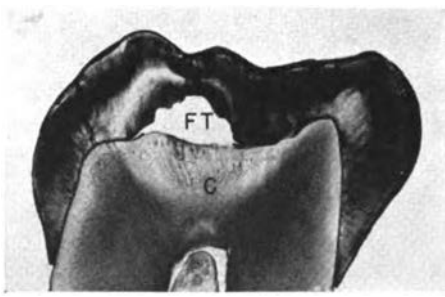


FIG. 56

Large undermining carious enamel fissure tunnel (FT) has involved a considerable area of dentin; strong enamel walls still cover cavity.

even involving the dental pulp, before treatment is instituted.

The different forms of enamel fissures and their varying reaction to caries are illustrated in photomicrographs. Figure 54 shows a deep, wide but highly food retentive fissure. In this case, in spite of the food-retentive character of the fissure, caries was barely active.

The difficulty of keeping many enamel fissures free from food debris is shown by comparing the size of two tooth brush bristles (B) with the width of a very narrow fissure (fig. 55). Evidence of unsanitary surroundings of this tooth is shown by the thick mucous plaque (p) of which one fragment (P^1) has become detached during the preparation of the specimen; caries, however, is not present (Bodecker 1931).

Enamel fissures sometimes have a tendency to tunnel in the body of the enamel or along the dentin-enamel junction (fig. 56, FT). This is a great menace to the tooth because of lack of salivary irrigation of this site.

The above illustrations of enamel fissures show that, in spite of their food-retentive qualities, dental caries is not acutely active. Some of the following photomicrographs of enamel fissures depict acute, deep caries penetration; in figure 57, for instance, extensive dentin destruction arising from a small fissure cavity has occurred. The irritation has resulted in the formation of physiologic secondary dentin (SD) and much protective secondary dentin (SD^1) which almost fills the pulp chamber (P). These structures have prevented pulp exposure.

The gravity of enamel fissure caries is noted in the two succeeding illustrations. The occlusal surface of a whole tooth is seen in figure 58, showing six very small carious cavities (X). An attempt to section this deeply carious tooth without the use of a special technic caused it to break into fragments. Another tooth, in a condition similar to that shown in figure 58 but having only one very small carious opening, is

shown halved in figure 59. The occlusal enamel appears intact in this section; yet caries entered through a fissure not visible in this plane, destroying the major portion of the crown dentin (C-C-C) and involving the dental pulp (P). The thickness of the enamel in this tooth is greater than normal, which explains the fact that the force of mastication did not break down the "roof" over the acvity and disclose the caries. If it had broken down earlier, saliva would have permeated the lesion and probably would have reduced greatly the rate of the destructive process.

The final illustration of a fissure tunnel is of particular interest (fig. 60) inasmuch as

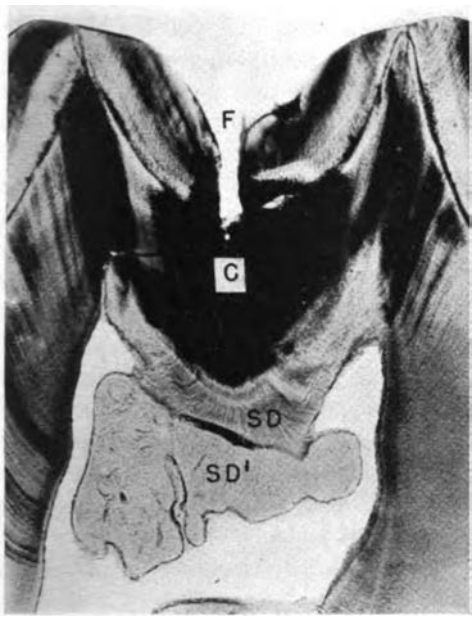


FIG. 57

Deep fissure caries (F), small orifice of cavity; considerable secondary dentin formation (SD), as a result of very extensive caries (C).

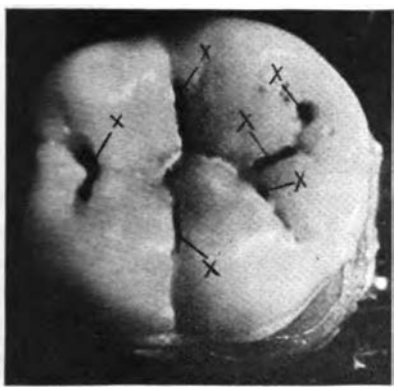


FIG. 58

Occlusal surface of an entire molar under low magnification, showing six small carious areas (X).

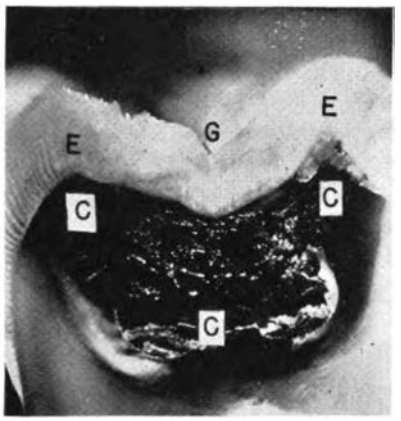


FIG. 59

Half a molar with fissure caries outside plane of section, shows great extent of carious dentin destruction (C, C, C) involving the pulp; groove (G) in enamel (E).

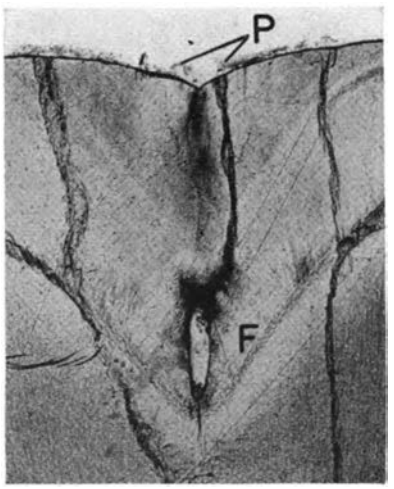


FIG. 60

A tunneling enamel fissure (F) close to dentin shows only slight caries of enamel (dark); tooth of an old person, judged by the marked attrition of occlusal surface; mucin plaques (P).

this tooth did not become carious, in spite of the presence of an enamel lamella connecting the fissure tunnel with the outside. The mouth from which this tooth was taken was in an unhygienic condition, as evidenced by the extensive plaques (P). The marked attrition of the occlusal surface shows that the tooth had been in position for a long time; therefore it had ample opportunity for becoming carious. The question arises: Why, in this and other cases (figs. 51, 54, 55, 60), did caries remain inactive or superficial, barely attacking the enamel and dentin, while other teeth (figs. 56, 57, 58, 59), showed such deep caries penetration? One

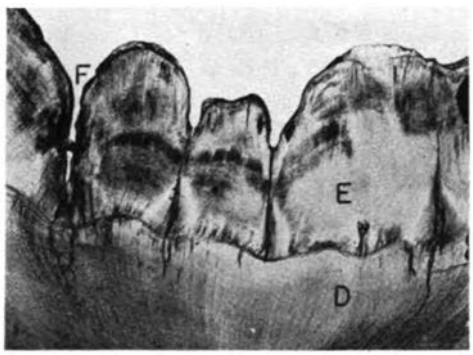


FIG. 61

Section of tooth of Pueblo Indian living more than 900 years ago, shows deep enamel fissures (F) but no caries; enamel (E); dentin (D) shows cracks due to drying of tooth.

answer is, of course, a difference in the oral bacterial flora; but this does not always explain dental caries activity, as shown by the subsequently described occurrence of unilateral proximal caries. Tooth resistance may be another explanation for the variable activity of caries in enamel fissures.

The manner of enamel fissure formation is known, but its cause is obscure. The crowns of teeth, i.e., both enamel and dentin, are calcified from a number of centers or lobes. If these enamel lobes do not coalesce perfectly, a fault or fissure remains. Some writers imply that fissure formation is due to improper diet during tooth formation. It has been shown, however, (Bodecker 1930) that enamel fissures are not a recent acquisition, as these faults were common in the teeth of the Pueblo Indians living more than 900 years ago (fig. 61); yet the caries incidence of these people appeared exceedingly low. This observation is based on the examination of 317 teeth, of which less than 3 per cent showed any signs of caries.

Statistical evidence that the first permanent molar is the most vulnerable of all of the teeth is proof of the serious menace of enamel fissures. Hyatt and Craig (1934) state: "At the ages of 20–24, for every 100 people, or a normal of 400 first permanent molars, the records indicate that 164 or 41 per cent of the molars are missing." Their figures for other ages do not quite correspond, which they attribute to the small number of cases for these particular age groups.

It may seem inconsistent to make enamel pits and fissures responsible for the high mortality of the first permanent molars, while the second permanent molars and the first and second premolars likewise show similar defects; yet these teeth have a far lower mortality rate. The answer to this paradox seems to be that the first permanent molars appear in the mouth at the age of six years, the beginning of the peak of caries activity of the permanent teeth.

Enamel fissures frequently are the site of fractures of the enamel, a factor particularly emphasized by Rosebury, Karshan and Foley (1933), and King (1936) as a cause of dental caries. The former showed that fractures develop a particular type of lesion in rat teeth, clearly distinguishable from true caries. The latter also suggests that fractures are the initial cause of caries in man. Applebaum and Adam (1938) on the other hand, came to the conclusion, on the basis of observations made with Grenz-rays (soft X-rays), that decalcification is more commonly found than fractures in enamel fissures and in smooth proximal surfaces of human teeth.

Conclusions drawn from the above illustrations show the treacherous penetration of caries through enamel pits and fissures, a process active in the great majority of the permanent molars and premolars of children and adolescents. It appears that the thickness of the enamel affects the caries rate; the thicker the enamel, the deeper and more rapid is the penetration of the caries process, probably because of the seclusion of the cavity from the saliva. It is an established clinical observation that pits and fissures predispose teeth to caries, if caries is at all active. However, should these teeth remain sound during the first 10 to 15 years, as, for instance, a first permanent molar in a person reaching 16 to 21 years of age, then these areas often remain immune throughout life in spite of caries activity in other teeth (figs. 55, 61). This clinical observation suggests that some physiological changes occur within teeth some years after their eruption, making them more resistant to attack by caries.

Smooth surface caries: This type of caries, as the name implies, is found on enamel surfaces having no pits or fissures. Here the enamel rods run parallel or even converge slightly from the entrance of the cavity to the dentin-enamel junction (fig. 52 B). As enamel caries penetrates principally along the axes of the enamel rods, lesions on smooth surfaces have large openings and can be diagnosed more easily, even though they are hidden between the teeth. This type of lesion does not proceed as rapidly as the pit and fissure type, because the carious focus, having a larger opening, is reached more readily by the saliva, which thus retards the destructive process. If the cavities are small, the proximal ones may be discovered, either by passing dental floss between the teeth, or, which is far better, by the use of X-rays.

When teeth first take their place in the mouth, they usually contact each other at a point, like two spheres. Slight vertical and lateral movement of the teeth during mastication (Bodecker 1936) soon wears the contact point to a contact surface (fig. 62). Beust (1935–1936) examined 287 extracted teeth, of which 246 showed facets due to proximal wear. Of these, 15 had caries both inside and outside of the facets; in the remainder, caries was restricted to the facets only. He concluded that the presence of acid-yielding plaques at the contact surface is mechanically impossible because of the friction caused by the movement of the teeth. Similar figures, though not quite so conclusive, are presented by van Huysen (1937). By means of a magnifying glass and explorer he found 405 facets in 344 extracted teeth. Caries was present in 393 proximal surfaces, of which 36 per cent were within the boundaries of the facets, 12 per cent outside this area, and 52 per cent indeterminate. F. W. Hinds (1942) concluded "that the friction, caused by the rubbing of the contact surfaces, prevents caries is not always true. It is apparent that retention alone cannot cause caries and rubbing or friction of contact surfaces cannot assure prevention of caries."

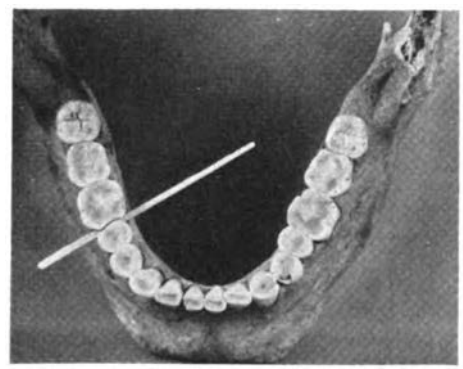


FIG. 62

Mandible of American Indian, about 300 years old, shows deeply worn proximal contacts of teeth, most marked between the right first molar and second premolar. All occlusal enamel is worn away including grooves and fissures by forceful mastication except right third molar.

The discussion of Beust's paper at the time of its presentation brought out the fact

that, in his examination of extracted teeth, he had no evidence that the facets were in actual contact at the time caries was initiated. This criticism seems valid, inasmuch as active eruption (Gamori and Orban 1938, p. 141) may extrude one tooth more quickly than its neighbor. This unequal eruption of teeth might change the relative position of the facets, permitting plaque formation and caries initiation.

The entrance to the cavity in this type of caries is comparatively large, making its diagnosis simple by means of dental floss or roentgenograms. Even though the majority are hidden between the teeth, they are not as treacherous as those originating in pits and fissures.

Histopathology of Enamel

Enamel caries: Normal, well-calcified enamel, viewed by transmitted light under the microscope, is highly translucent, showing only the faintest indication of its rod structure. The first effect of caries is to make the rods more visible. This change is caused by the abstraction of the more loosely combined mineral salts located in the enamel rod sheaths. The enamel then appears more opaque, often having a yellowish hue by transmitted light; by reflected light, it is a chalky white. Enright, Friesell and Trescher (1932) conducted a study of dental caries, involving the bacterial phase of the problem and the *in vitro* decalcification of teeth in relation to the caries lesions. Beust, according to their report, in a discussion of a paper by Bunting, remarked: "If you take a tooth with incipient caries that as yet exhibits no break in surface continuity and immerse it in lactic acid, you will be surprised to find that the sound enamel will be decalcified and the decayed part remains." Enright *et al.*, having corroborated this statement by decalcifying 30 teeth, state: "Whether the increased organic material in these carious areas consists of bacteria, parts of bacteria, bacterial products, mucous or what not, it seems that the ability of the mass to protect the inorganic elements of the enamel from the action of acids explains why the natural carious processes often come to a halt, even when acid-producing bacteria are contiguous to the lesion." Bodecker (1927;—and Bodecker 1929) Gottlieb (1944 and personal communication) and Nuckolls and Frisbie (1946) observed a similar increase of the slightly affected organic matrix; it becomes more acid-resistant, takes on greater staining qualities (Bibby, 1932, 1935) and is not composed of bacteria. Gottlieb, Diamond and Applebaum (1946) in discussing this subject suggest: "The possibility or probability that the enamel at the site of caries was originally in a deficiency state of calcification of varying degrees becomes a very important consideration."

Applebaum (1935) in extensive studies of enamel caries showed that these lesions are not identical in all teeth. He concurs with Beust (1934a) that sclerosis of dentin and enamel is part of the mechanism of caries resistance, reducing permeability, and regards this characteristic as one of the controlling factors in tooth resistance to caries activity. Applebaum adds that artificial caries produced by Enright *et al.* (1932) did not show the same reaction zones noted in caries of vital teeth. This subject is discussed in greater detail below. Applebaum (1938) also believes that a mechanism of resistance is indicated which may operate through the saliva or the pulp when a reduction of caries occurs with a protective diet. However, he feels that his roentgenographic studies cast doubt on the possibility of salivary remineralization of incipient caries lesions. He presented evidence of variable tissue resistance, having a wide variation in appearance of carious enamel, and concludes that passive resistance to acid may be due to enamel sclerosis.

Mummery (1926) observed that whatever the alteration in the enamel may be, there appears to be a distinct change of structure which is not due to decalcification and is

intimately associated with the decayed area, indicating that enamel cannot be a dead, inert substance, but is capable of reaction to stimuli and is therefore, like other tissues of the body, endowed with vitality.

Enamel proteolysis: The destruction of the mineral structure of the enamel by an acid, first described by Miller, has been mentioned previously; here the newer concept of the bacterial destruction of the organic enamel matrix will be considered in some detail. The foundation of this concept is the presence and condition of the organic enamel matrix. Hodge (1944, p. 55) places it at 1.7 per cent plus 2.3 per cent water. Deakins and Volker (1941) state the average amount "based on total nitrogen determination was found to be 0.49 per cent–1.95 per cent." This small percentage may seem negligible in comparison to the bulk of mineral elements of the enamel. However, Bodecker (1924–1926) showed that when comparing the organic with the inorganic constituents of the enamel by volume instead of by weight, it is evident that the organic can completely permeate the mineral elements, just as is the case in silicious sponges. This fact is demonstrated by taking a stone (33.72 grams) and a sponge (0.21 grams) of equal size, representing respectively the mineral and the organic elements of the enamel (fig. 63). An analogy has been drawn (Bodecker 1924–1926) between enamel and a brick wall, in which the bricks represent the enamel rods and the mortar represents the cement substance. Let us assume that some agent is able to destroy the mortar: the result would be the crumbling of the wall. A similar condition may occur in the enamel by the destruction of the organic matrix.

A review of the literature of the organic enamel matrix shows that for many years after Miller's chemico-parasitic theory of dental caries was set up, the enamel was presumed by most investigators to be an entirely mineral structure (Williams 1896) in spite of the chemical analyses which at that time fixed the quantity of organic material at about 3 to 5 per cent. Even so, the existence of the organic enamel matrix was not accepted for some time, in spite of the fact that Bodecker isolated it in 1906.

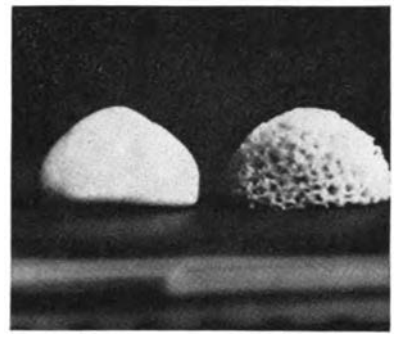


FIG. 63

Comparison of the volume of organic and inorganic elements in enamel to a stone and sponge of *equal* size. Stone weighs 33.73 gms; sponge: 0.31 gms, i.e. less than one per cent of stone. Hence 2 to 3 per cent of organic enamel matrix is equal in volume to mineral elements.

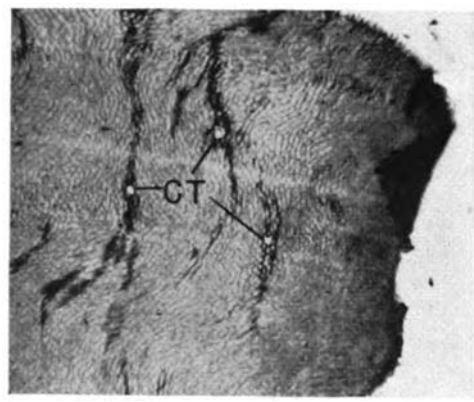


FIG. 64

Section of organic enamel matrix prepared by celloidin decalcifying method; carious tufts (CT) honeycombing enamel in advance of actual cavity formation.

This opened up the possibility that not only is acid a factor in enamel caries, but also proteolytic bacteria may destroy the organic matrix (fig. 64). An observation was made at this time (Bodecker 1906) that the organic "enamel lamellae frequently being the

point of entrance of bacteria, are pathologically significant in the origin of caries" (fig. 65). Figure 66 shows a permeable enamel lamella through which silver nitrate penetrated from the surface of the tooth

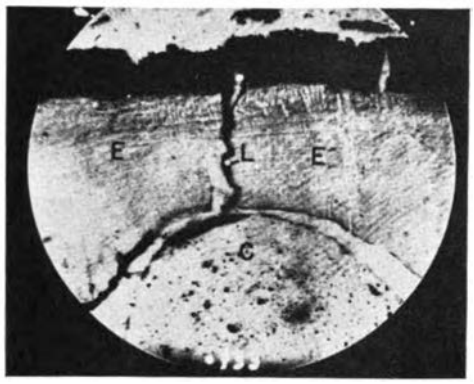


FIG. 65

Bacterial penetration through enamel lamella (L) leaving enamel (E) unaffected, causing deep extensive caries (C) in dentin. (Courtesy *Dental Review*, 1906.)

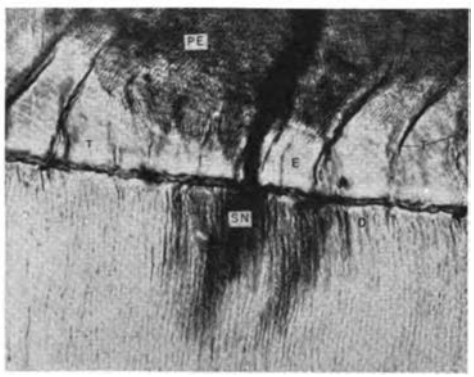


FIG. 66

Ground (undecalcified) section showing highly permeable enamel lamella through which silver nitrate (SN) has penetrated the enamel from the surface to dentin (D). Surrounding enamel is permeable (PE) making this area vulnerable to caries; tufts (T).

into the dentin, demonstrating the danger to the tooth if these structures are permeable. Baumgartner (1910) observed a type of protozoa and cocci in caries and concluded that these are active in the destruction of the enamel. Fleischmann (1921) regarded the enamel lamella as an entrance for coccal forms of microorganisms and believed that these are the only true cause of enamel destruction.

The investigation of enamel caries was carried on (Bodecker 1927) and the following observation made: "The organic matrix plays a role in the caries of the enamel. How great or how important this role is, further research will decide. It is, however, clear that the disintegration of the enamel is not such a simple matter as previous investigators had thought. Past theories

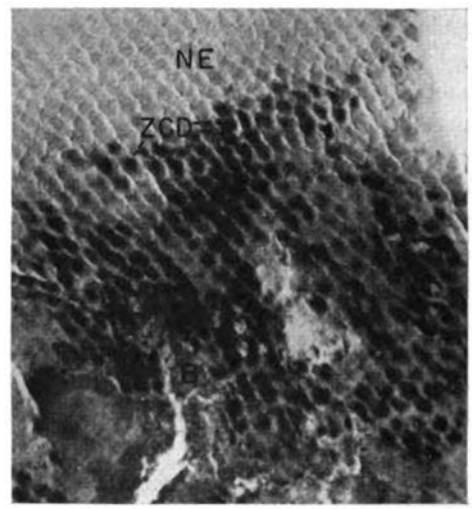


FIG. 67

Decalcified section (4 microns) of carious enamel; bacterial mass (B); zone of carious degeration (ZCD); normal enamel (NE); stain Heidenhain ferric haematoxylin.

considered the carious destruction of the enamel to be analogous to the melting of a lump sugar in a cup of tea." Further, it was shown (Bodecker and Bodecker 1929) that streptococci or diplococci sometimes penetrate in advance of actual cavitation during enamel caries. Figures 67 and 68 are photomicrographs of specimens from the original articles (Bodecker 1927; —and Bodecker 1929). These are exceedingly thin sections (3–4 microns) of carious organic matrix. It was observed (Bodecker 1927) that "the enamel in the immediate vicinity of the

bacteria does undergo some change preparatory to its destruction by these agents." This affected area was named the "zone of carious degeneration." After noting the progressive changes, up to complete destruction, in the enamel rods and rod sheaths on the approach of the bacteria, the process was divided into four stages. At this time also, attention was called to the role of the organic enamel tufts (fig. 69), which offer channels for bacterial penetration of the enamel from the dentin, thus facilitating backward or secondary caries. The authors concluded: "Enamel is destroyed during dental caries by two agencies:

(a) by acid, in all probability lactic acid, formed by *B. acidophilus*, and

(b) by proteolytic action of bacteria (streptococci or diplococci ?) on the protein content."

This concept was so different from accepted views that these observations received no consideration.

Pincus (1935) then took up the subject and maintained that the destruction of the organic enamel matrix was the sole factor in enamel caries, presenting the following evidence. He (1944) subjected non-carious teeth to proteolytic bacteria in a medium of cooked meat, observed that "enamel becomes deeply pitted," and concluded that "(1) bacteria capable of attack on enamel without the aid of acid do exist in the mouth; (2) they are found in carious lesions; and (3) they can produce lesions resembling clinical caries". He emphasized further that "the medium used—cooked meat—was tested at the start of the experiment and each day thereafter at least once. The hydrogen-ion concentration remained throughout at 7.4 and 7.8, that is, alkaline. Acid formation could of course follow bacterial attack on carbohydrates, and work on this point showed that the carbohydrate in the media was low in the first place, and any present was destroyed in the preparation of the meat for use as a bacteriological medium." He states further (pp. 159, 238), "anerobic bacteria appear responsible for the destruction of the organic enamel matrix."

Gottlieb and E. C. Hinds (1942) continued the investigation of enamel caries and observed: "Two groups of microorganisms seem to cooperate in producing caries, proteolytic groups producing a yellow pigment, and acidogenic groups showing up as an acid (in enamel, transverse striations; in dentin, shrinkage)." Gottlieb (1944b) reported also on the proteolysis of the enamel

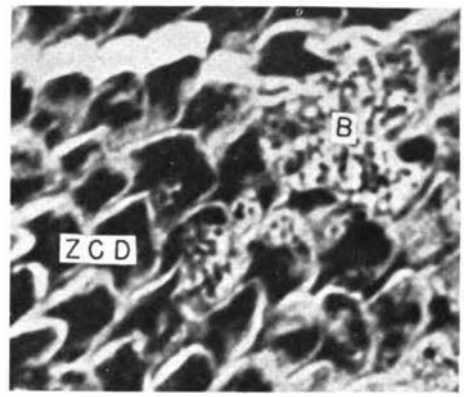


FIG. 68

A small confluent zone of bacteria (B); enamel rods have undergone carious degeneration (ZCD); 4 micron thick section; Heidenhain ferric haematoxylin.

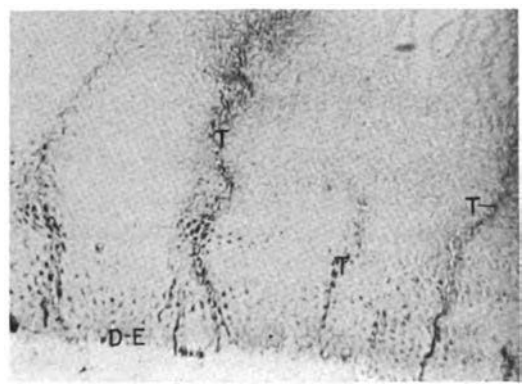


FIG. 69

Backward or centrifugal caries, passing from dentino-enamel junction (D–E) outward along organic channels, the tufts (T); 3 micron thick section, Heidenhain ferric haematoxylin.

rods and rod sheaths, dividing the process into four stages. Previously a similar classification was made (Bodecker 1906; and Bodecker 1929). However, these authors differ on certain details. Further light was thrown on the enamel lamella and its role in dental caries (Gottlieb, 1947). This structure was shown attached to a bacterial plaque on the surface of the enamel. The conclusion was reached that "the tenacious plaque does not cause caries, but a plaque becomes tenacious after caries has begun." Some further discussion of enamel caries will be included below under dentin caries (Gottlieb 1944b, 1947; — *et al.*, 1946).

Frisbie, Nuckolls and Saunders *et al.* (1944, 1947; Hurst *et al.* 1948) also draw definite conclusions as a result of their extensive observations of carious and noncarious organic enamel matrix. They find that gram-positive microorganisms of spheroidal shape are exclusively present in the enamel matrix at a distance from the actual carious break in the enamel. On the basis of their observations, they offer this working hypothesis: "Caries is primarily a degradation of the organic matrix, resulting from the enzymatic action of microorganisms, which is followed by a subsequent physical disintegration of the inorganic salts."

It is evident that the observations of ten investigators, listed chronologically, viz., the Bodeckers (1906, 1927, 1929), Baumgartner (1910) Fleischmann (1921), Pincus (1935, 1944), Gottlieb and Hinds (1942; Gottlieb 1944a, b, 1947) Frisbie, Nuckolls and Saunders (1944, 1947; Hurst *et al.* 1948) on enamel proteolysis open up an entirely new field of research in dental caries. This problem is not solved by an means, and much could be added concerning controversial points. Sufficient evidence has been presented, however, to establish the possibility of proteolytic destruction of enamel as a factor of enamel caries.

Enamel cuticle: Kronfeld (1933, p. 71) states that the presence or lack of a well-developed, hornified cuticle (figs. 7, 8) on the enamel surface seems to be an important contributing factor in the varying susceptibility of different individuals to dental caries. This subject is also discussed by Bibby and Glickman (1941) in noting caries of the cementum stopping short of the closely adjacent enamel, which suggests that the cuticle offers greater resistance to attack than does the cementum. This observation may be true, even though the cuticle is soon worn from the occluding surfaces of the teeth and from areas subject to vigorous brushing. A well keratinized enamel cuticle may well offer important protection to the most susceptible areas where it is long retained, i.e., enamel fissures and proximal tooth surfaces.

Enamel lamellae: Enamel lamellae were mentioned in connection with enamel proteolysis; brief consideration must be given here to their physical character. These are important organic structures in dental caries activity which, when permeable, may greatly weaken enamel resistance. Bodecker (1906) (fig. 65), Gottlieb (1921) and Fleischmann (1921) called attention to the role of the enamel lamellae in the progress of caries from the tooth surface to the dentin. Furthermore, Gottlieb, as well as Bibby (1932) emphasized the importance of the hornification of the cuticle as a protection against dental caries. Since then, the role of the enamel lamellae in the caries problem has also been studied and emphasized by Orban (1928), Barker (1931) and more recently again by Gottlieb (1944a, b). The physical state of the enamel lamella appears to determine its reaction to the penetration of acid or bacteria and their products. Lamellae are keratinized to varying degrees, making some highly resistant and impermeable to carious penetration while others are permeable and vulnerable, thus weakening the enamel.

Enamel resistance to caries: Bibby (1932) recalls the fact that keratin is a relatively indestructible substance and that biologically it is destroyed actively only by the

action of certain fungi and actinomyces. He showed by experimental evidence that enamel keratin shares this property of resistance to bacteriological destruction, and he suggests that this, with other factors, at times limits the advance of enamel caries. He observes further (1935) that hypoplastic teeth often outlast normal neighbors and concludes that the organic cuticle, lamellae, and rod sheaths, rather than the content of inorganic salts, are significant.

E. C. Hinds (1942) suggests the possibility that lamellae may also be impregnated with some protecting element of the saliva. He discriminates between the different kinds of resistance of enamel against invasion of microorganisms: (1) transparent enamel, a resistance inherent in the rod sheaths, and (2) conditions of lamellae, permeable and impermeable.

Kitchin (1941) attributes the varying rate of enamel destruction to areas of lesser calcification; these present less alkaline material which might neutralize acid activity. He lists them as enamel tufts, lamellae, spindles, and striae of Retzius, and states that enamel, especially the tufts, is more poorly calcified in young teeth, at which time caries is also more rapid.

Gottlieb (1944a) likewise points out that enamel caries advances faster in the less calcified areas. The alternately more and less calcified incremental lines of Retizus offer a varying resistance to caries. Hodge and McKay (1933) observed that the surface zone of the enamel is hardest, while the more internal ones are softest.

Histopathology of Dentin

The microscopic examination of carious lesions of the dentin prominently shows the dentinal tubules filled with microorganisms (fig. 70). These are regarded as acid-forming because of the decalcification of the surrounding dentin matrix. As this becomes softened, the dentinal tubules increase in diameter as a result of the steady growth of microorganisms. The tubules continue to enlarge, coalescing with neighboring ones and eventually breaking down the dentin matrix. The destructive process proceeds principally towards the pulp; however, at the dentin-enamel junction it progresses laterally, attacking and undermining also the enamel ("secondary or backward decay") causing its fracture and thus enlarging the entrance to the cavity.

Caries of the dentin has been given more attention than that of the enamel ever since the time of W. D. Miller (1890). In this connection, Gottlieb (1944a) supports the concept of two types of dental caries and states: "Caries is apparently the result of

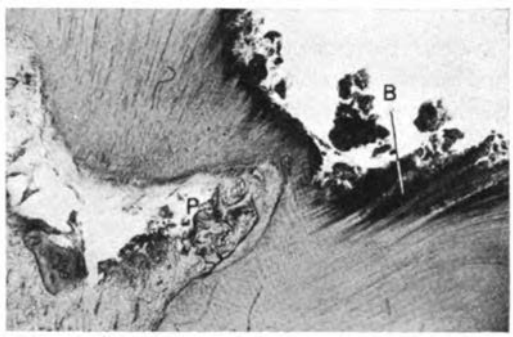


FIG. 70

Dentin caries of a maxillary central incisor with bacteria (B) penetrating towards the pulp (P) along the dentinal tubules; only little secondary dentin has been formed.

action of invading proteolytic microorganisms along the protein roads of the tooth tissues. Their presence is indicated by the appearance of a yellow pigment. Acid-producing microorganisms may accompany them to a varying degree. Acid producers can be located in the enamel by appearance of transverse striations, and in the dentin by shrinkage. Invading a lamella, the yellow pigmentation forms the spearhead, spreads into the dentin undermining the enamel. The enamel, being more resistant to invasion, may exhibit a layer of intact tissue outside the yellow dentin (undermining dentin caries proper). If, however, the micro-

organisms invade the narrow roads of the prism sheaths, the acid producers form the spearhead. When they reach the dentin, they apparently increase in number, due to better living conditions, and produce enough acid to make the dentin shrink away from the enamel. If the overlying enamel closes tightly, a vacuum is created. The circulating dental tissue fluid is here the only material which can be aspirated. It stagnates and coagulates. That offers excellent living conditions and the invasion of enamel and dentin starts from that breeding place. In the beginning, dentin and enamel show a smooth borderline towards the coagulated tissue fluid and only a trace of yellow pigmentation. The formation of a necrotic cavity is the final stage. The enamel breaks and the cavity is exposed. The enamel remains long unbroken because the dissolved calcium salts are replaced by the protein bodies of the microorganisms, making the enamel more resistant to acid (undermining dentin caries due to shrinkage)."

Differences in hydrogenion concentration in sound and carious dentin were defined by Grossman (1940), who established carious dentin as having 6.51 pH and noncarious dentin, 8.16 pH. He found only insignificant differences among the pH values of sound dentin, whether taken from carious, sound, or filled teeth.

Light has also been cast on the changes in carious lesions through microanalyses performed by Manley and Deakins (1940). They observed: "Early workers discovered that carious dentin is extensively decalcified, but absolute changes in water, organic and inorganic content have never been measured. The principal difficulty has been the small size of samples obtainable; entire carious lesions weigh only a few milligrams. Suitable micromethods are now available for this investigation." They summarize: "Microdeterminations of volume, water, organic and inorganic constituents have been carried out on normal dentin and certain parts of carious dentin lesions. When calculated on the *volume percentage* basis, the results indicate that there are 3 physically and chemically distinct zones within the carious area, which are characterized by:

"1. Normal hardness and decrease in organic volume amounting to $\frac{1}{8}$ of the original;

"2. softening and inorganic loss varying from $\frac{1}{5}$ to $\frac{1}{3}$ of the original with but slight decrease and constant organic volume;

"3. sponginess and major losses of both organic and inorganic material.

"In all zones a gain in moisture compensated for the volume losses in the other constituents."

Mention was made that Tomes (1859) occasionally observed a transparent zone between the carious lesion and the vital dental pulp (the transparent zone of Tomes); thus he appears as a pioneer in describing dentin changes resulting from the irritation of caries. Miller (1903) also perceived a transparent area in the dentin immediately under superficial caries of the enamel. Kronfeld (1933, p. 89) observes that "in the periphery of the carious lesion, transparencies are always found, if the tooth under consideration has a vital pulp (fig. 38). The amount of transparency depends wholly on individual circumstances, age of tooth, condition of pulp, rate of progress of caries. In that respect, transparency of the dentin is rather similar to secondary dentin formation: both occur as a reaction to irritation or injury; both consist of the formation of a calcified barricade; both occur more extensively if the tooth is young and the progress of irritation is slow."

Penetration of the carious process in dentin causes the development of a number of zones, in addition to that of transparency, between the base of the carious cavity and the vital pulp; (Kronfeld 1933, p. 93; Williams 1896; Bodecker and Applebaum 1930, 1931, 1933; Beust 1933, 1934a, b).

The FIRST zone at the base of the cavity,

farthest removed from the pulp, is completely decalcified.

The SECOND zone is partially decalcified and may be slightly opaque or transparent when observed by transmitted light under the microscope.

The THIRD zone is hypercalcified and transparent.

The FOURTH zone contains dentinal fibers which have undergone sclerosis or protective metamorphosis.

The presence of the sclerotic zone surrounding a cavity in a carious, vital tooth has usually been established on the basis of stain penetration (Beust 1931a; Bodecker and Applebaum 1930). This method is not infallible. As demonstrated previously, metamorphosed areas in dentin which often are not hypercalcified may be impermeable. But evidence is also presented concerning the sclerotic nature of this zone by means of the Grenz-rays (figs. 71, 72). Figure 71 is a photomicrograph of a tooth with caries entering outside of the plane of the section. A sharply localized zone of metamorphosis (M) or opaque dentin is noted near the dentin-enamel junction. Figure 72, a Grenz-ray photograph of this section, shows a definite radio-opacity, corresponding to the metamorphosed zone of figure 71. This radio-opacity indicates a zone of hypercalcification. A true conception of the form of this sclerotic zone may be obtained by its visualization in three dimensions, in which case it is a calcified sphere surrounding and walling off the carious area from the sound dentin.

Still further evidence is presented by Hodge and McKay (1933) who made extensive tests of the varying hardness of different areas of dentin and observed that immediately beyond the region of caries, in the transparent zone of Tomes, the hardness is twice that of normal, healthy dentin.

Beust (1931a, 1934a) differentiated between two translucent areas in dentin: one just described, due to hypercalcification resulting from pulp activity, which retards the advance of caries; and another, nearer the carious lesion, which is greatly decalcified. Even though both are translucent, Beust showed by *in vitro* stain penetration

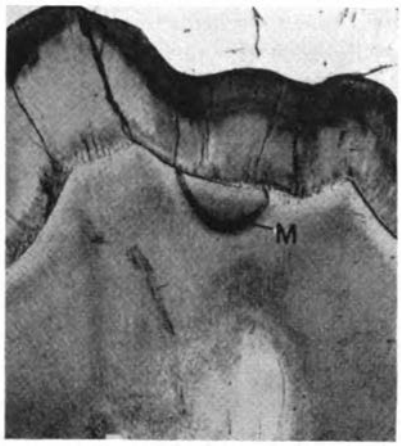


FIG. 71

Localized metamorphosis (M) caused by carious cavity outside of plane of section. (Courtesy of E. Applebaum).

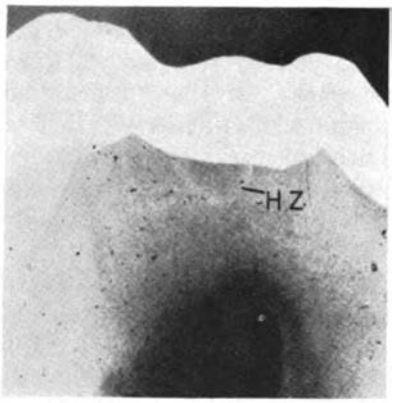


FIG. 72

Grenz-ray (soft X-ray) of specimen (fig. 71) has radio-opaque ring indicating hyper-calcification (HZ), the fourth zone of caries. Under high magnification dentinal tubules show a precipitation of mineral droplets.

that the former does not take a dye ("chromophobic") and the latter area stains deeply ("chromophillic").

Concerning the formation of the various zones around carious lesions, Kronfeld (1933,

p. 94) states that "stages 3 and 4 are the result of disturbed metabolism in Tomes' fiber as a reaction to the irritation; stages 1 and 2 are the result of the actual bacterial invasion and decalcification of the dentin." Kronfeld makes another observation which throws light on the physiologic activity of the dental pulp: "It is interesting to note that stages 1 and 2 can be observed in pulpless teeth. The fact that stages 3 and 4 are absent is evidence that changes observed in the last-named stages are due to a vital reaction of the dentin to external irritation." Beust (1931b) likewise believes that the reaction of the dentinal fiber to external irritation shows that caries cannot be regarded as a simple proteolysis of dead tissue and that dentin sustains certain phases of metabolism.

Variable Resistance of Teeth to Caries

The rate of caries penetration differs in both enamel and dentin, dependent, to some extent, on their permeability. This factor appears determined by secondary dentin formation and by sclerosis and protective metamorphosis of the primary dentin. Applebaum (1935) observed that enamel caries does not progress with equal rapidity in all teeth and adds the translucent band around incipient lesions as a retarding factor in enamel destruction.

Beust (1934b) observes that "sclerosis is an expression of factors of resistance (to decay) possessed by a tooth. . . . A tooth's resistance to caries or other tooth-destroying agents increases in proportion to the amount of its sclerosis."

Bibby and van Huysen (1933) state: "The organic structures of the enamel surface seem to undergo a change which results in their pigmentation and in an apparent alteration in the density of the enamel rod sheath;" also that "a thick, somewhat structureless crown layer of unknown composition" and "a bacterial mat" are formed.

"As a result of the superficial changes described, the surface becomes resistant to the destructive action of acids and bacteria. This resistance probably protects the deeper portions of the enamel from decalcification and thus could act as a defense against dental caries."

Younger and Gottlieb (1946) observe that some untreated carious cavities do not extend to the pulp for years: others reach the pulp in a short time. They ascribe this difference in rate of penetration to the hypercalcified periphery of the lesion, formed in the presence of acidogenic microorganisms.

The permeability of primary dentin remains comparatively high during the first 10 years after the tooth appears in the mouth (Beust 1931a; Bodecker 1931; Lefkowitz 1942). Coincidental with this high permeability is a high activity of dental caries in the teeth of children and adolescents. This may be no mere chance, as permeability and dental caries appear interrelated. High permeability of the dentinal tubules results in rapid penetration from without; low permeability reduces the rate of penetration. Fish (1930) observed a definite checking of caries penetration at the junction of the primary and secondary dentin. Frequently the carious process proceeds so rapidly, as a result of an insufficient reduction of permeability of the secondary dentin, that pulp involvement occurs. On the other hand, caries may approach so gradually that sufficient secondary dentin of a less permeable character is laid down for its protection. Kronfeld (1933, p. 88) states, relative to the progress of caries: "It seems very reasonable that the slower progress of caries in dentin in old teeth may have something to do with the renewed calcification and decreased organic content of old dentin". (This concept is still controversial.)

Benzer and Bevelander (1942) examined 77 carious lesions for the purpose of determining the frequency of secondary dentin formation. One of their conclusions is: "When the incidence of secondary dentin is examined in reference to the relative depth of the lesion, we find that the deep, medium

and shallow lesions reveal the presence of this tissue in the ratio of 72 per cent, 52 per cent, and 36 per cent respectively. These figures indicate a definite relation between the depth of the lesion and the probable presence of 'pathological' secondary dentin."

The relative caries activity in vital and pulpless teeth was examined by Feldman and Lefkowitz (1942). They state: "A roentgenographic examination of teeth of 112 individuals, aged 30 to 74, was made for cervical proximal caries in vital and pulpless teeth. Cervical proximal caries was the lesion of choice because the element of error in diagnosis from the roentgenograms is minimal. The results indicate that the incidence of cervical caries is significantly less in pulpless teeth." It seems evident that the eventual decrease in permeability of pulpless teeth is one retarding factor in their carious destruction.

Mellanby (1927) observed experimentally that in dogs a diet rich in vitamin D results in the formation of a large amount of well calcified, secondary dentin and that the amount and character of secondary dentin formed is a measure of resistance of the living tooth. She observed that, where the teeth are well calcified and yet carious, 47 per cent have either no secondary dentin or badly calcified secondary dentin, indicating *increased resistance* to external stimuli after eruption during the period of some external stimulus. It appears evident, therefore, from this and other observations, that the thicker the layer of secondary dentin and the fewer its tubules, the slower is the penetration of the carious process. Physiologic changes in dentin, hastened by mild external irritation (such as chronic dental caries, attrition and abrasion) obviously impede the progress of dental caries. These changes include dentin sclerosis and protective metamorphosis, as well as the formation of secondary dentin.

The recent observation of Ellison and Halpert (1947) may throw further light on the caries resistance noted in metamorphosed dentin. By micro-methods they analyzed young and metamorphosed dentin of 18 permanent teeth and one deciduous tooth and found the following: "From the analytical results, it appears that some inorganic substance which is not calcium or phosphorus is being incorporated into the dentin to produce the change which has been noted above. The nature of the substance which is being added is not indicated." The identification of this unknown material may explain why metamorphosed dentin is more resistant than young dentin to caries over and above the resistance caused by its reduced permeability.

The significance of symmetrical incidence of caries is stressed by Strusser and Dwyer (1932). In an examination of 942 school children, ranging in age from 5 to 12 years, they observed some evidence to substantiate the view that pairs of simultaneously formed teeth had a similar reaction to caries. This observation appears to stress the importance of a good nutritional status during tooth formation.

Mention was made that caries occasionally attacks only one of two closely contacting teeth. It was observed (Bodecker and Ewen 1937) that 179 unilateral carious lesions were noted in 516 mouths. One explanation is that, since some of these teeth had been calcified at different periods, one might be improperly formed as a result of nutritional disturbances, while the other might be calcified normally during a period of good health. However, the five pairs of maxillary incisors (figs. 73, 74) were developed simultaneously and the above explanation cannot apply. The fact that the pulp of the carious tooth (fig. 74) is well-defined, while the non-carious one appears almost obliterated with secondary dentin, again suggests that this structure increases tooth resistance to caries.

Another factor in the activity of unilateral dental caries is that the enamel of one tooth may have a permeable lamella, causing it to become carious readily, while the closely adjoining tooth remains intact.

Still another factor in caries resistance of individual teeth is presented by the observations of Crowell, Hodge and Line (1934)

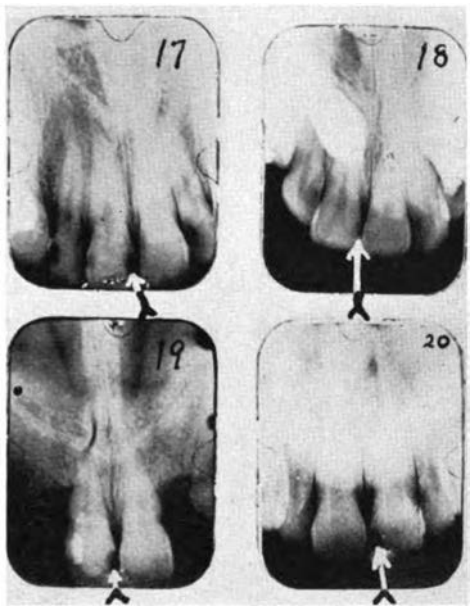


FIG. 73

Roentgenogram of four pairs of central incisors with unilateral caries (arrows).

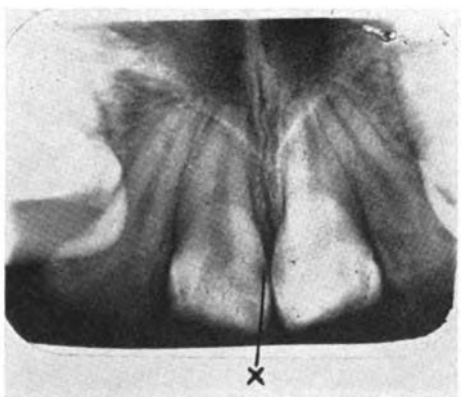


FIG. 74

Two incisors with unilateral caries suggest benefits of secondary dentin; the non-carious tooth shows pulp calcification while in the carious one (X), pulp is clearly visible.

who analyzed 130 teeth for "percentage values of water, inorganic residue, P, Mg, and Ca, as well as 'basic excess' ". They observed that "variations in composition of teeth from the same mouth were as wide as for teeth from different mouths." These variations appear indicative of the effect of systemic conditions during tooth formation and of differences in pulpal activity in individual teeth after their eruption; the latter has not been substantiated.

Previously presented evidence shows that reactions in dentin and enamel occur only in the presence of a vital pulp. The stimulation of the forceful chewing of detergent foods increases the blood supply of a normal pulp, therefore its function should also improve. However, no experimental evidence is available in support of this concept.

Extreme examples of forceful chewing of food are recorded by Waugh (1930) in his gnathodynamometric studies of the primitive Eskimo. He reports a biting force up to 330 pounds, while Brekhus, Armstrong and Simon (1937) recorded the average of only 126 pounds in athletes of the University of Minnesota. The attrition of the teeth of the Eskimo is excessive and it is possible that their low caries incidence may be explained in part by energetic mastication.

Wallace (1934) as mentioned previously stresses the physical cleansing of teeth of food debris by forceful mastication, but a consideration of the anatomy of the teeth and their relative position in the arch demonstrates clearly that mastication, no matter how forceful it is, can remove food debris from the free surfaces only. The contacting proximal surfaces, on the other hand, cannot be cleansed in this manner.

Turesky and Bibby (1944) advanced experimental evidence on this subject. They state in their summary: "Tests on the elimination from the mouth of artificially introduced yeast indicate that, in descending order, chewing apples, sticky candy, chewing gum, paraffin and peach were more effective than tooth brushing in cleansing the mouth.

"Preliminary tests indicate that carrot and bread and butter greatly aided, but that prior use of mineral oil interfered with

the cleansing mechanism of the mouth. Intrinsic factors possibly including the rate of salivary flow seemed to be of more importance in determining the rate of removal of yeast than the nature of the cleansing agents used on a tooth brush."

Heredity and Dental Caries

There is wide variation in the degree of caries susceptibility. Clinical observations show that one family has an exceedingly high rate of caries, while another has an average or a low one. These observations have given rise to the impression that caries susceptibility or immunity may be inherited. Bunting (63) believes that in a small percentage of cases inherited tendencies or inherent characteristics may be more important factors in caries susceptibility than any ordinary dietary consideration. Hunt, Hoppert and Irwin (110) find heredity a factor in dental health of rats. They state: "A caries-susceptible strain and a caries-resistant strain of rats have been produced by selection, progeny testing, and close inbreeding. Heredity therefore is an important factor in the development of rat caries. . . . The susceptible line has become progressively more susceptible and the resistant line more resistant."

The above observation applies only to rats, but Klein (1946) has shown that the teeth of man may likewise be affected by heredity. He observed that in 1700 married couples of Japanese ancestry, "parents who have experienced the largest amount of caries have offspring who have approximately three times more caries experience than do the offspring (of same ages) of parents with the smallest caries experience."

Clinical and Histological Classification of Carious Lesions

Dental caries activity is commonly divided into acute, chronic and arrested caries. Meticulous observations of caries lesions disclosed the fact that they may vary considerably, not only as to form and location of the lesions and age of the person, but also as to the physical character of the partially destroyed dentin. Bodecker (1937) classified caries as (1) acute dental crown caries, (2) chronic dental caries, and (3) acute dental root caries.

Acute dental crown caries: This type (fig. 75) affects principally children and young adults up to about the age of 25 years. After this time, the intensity of the disease usually is reduced, even if caries previously has been rampant. The orifice of a lesion or cavity in a tooth affected by acute dental crown caries remains small for a considerable time after its inception (fig. 75c), giving no sign of the rapid destructive process within the tooth, which can soon involve a large part of the interior of the crown. A thick zone of decalcified dentin surrounding the cavity (fig. 75 DD) is another criterion of acute dental crown caries.

The conformation of the acute dental crown lesion in the dentin is that of a cone. The apex is directed towards the pulp with its base at the previous dentino-enamel junction (figs. 52 and 76) and resembles an infarct in bone. The shape of the enamel lesion also is that of a cone, but its orientation is different, depending on the character of the tooth surface on which the lesion occurs. In the smooth tooth areas, as those found on the proximal surfaces (fig. 52 B), the base of the carious cone is on the surface of the enamel and becomes narrower as it approaches the dentino-enamel junction to join the dentin infarct. On the other hand in enamel pits and fissures, commonly found on the occlusal surfaces of the posterior teeth and on the lingual surfaces of the maxillary incisors, the carious enamel cone is inverted and has its apex on the surface of the tooth (figs. 52 A, 76). Usually the dentin situated between the caries lesion and the pulp shows only slight metamorphosis (figs. 75, 76), permitting a rapid penetration of the destructive process through this permeable dentin.

Figure 77 illustrates the protective quality of secondary dentin (SD). This was formed

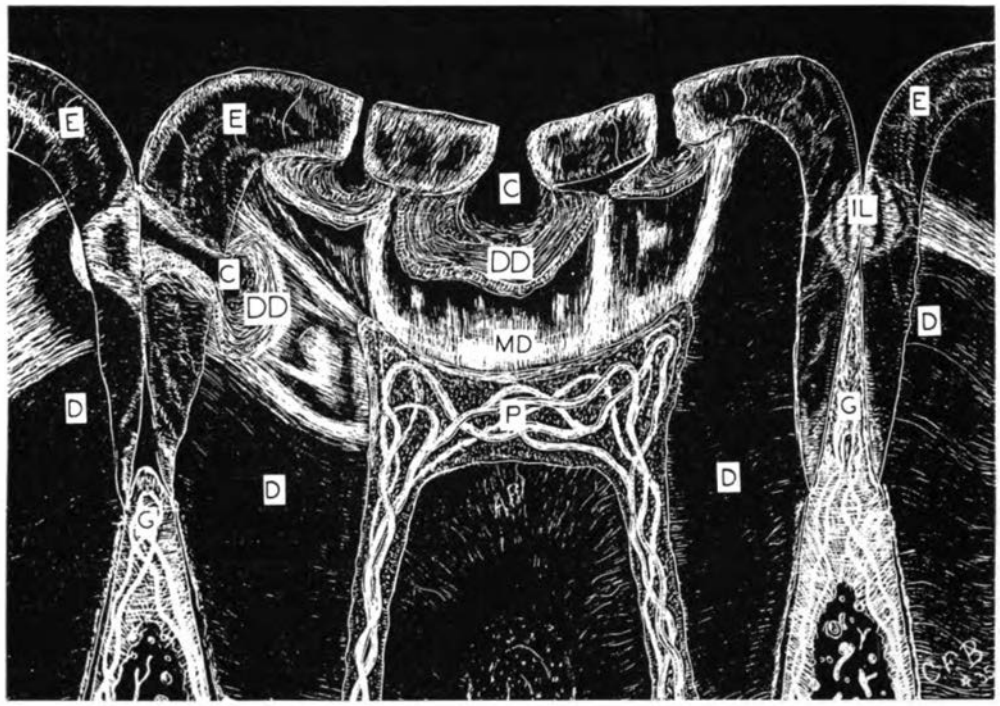


FIG. 75

Diagram of acute dental crown caries; cavity (C) has small orifice; thick decalcified dentin (DD) lining the cavity; only slight metamorphosed dentin (MD); pulp (P) with but little secondary dentin; enamel (E); incipient carious lesion (IL) at the proximal contact of neighboring tooth; gingiva (G).

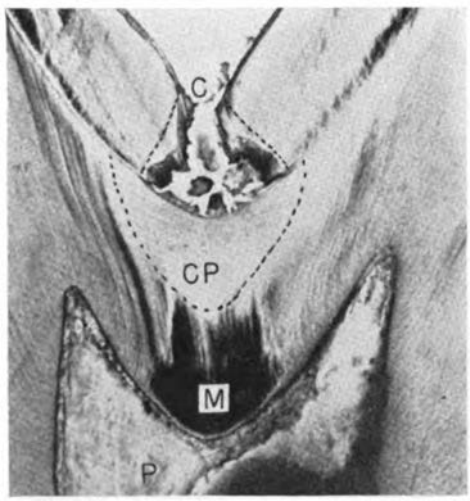


FIG. 76

Photomicrograph of acute crown lesion of caries; orifice of cavity (C) is small; carious penetration (CP) is deep, only slight metamorphosis (M) near the pulp; extent of dentin caries shown by dotted line.

as a result of long-continued and slowly progressing attrition (A), filling the pulp chamber. However, but little secondary dentin has been formed in the lingual area near the cervix, which thus lacks similar protection, and acute caries could therefore penetrate deeply, involving the pulp.

Acute dental crown caries affects first the enamel pits and fissures of the maxillary and mandibular molars and premolars, later the proximal surfaces of the teeth, then the maxillary incisors and canines, and lastly the anterior mandibular teeth. The appearance of carious lesions on the lower anterior teeth is a sign of rampant dental caries. As mentioned previously, if the cervical areas of the teeth become affected, particularly when this occurs before middle age, it indicates a high activity of dental caries.

Chronic dental caries: This type affects the surfaces of the teeth in the same order

as does that of the acute type but is far less severe, starting at the very beginning as a slow destructive process. The form of the lesion, shown in a diagram (fig. 78), is radically different from that of acute crown caries. It has a larger orifice even in its incipiency, which precludes undisturbed food retention; hence, saliva flushes out the cavity more readily, retarding acid formation in this type of lesion. The zone of decalcified dentin (fig. 78 DD) is thin, covering the hard calcified tissue below. The tubules in this area have undergone marked protective metamorphosis (MD), resulting in a great reduction of permeability. Consequently, caries proceeds so slowly that the pulp has ample opportunity to form a considerable amount of secondary dentin (SD), thus sealing many tubules leading from the lesion to the pulp. When chronic dental caries affects the proximal surfaces, contact with the neighboring tooth is often broken or lost, so that the patient becomes uncomfortably conscious of food impaction.

Extracted teeth with chronic dental caries affecting the occlusal surfaces are rare for a number of reasons: their removal is not indicated because fillings in such teeth are most permanent, caries not being active around their margins; or the surfaces have been worn down through attrition, so as to make them self-cleansing. Chronic caries on the cervical areas, on the other hand, is common (fig. 18). This tooth, an old one judging by the considerable attrition (A) and cementum formation (C), shows caries on the labial and lingual surfaces. In both instances, the openings of the cavities were large and secondary dentin formation protected the pulp.

Figure 79, a section of a tooth with acute enamel hypoplasia, shows two superficial carious areas (C, C′) and one somewhat deeper cavity (C″). The lesions are markedly non-food-retentive, a characteristic of chronic dental caries. The zone of decalcified dentin is very shallow in two lesions (C, C′) and somewhat deeper in the third (C″). Marked formation of interglobular dentin (ID) is present, which, in this case, did not hasten the destruction of the tooth.

Acute dental root caries: This type is located on the cervical areas of teeth of persons past middle age and affects almost exclusively the cementum and dentin in this area. The enamel may be destroyed by undermining, causing it to fracture. Occasionally the crowns of such teeth may either

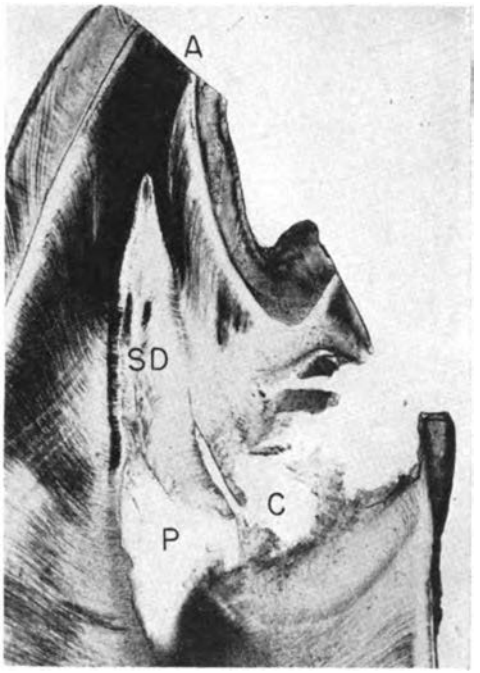


FIG. 77

Attrition (A) caused formation of secondary dentin (SD) in pulp chamber, but very little near cervix of tooth, permitting rapid and deep penetration of acute caries (C) involving pulp (P).

be completely non-carious or at most have small occlusal fillings. Figure 80 shows the form of the lesion in a diagram and indicates the insidiousness of its penetration towards the pulp. If persons chew their food thoroughly, attrition of the occlusal surfaces of the molars and pre-molars is so extensive that the proximal contacts may be lost, permitting the food impaction between the teeth (fig. 80). This forces the gingiva or

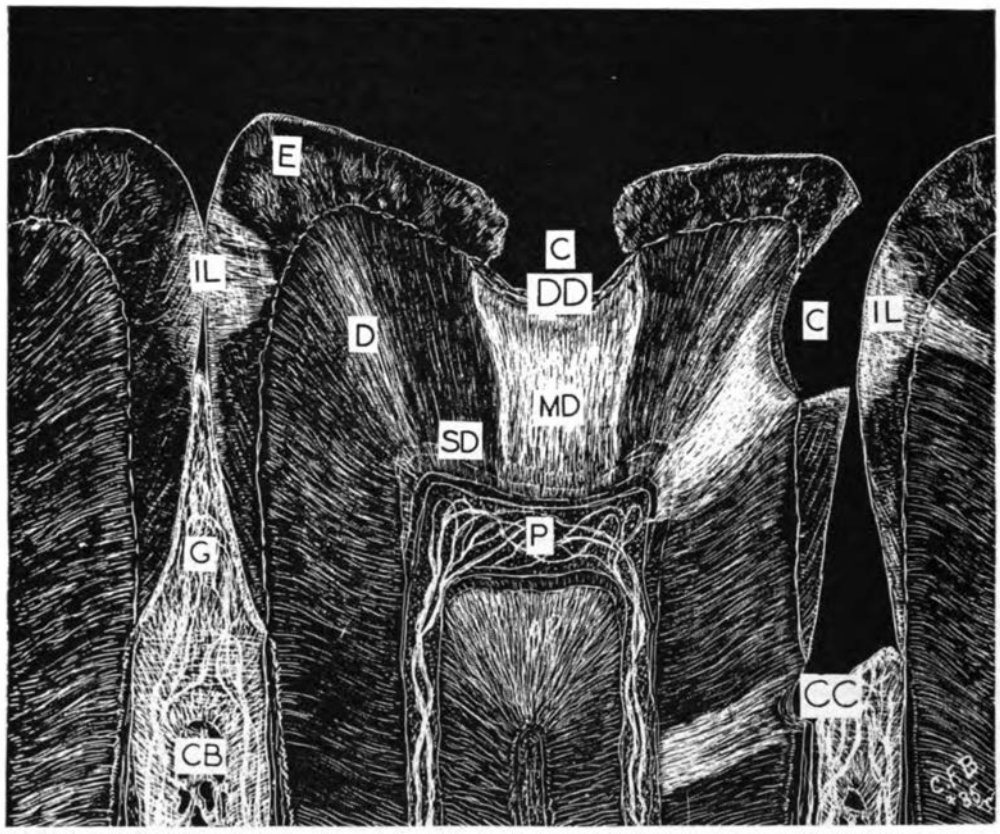


FIG. 78

Diagram of chronic dental caries. Orifice of cavity (C) large; layer of decalcified dentin (DD) thin; metamorphosed dentin (MD) is extensive between cavity and pulp (P); secondary dentin (SD); beginning of cervical caries (CC); initial lesion of caries of enamel on adjoining tooth (IL); gingival tissue or gum (G); cervical bone (CB).

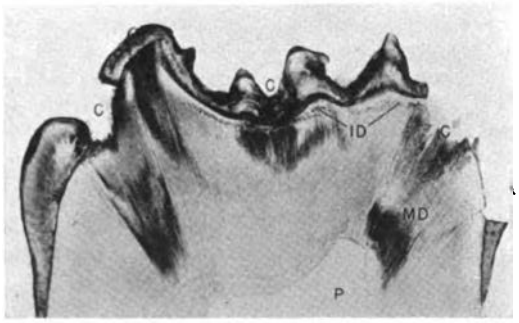


FIG. 79

Chronic dental caries (C, C, C) in hypoplastic tooth; metamorphosis between cavity and pulp fairly marked (MD); thin layer of decalcified dentin; interglobular dentin (ID).

gum tissue away from the cervices of the two teeth, permitting the carious destruction of the exposed cementum and dentin. Acute dental root caries penetrates faster than chronic caries and somewhat more slowly than the acute dental crown type.

The conformation of the lesion of acute dental root caries is similar to that of the acute crown type, showing deep penetration and a fairly small opening. Sometimes it is even difficult to discover these lesions, as the orifice of the cavity may be well hidden in the proximal area.

Figure 81 shows a small acute root caries lesion (C) while the crown dentin is non-

carious and highly metamorphosed. The pulp nodules (PN) indicate an abnormal pulp, due possibly to carious irritation.

An enamel fissure tunnel (fig. 82 F) was previously carious but later probably arrested, as indicated by a localized zone in the metamorphosed dentin. Of the two acute root lesions (1, 2), one is incipient, the other more advanced, as indicated by the decalcified dentin (DD) which has undermined the enamel. Secondary dentin (SD) has been formed as a result of the irritation.

Arrested dental caries: The carious process sometimes comes to a standstill as noted in figures 54, 60, and 82 F. It is not possible to determine the activity of dental caries from an examination of tooth sections. However, when whole teeth are studied, arrested caries can be determined more readily. Various reasons are ascribed as causing the arrest of dental caries, such as dietary improvement, change in bacterial flora, or improved oral hygiene.

The morphologic changes which occur in

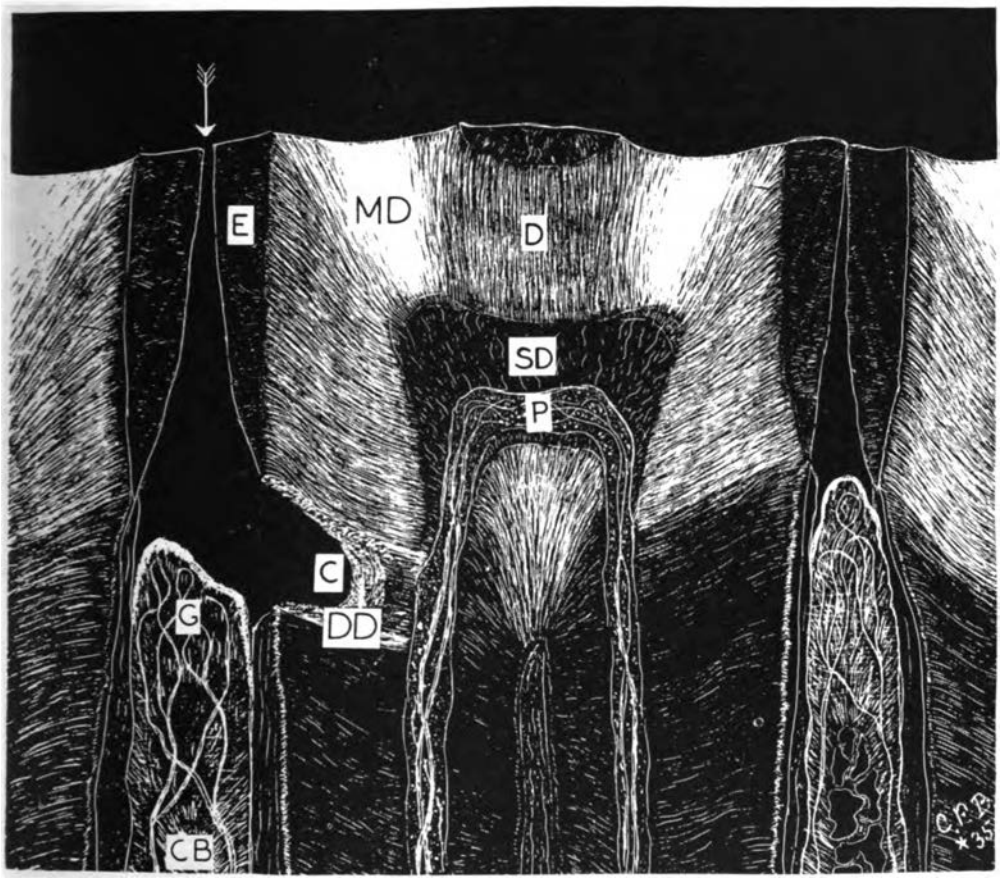


FIG. 80

Diagram of acute dental root caries; crown dentin highly metamorphosed (MD); extensive attrition of occlusal surface, also contact is destroyed with neighboring tooth, allowing food impaction resulting in recession of gingival tissue (G) from cementum at cervix, causing caries (C); decalcified dentin (DD) is fairly extensive; very little secondary dentin formation in this area; metamorphosed dentin slight between pulp (P) and lesion (C); in pulp chamber great mass of secondary dentin (SD).

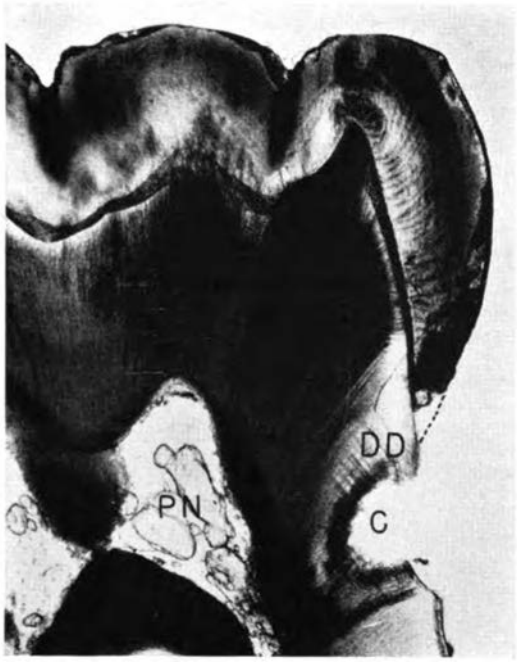


FIG. 81

Beginning of acute dental root caries (C); decalcified dentin (DD) undermining enamel (dotted line); very little secondary dentin formation, except pulp nodules (PN); excessive metamorphosis of crown which is non-carious.

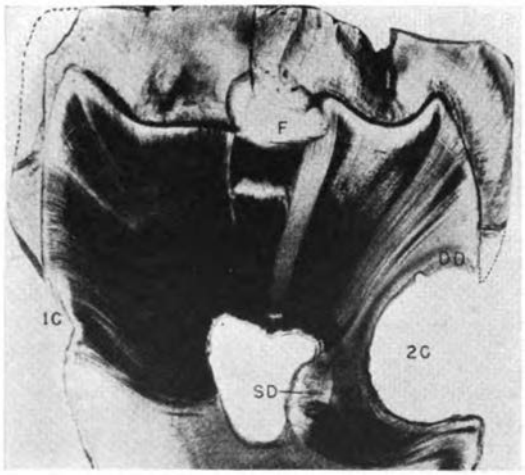


FIG. 82

Enamel fissure tunnel (F), with arrested caries; incipient (1 C) and advanced dental root caries (2 C) with resultant secondary dentin formation; crown highly metamorphosed; decalcified dentin (DD); secondary dentin (D).

arrested carious lesions have been under discussion for many years. The generally accepted view is that when active destruction stops, the zone of decalcified dentin is worn away by the force of mastication, thus exposing the underlying calcified dentin. Another view is that as a result of dietary improvement, the previously decalcified dentin recalcifies. Further evidence must be presented to substantiate this hypothesis.

A tabulation has been set up (Bodecker 1934) which shows in brief form the fundamental differences between the three types of dental caries from both a clinical and a histological standpoint (fig. 83).

Evidence has been presented of two groups of factors active in dental caries: exogenous and endogenous. These two factors are considered by some as being antagonistic, i.e., the exogenous are the attacking factors and the endogenous are the protective ones. This concept, commonly applied to infectious diseases, is illustrated in figure 84.

Distinction of Caries from Hypoplasia, Attrition, Abrasion, Erosion

Hypoplasia requires a brief discussion as it seems to have an indirect bearing on dental caries activity. This condition appears to be due to a disturbance of enamel and dentin formation, causing small pits or grooves on the surfaces of the enamel (fig. 85) or, in extreme cases, highly malformed teeth (fig. 86). The cause of hypoplasia has generally been ascribed to nutritional disturbances or exanthematous diseases active during tooth formation. Sheldon, Bibby and Bales (1945) examined 95 teeth microscopically from 34 patients with detailed medical histories and observed: "In more than 70 per cent of the cases there was a positive correlation between the time of formation of a band of definitely defective enamel and the existence of some systemic disability." They conclude: "Deficiencies of vitamins A, C and D and also of calcium and phosphorus were the commonest cause of defective enamel formation. Enamel defects were also noted in rela-

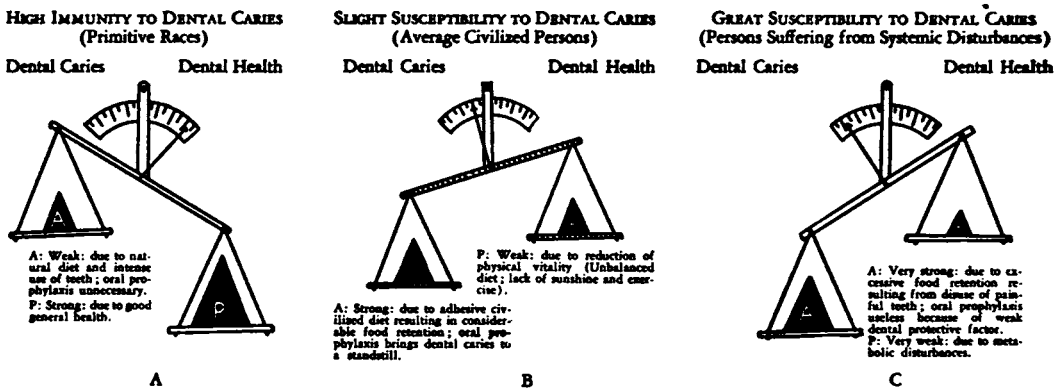
TYPES OF DENTAL CARIES*

	Acute Crown	Chronic	Acute Root
Frequency.	Exceedingly common.	Fairly common.	Not common.
Age at which most active.	During childhood and up. Fifteen to twenty-five years.	After twenty to thirty years.	After fifty years.
Distribution.	Chiefly in crowns of teeth at areas of food stagnation. In highly rampant cases also on free surfaces.	Usually in crowns but also in roots if these areas are accessible through detachment of gingiva.	Principally in roots; the crowns often have few or no fillings and are now more nearly immune to caries.
CONFORMATION OF LESION			
Occlusal surfaces.	Show very small orifice of cavity, slight destruction of enamel; deep penetration.	Show wider orifice, greater enamel destruction, shallow penetration.	Does not occur.
Approximal surfaces.	Show somewhat wider orifice of cavity but deep penetration.	Show extreme surface destruction, shallow penetration.	Rarely occurs.
Root surfaces.	Has not been observed to show acute caries.	Show extensive but shallow lesion.	Show small orifice and very deep penetration, usually in secluded areas at the cervices of the teeth.
DENTIN			
Rapidity of progress.	Rapid penetration, often with pulp involvement.	Slow penetration, pulp involvement more rare.	Fairly rapid penetration; pulp involvement frequent.
Sensitivity to instruments.	Very great.	Slight.	Moderate.
Thickness of decalcified zone.	Considerable.	Very slight.	Moderate.
Character and color of carious dentin.	Soft cartilage-like; a yellowish-white color; easily removed with spoon excavators.	Less soft; of a dark yellow, brown color or black.	Soft, leathery; of a brownish color.
ENAMEL			
Extent of partial decalcification.	Great; very friable and easily removed.	Slight.	Rarely occurs.
Configuration of cavity walls.	Extensive overhanging walls.	Slight, if any, overhanging walls.	

* The clinical data incorporated in this classification have been corroborated and approved by the following: L. R. Cahn, Associate Professor of Dentistry (Oral Pathology); H. Holliday, Assistant Professor of Dentistry (Periodontia); H. J. Leonard, Professor of Dentistry (Oral Diagnosis, Periodontia); E. C. McBeath, Associate Professor of Dentistry (Children's Dentistry); A. T. Rowe, Professor of Dentistry (Prosthetic Dentistry); L. M. Waugh, Professor of Dentistry (Orthodontia); D. E. Ziskin, Assistant Professor of Dentistry (Oral Diagnosis).

FIG. 83

tion to chickenpox, measles, pneumonia, pertussis, intestinal and gastric disturbances and upper respiratory infection in the children and toxemia of pregnancy in the



Diagrams to Illustrate Two Opposing Factors Active in Dental Caries

The exciting cause of the disease is fermentation of food débris and other factors affecting the teeth from the *exterior*. This is shown in the diagrams at the attacking factor (A). Opposed to this is the protective factor (P), a defense mechanism active through the dental pulp and saliva, dependent on systemic conditions. The scale demonstrates the balance between dental caries and dental health.

Fig. 84

(Courtesy: Nelson New Loose-Leaf Medicine, Chapt. I-A, pp. 15–20G, Fall 1935)

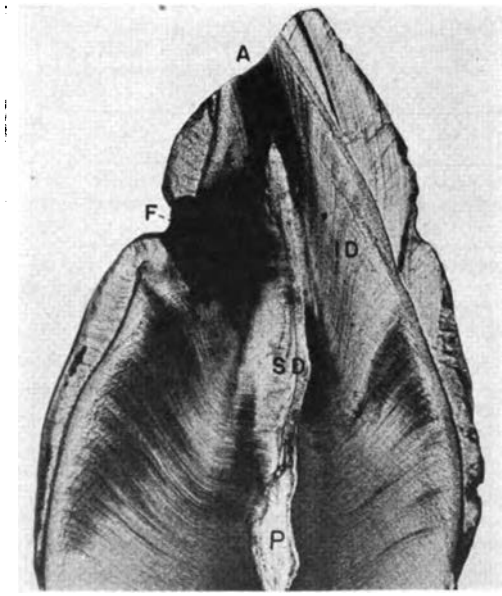


Fig. 85

Old hypoplastic anterior tooth, pitted labial surface, interglobular dentin (ID); extensive attrition (A) causing secondary dentin formation (SD); small filling (F) in lingual pit.

plements this with the following pertinent information: "Clinically these lesions (hypoplastic) affect the deciduous dentition as well as the permanent anterior and first molar

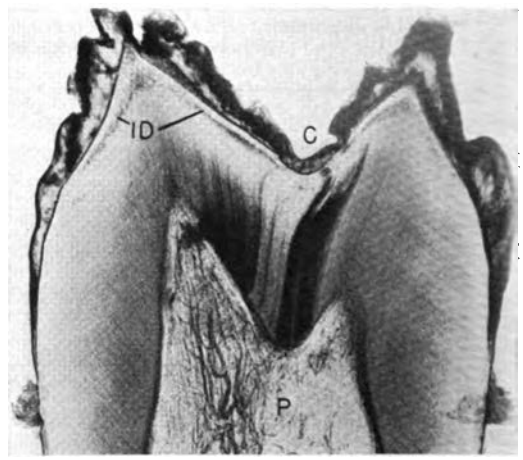


Fig. 86

Excessive hypoplasia of premolar with thin deeply pitted enamel and interglobular dentin (ID); only small carious area (C); pulp (P).

mother, but in practically all instances these conditions were superimposed upon an inadequate nutritional state."

Diamond (personal communication) supteeth. Rarely are the bicuspid or second and third permanent molar teeth involved. The histologic mechanism of hypoplasia reveals it to be a destruction of a group of

ameloblasts functioning at the time of a metabolic disturbance.

"The chronology of enamel formation of the particular teeth which are commonly affected by hypoplasia extends from the beginning of the second half of the intrauterine state to the 12th or 13th month of life. Metabolic disturbances must therefore operate during this particular period to produce the hypoplastic lesion. The usual exanthematous children's diseases occurring after the 13th month of age do not produce these lesions. From present accumulated knowledge it would appear that the two main groups of metabolic disturbances responsible for the larger body of these lesions are congenital syphilis and nutritional deficiency diseases. The maxillary first premolar generally shows no evidence of hypoplastic lesions although its enamel begins formation at about the 16th month. During this 3- or 4-month interval from the 12th to the 16th month of life, some important metabolic change must occur to increase constitutional resistance. In the exceptional instance when these lesions occur with the premolar and second and third molar teeth, the constitutional disease must be of a continuing and severe nature."

Mellanby (1934, p. 108), who has made a very extensive study of the subject, classified hypoplasia as gross and microscopic. She voiced her opinion as follows: "It can therefore be stated as a general hypothesis that there is a close, direct association between structure and caries." Bibby (1943) states: "To check the claims of Mellanby and her associates that minute differences in the roughness of the enamel surface (microscopic hypoplasia of Mellanby) are related to susceptibility of teeth to caries, a correlation was made between the enamel surface texture and the amount of caries in the teeth of 200 children. Half of the children age 10 to 13 lived at home, and half age 6 to 16 were in an orphanage. . . . The findings support the claims of Mellanby and others that there was a correlation between the minute surface structure of the teeth and the liability of the teeth to decay."

Diamond adds, "Reference to Mellanby's classification of hypoplasia as gross and microscopic: At the time of Mellanby's interesting study in 1934, the phenomenon of hypocalcification and its mechanism was as yet not understood as a disturbance in the calcification process of enamel. This is differentiated from hypoplasia which is a disturbance during the formation stage. A careful study of Mellanby's 'microscopic hypoplasia' is in reality evidence of hypocalcification."

Staz (1934) found that dental hypoplasia occurs more often as a result of exanthematous than of deficiency diseases. In a group of 73 Indian children, 39 had hypoplastic teeth in varying degree. His observations showed that these are less susceptible to caries than normal ones.

Congenital syphilis is regarded as a factor affecting enamel development. The pits and furrows on hypoplastic teeth are ideal for dental caries, because food retention is more likely in these areas. Clinical observations, however, indicate that often these areas are no more susceptible to dental caries than normal surrounding enamel.

Retarded development and defective calcification of the dentin occur also as a result of severe vitamin C deficiency (Wohlback and Howe, 1925) but there is no convincing evidence that this condition is related directly to caries activity.

Opinions apparently differ concerning the interrelation of hypoplasia and dental caries. It seems evident that hypoplasia *per se* is not a cause of caries. Figure 85 is a section of an anterior tooth with hypoplasia of the labial plate of enamel and interglobular dentin (ID). Judging by the degree of attrition (A), the extensive secondary dentin formation, and considerable metamorphosis, this is a tooth of an older person; yet only one small filling (F) was inserted early in life. Furthermore, a hypoplastic crown of a premolar (fig. 86) which, before sectioning,

had the appearance of a cauliflower, shows deeply pitted enamel and an extensive zone of interglobular dentin (ID). Even so, in spite of the inferior structure of the tooth, only a very small carious area (C) is present on the occlusal surface and this does not appear to have penetrated to the dentin. Whether or not caries develops probably depends on other factors such as the oral bacterial flora, food retention, character of the saliva, tooth resistance to attack, etc.

Attrition is the wearing away of the incisal edges of the anterior and the occlusal surfaces of posterior teeth, resulting mostly from the nervous habit of grinding or gritting the

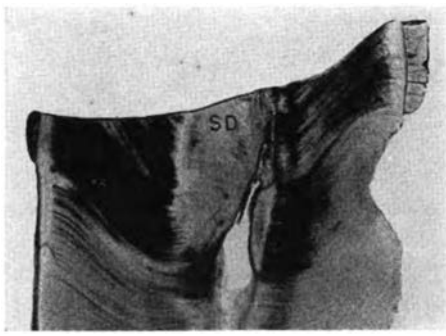


FIG. 87

Excessive attrition of upper anterior tooth through occlusal neurosis, involving original pulp chamber; pulp would have been exposed if not protected by secondary dentin (SD).

teeth (bruxomania or occlusal neurosis); also, the energetic mastication of food and the persistent chewing of tobacco cause extensive attrition, particularly if the teeth are malposed. Frequently the entire enamel is destroyed on the lower anterior teeth as a result of occlusal neurosis, exposing the dentin which then becomes exceedingly hard and dense with a brown, glass-like surface. In exceptional cases, attrition may be so extreme that more than half of the crown of the tooth is destroyed, involving the original pulp chamber. Usually this organ protects itself by the formation of secondary dentin (fig. 87). Dentin exposed by attrition is only rarely susceptible to dental caries; on the contrary, attrition of the posterior teeth is beneficial, inasmuch as the enamel fissures are eliminated by the wearing away of the occlusal surfaces.

Abrasion and attrition result in a loss of tooth structure, but their causes are different. Attrition, as just noted, is the wearing away of the occluding surfaces of the teeth by mastication of food and gritting of the teeth, while abrasion is caused by abnormal means such as the incorrect use of the tooth brush with gritty dentifrices. The areas affected by tooth brush abrasion are principally the labial and buccal crevices or necks of the teeth into which notches of varying depth and size may be cut. In exceptional cases, notches have been observed of sufficient depth to involve the dental pulp. Here again, this organ usually forms secondary dentin for its protection. Even though abrasion exposes the dentin, rarely if ever are these areas attacked by dental caries.

Erosion also affects the cervical areas of the teeth, usually very close to the gingival margin. For many years the cause of this destruction of enamel and dentin was believed to be due solely to tooth brush abrasion. Recently (Bodecker 1945) the presence of a slightly acid fluid which can cause the chemical destruction of enamel and dentin was shown in these areas. Tooth destruction through erosion often is hastened by abrasion; the tooth brush repeatedly removes the slightly decalcified organic dentin matrix, thus extending the lesion and keeping the surface hard and highly polished. The origin of the acid is uncertain, but when present it usually is restricted to only a few teeth in the mouth, suggesting that erosion activity is related to localized gingival conditions. These lesions, in contradistinction to those caused by abrasion, frequently become carious.

Conclusions

Dental caries seems to be due to exogenous and endogenous factors.

Acid and proteolytic microorganisms comprise the exogenous factors. Of these, the former destroy mineral structures and the latter destroy the organic matrix of dentin, cementum and enamel. Variations in caries activity may depend upon differences in profusion of aciduric micoorganisms, consumption of fermentable carbohydrate foods, the character and quantity of the saliva, or oral hygiene.

Variable resistance of teeth to attack appears to be linked with endogenous factors; their presence seems to be responsible for reduction of dental caries susceptibility, whereas their absence tends to increase caries activity. Certain of these factors, and their effects, are:

(a) An optimal amount of fluorine in enamel retards solution by acid and may block previously permeable enamel lamellae and have a bactericidal effect.

(b) An optimum diet containing sufficient quantities of vitamin D and calcium provides for development of sound teeth.

(c) Well keratinized, impermeable enamel lamellae may prevent the most dangerous type of caries, i.e., rapid penetration of the enamel through minute channels, resulting in the deep involvement of the dentin.

(d) Well keratinized enamel cuticle may retard formation of initial caries lesions.

(e) Well keratinized enamel rod sheaths and tufts may reduce the rate of carious destruction of enamel after initiation of the lesion.

(f) Secondary dentin formation, enamel and dentin sclerosis, and dentin metamorphosis reduce permeability, which results in a lowering of caries activity and a protection of the dental pulp.

(g) An adequate supply, quantitatively and qualitatively, of tissue fluid (dental lymph) is necessary for the normal mineralization of enamel and dentin after tooth eruption.

ORAL ENVIRONMENT AND DENTAL CARIES

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CONTENTS

Other sections of this survey of the literature on dental caries provide information related to oral environment as follows:

In Experimental Dental Caries in Animals it is shown that acidogenic bacteria are normal inhabitants of the rat mouth and that such organisms are found in carious rat teeth. The relation of rat caries to fermentable carbohydrates is discussed and the point is made that the carbohydrates and acidogenic bacteria do not initiate caries in rats but do promote the development of cavities after initiation. This has been used as the positive evidence that the process of initiation is different from that of enlargment of cavities, and it is the basis of the assertion that there is as yet no proof that bacteria and carbohydrates initiate caries in man. In other words, the assertion is made applicable to man in a negative way, that is, in lack of knowledge.

King (1937) developed a theory of the initiation of caries from observation of caries in rat teeth as follows: "I am, however, perhaps more interested, at the moment, in how and why the carious process starts and recently have begun to wonder if decalcification of the enamel is really the very first stage in caries, as is inferred in the chemico-parasitic theory. Experiments on rats indicate that physical injury of the enamel precedes any chemico-parasitic reaction. Caries-like lesions in the molar teeth of rats appear to be initiated by cusp fracture and by physical injury of the enamel at the base of deep fissures. The fermentation of food debris alone appears to have no ill effect on the teeth of these animals, nor indeed does mechanical injury such as attrition do any harm, but if the mechanical injury occurs in areas where food stagnates, then the 'carious' process takes place. Although, therefore, the rat lesions seem to be initiated by physical rather than by bacterial agencies, the latter are of great importance in the later stages of the disease.

"As regards the human carious process, the late Mr. Malleson showed very clearly, in 1925, that certain enamel defects such as cracks play a significant part. But it may be that only a small break in the enamel surface—perhaps even in Nasmyth's membrane—is needed to give the products of bacterial action a footing, since the outer part of the enamel is distinctly different from the deeper parts."

In the review Fluorine and Dental Caries it is shown that the counts of *L. acidophilus* are positively correlated with the caries rate. The work at Bauxite, Arkansas, is cited, showing correlation with caries and with the past exposure to fluoride waters. The lack of correlation of *L. acidophilus* with present fluorine is discussed. These findings are in accord with the view that *L. acidophilus* is related to the condition of cavitation but probably has nothing to do with initiation of caries.

Bunting, Nickerson and Hard (1926) said of the incidence of dental caries in a Toledo orphanage:

"The most striking observation which we made is that of the very low percentage of caries found in a Toledo orphanage among children ranging in ages from seven to thirteen who had been inmates of that institution for a continuous period of four to ten years. Among them there were but approximately 35 per cent who had caries while 65 per cent showed no evidence of the disease. There were many children in whom there was severe dental hypoplasia but no evidence of caries. This is remarkable in that so far as it has come to our knowledge, no such percentage of dental caries has been reported in any similar group of children."

They reported 35.29 per cent of 34 such children as having caries, compared to 73.81 per cent of 42 in a Detroit orphanage and

81.25 per cent of 48 in a Grand Rapids orphanage. Since the mottled enamel district of Ohio observed by Houser and Knox (1939) was centered about Toledo, it is quite likely that the hypoplasias observed by Bunting, Nickerson and Hard were mottled enamel and the low caries rate a result of fluorine. Their conclusions with respect to diet in this orphanage as well as in an orphanage at Maumee, Ohio, about 10 miles distant from Toledo, are subject to scrutiny for the influence of fluorine. (See Bunting *et al.*, 1930). The same may be said of the work of Snyder (1939), Koehne and Bunting (1934) and Jay, Hadley and Bunting (1936). Conclusions drawn from the observations by Day (1934) on plaques should be modified by the fact that his subjects had mottled enamel.

It is pointed out that the solubility of enamel powder is *reduced* by treatment with sodium fluoride solutions but the question is raised by the present author as to whether the initiation of caries is a matter of solution of enamel in acids. This is pertinent to the fact that the anterior teeth in fluoride areas do not decay except in rare cases; i.e., not that they show *reduced* decay, but rather, they do not decay at all.

In the fluoride review the success of topical application of fluorides is discussed as a possible result of sterilization of tooth surfaces, a possibility not noted by any of the authors of the three papers cited.

The work of W. D. Miller has been thoroughly examined for a statement that microorganisms initiate caries. Likewise one may seek without finding any disciple of Miller who has ever committed to print the idea that microorganisms effect the transformation of intact enamel to a cavity. The fact that bacteria can excavate, once the ground is broken, cannot be successfully combatted, but that they break the ground has not been proved; throughout the literature on caries since Miller, however, the undiscriminating implication is to be found that microorganisms do cause caries, "cause" signifying production of all that is necessary for the development of a cavity.

McIntosh, James and Lazarus-Barlow (1922) "were of opinion that if bacteria played an important role, then it was necessary to find microorganisms which were capable of softening enamel, which process histopathological research has shown to be the initial lesion in caries." The first part of this opinion is logical but there is no proof then or now that the *initial* lesion of caries is produced by softening of the enamel.

In contrast to the view of McIntosh, James and Lazarus-Barlow, who were principally responsible for the aciduric hypothesis, is that of Florestano, Faber and James (1941) who remarked, "Although the exact cause responsible for the initiation of the carious lesion is still unknown, it is generally conceded that the decalcifying process is due to acids produced by certain oral microorganisms."

The view that caries is solely a matter of local environment is presented by Bunting (1933) who said: "The belief that susceptibility to dental caries is determined or largely controlled by the quality or resistance of the tooth is based solely on hypothetical opinion unsupported by scientific experimentation or evidence and is definitely refuted by so great a preponderance of clinical and experimental observations that it can no longer be considered tenable. At the present time, there is practical agreement among the students of the subject that all teeth, irrespective of their quality, are susceptible, in varying degrees, to dental caries and that the forces which determine this disease are resident not in the tooth itself, but in its environment. In the early stages of caries at least, the tooth plays a passive role. The rate and extent of the disease depend somewhat on the integrity and quality of the enamel substance, but more on the intensity of the attacking force and the environmental conditions under which it operates. In view of this fact, there is no scientific justification

for the admonition, 'feed your teeth' as a specific preventive measure against dental caries."

This view of Bunting is difficult to defend in the face of certain clinical evidence. Hanke, *et al.* (1933) wrote: "An examination of hundreds of dental roentgenograms shows that an approximal cavity may develop below the contact point in only one of two contiguous teeth. Both of the teeth are in a common zone of bacterial activity. They should be subjected to identical concentrations of acid, but the action of this outside influence is not identical upon the two teeth. The question is, why do not both of these teeth decay?"

Bödecker and Ewen (1937) defined as unilateral caries, attack on only one of two approximating surfaces. They found 179 such lesions in 516 full mouth roentgenograms. They discussed variation in environment as influencing development of unilateral caries and found such explanations inadequate for most cases. Bacterial plaque affecting one tooth and not the other because of cleansing action of food was ruled out, as in many cases the contact was too close for any passage of food. Retention of a carious deciduous tooth to induce caries in a permanent tooth was excluded by observation of caries in the distal surface of the second premolar with none in the mesial surface of the first molar. Caries of the mesial surface of first molars was deliberately excluded from the study as possibly induced by caries of the distal surface of the second deciduous molar. Since Bödecker and Ewen could find no explanation of unilateral caries in oral environment they examined the effects of variations in calcification and structure. Conditions during the time of formation of approximating teeth could result in defective calcification of the enamel of one of the teeth and thus account for unilateral caries except for the case of caries in only one of the approximating surfaces of the central incisors. They offered an explanation in this case based on lamellae, saying: "These structures resemble cracks in the enamel and were first described and named by the author (1906). Some of them, in fact, may be cracks caused by trauma which have filled in with organic material. Such structures, probably being more permeable than the surrounding tissue, can well be, under certain circumstances, an entrance point for the external attacking force of dental caries." They pointed out that the second upper incisors "seem to be particularly prone to proximal caries, leaving the adjoining distal surface of the central and mesial aspects of the canine intact.... This observation indicates that, in all probability, their caries resistance is lower than that of the first incisors and the canines. Whether a deficient calcification or structural fault makes them more prone to caries is yet unknown." If Bödecker and Ewen had given the frequency of occurrence of unilateral caries in homologous sites in opposite jaws, their argument for resistance to caries by the teeth would be even stronger.

In contrast to unilateral caries which indicates resistance to caries inherent in the tooth, is the clinical evidence of decay of rotated teeth, indicating that any tooth will decay if the environment is conducive to caries. For example, Enright, Friesell and Trescher (1932) said, "In cases where the teeth have a quarter-turn in the arch, and the normal lingual surface of the tooth is in contact with an approximating tooth, caries readily begins on that lingual surface." A possible explanation of the above fact is that the teeth are susceptible to decay on any surface but that *development after initiation* is possible only where environment favors development of a cavity.

Bradel and Blayney (1940) have succinctly stated the types of approach to the search for specific organisms, if any, in dental caries: "Study of the bacteriology of dental caries may be approached: (1) by studying the bacteria removed from localized areas on the teeth; (2) by culturing selectively certain specific organisms from the

mixed flora and determining their association with caries activity, and (3) by studying all the organisms found in representative samples of the mixed oral flora and comparing the differences in caries-active and caries-free mouths." The papers of Blayney and his associates provide some of the best evidence that there is no specific organism of caries.

Sullivan, Still and Goldsworthy (1939) said of classification of lactobacilli: "After reviewing the more accessible papers, these three points seem to emerge:—1. That classification of strains from whatsoever source or site has presented great difficulties which have not been lessened by the different nomenclatures and criteria used by various authors. 2. That there are two diametrically opposed schools of thought on the identity of the varieties of lactobacilli occurring in different sites, for example, the intestine and the oral cavity. 3. That there is fairly general agreement that among lactobacilli from all sources there are two main groups, the boundaries of which are somewhat ill defined."

This review is concerned with the general role of bacteria, and classification of the alleged causative organisms is not undertaken.

ORIGIN OF THE CHEMICO-PARASITIC THEORY OF MILLER

Predecessors of Miller

Robertson (1835) wrote, "The only cause capable of explaining the partial operation and the particular situations of decay, is the corrosive or chemical action of the solid particles of the food which have been retained and undergone a process of putrefaction, or fermentation in the several parts of the teeth best adapted for their reception." And he said that saliva "by its decidedly antiseptic properties, prevents the process of putrefaction." Of this, Black (1886a) said: "Robertson in 1835 said distinctly that caries is produced by fermentation at the particular spot at which the caries occurs, but he could not explain fermentation. He knew from observation that acids were produced by what was known as fermentation. Ever since that time the dental profession has been in search for the source of the acid which produces caries." But Robertson spoke only of "chemical or corrosive action" both in the above and in other places. In deploring the use of acids to remove tartar he said: "The effect produced by acids upon the teeth, and that produced by caries are distinguished by very different characters; the latter is partial in its attacks . . . with the former it is quite the reverse, the effect produced is apparent upon the whole range of the front teeth which come in contact with the acid, and those parts of them too which are the least subject to decay, namely, their smooth front surfaces."

Buehlmann (1840) reported seeing thread-like forms in saliva, on teeth and especially in tartar.

Erdl (1843) removed membranes from teeth by means of dilute hydrochloric acid and found them made up of microorganisms. Some of these membranes were from enamel and were probably Nasmyth's membrane, but others were undoubtedly from dentin as his illustrations show that there was penetration into the dentin.

Ficinus (1847) observed microorganisms in material from teeth and called them "Denticola". He found them nested in indescribable masses in carious teeth and concluded there was a reciprocal relationship. He considered caries a putrefactive process related to infusoria that first attacked the Nasmyth membrane, then penetrated the enamel and spread through the dentin to final destruction of the tooth.

Leber and Rottenstein (1867) (Chandler translation, 1873) said beginning caries presented "a chalky mass, analogous to that which forms the congenital white spots of enamel, which are also a frequent seat of dental caries." "Great importance

has been generally attributed to the enamel cuticle, discovered by Nasmyth, in the production of caries, some regarding it as a protecting covering, others as the seat of the disease." Leber and Rottenstein were particularly interested in a fungus, *Leptothrix buccalis*. They wrote: "In our opinion the progress of caries in enamel is this: By the action of an acid the enamel becomes porous at some point and loses its normal consistence. At the same time there is seen to appear a brown color, in consequence of the change which has taken place in its organic structure. There is formed at the surface a bed of leptothrix, which probably penetrates the dental cuticle, if it still exists, and destroys it. Chinks and fissures are opened in the enamel, which has become less consistent. Acid liquids and granulations of leptothrix penetrate there, while minute fragments become detached and are promptly enveloped by the elements of leptothrix, which, joined to the continued action of the acids, hasten the dissolution."

In the dentin they found thickening of the walls of the tubules, which they ascribed to mechanical pressure by the growth of leptothrix. They found no evidence of inflammation.

"From what has been said it results that *two principal phenomena* manifest themselves in the formation of dental caries, viz., *the action of acids* and the *rapid development of a parasitic plant*, the *Leptothrix buccalis*." They did not consider *Leptothrix buccalis* as the acid former, and this designation of microorganisms *acting upon carbohydrates* as the source of acids remained for Miller (1882) to demonstrate.

Clark (1871) indicated a belief that fermentation acids were active in caries. Later, with more assurance, he (1879) stated his belief "that acids taken into the mouth and formed by fermentation act on tooth structure, thus producing caries." In connection with fermentation he discussed "dental bacteria" as a specific type and said, "They are of a half U shape, are from one and a half to three micrometers long by one-half wide."

Underwood and Miller (1881) illustrated "micrococci, oval and rod-shaped bacteria" in sections of decayed dentin. They showed a diminution of the bacterial population toward the deeper layers of a carious lesion. They concluded: "We consider that caries is absolutely dependent upon the presence and proliferation of organisms; that those organisms attack first the organic material, and, feeding upon it, create an acid which removes the lime salt; and that all the difference between caries and simple decalcification by acids is due to the presence and operation of germs. This view we propose to call the 'septic theory'." On the basis of this theory they proposed and practiced antiseptic procedures in the treatment of caries.

It may be said, therefore, that up to 1881 a relationship between oral microorganisms and caries was recognized and that action of acids was suspected as the active demineralizing mechanism, but the source of the acids was not clearly demonstrated.

Miller's Investigations

Miller, (1882, 1883a) in two papers which were essentially the same, advanced the theory that the source of the acids which decalcify the teeth in caries is in the activities of oral microorganisms flourishing on carbohydrates. He said, "A mixture of 68.0 grams saliva + 1.0 bread + 0.5 meat + 0.5 sugar, kept for forty-eight hours at the temperature of the human body, generated more than sufficient acid to decalcify the entire crown of a molar tooth.

"Pieces of sound dentine, placed in a mixture similar to the above, became in ten days decalcified to the depth of half a millimeter." He excluded meat as sustaining acid formation as, with meat alone, mixtures quickly became alkaline and no decalcification occurred. He showed that the bacteria alone, that is without bread or other carbo-

hydrate, effected no decalcification of dentin. His illustrations showed various forms of microorganisms in dentinal tubules and he said:

"1. The first stage of caries of the teeth, *i.e.*, the extraction of the lime-salts, is for the most part caused by those acids which are generated in the mouth by fermentation.

"2. Decalcification of the enamel signifies total destruction of that tissue; of the dentine there remains after decalcification a tough, spongy mass, which becomes subject to the invasion of enormous numbers of fungi (leptothrix-threads, bacilli, micrococci, etc.).

"3. The leptothrix-threads are found, with rare exceptions, only upon the surface, or in the superficial layers of the softened dentine, and appear to take but a small part in the invasion. The bacilli, on the other hand, penetrate far into the dentine, even into the finest branches of the canaliculi. Micrococci penetrate furthest.

"4. In the separate tubules is frequently to be seen a gradual change from leptothrix-threads to long bacilli, from long to short bacilli, and from the latter to micrococci.

"5. The fungi produce anatomical and pathological changes in the deeper layers, stop up the canaliculi, and necessarily lead sooner or later to the death of the dentinal fibrils. The outer layers of dentine, thereby deprived of nourishment, die and fall a prey to putrefactive agents.

"6. The invasion of the fungi is always preceded by the extraction of the lime-salts.

"7. The fungi have not the power either to penetrate or to decalcify sound dentine, so that the infection of a perfectly sound tooth by a carious one seems to be excluded.

"8. We may accordingly look upon caries of the teeth as consisting of three stages: (1) decalcification; (2) infection and devitalization of the decalcified dentine; (3) putrefaction of the devitalized dentine, though it would not be easy to say just where one stage ceases and the other begins.

"9. I have in a number of cases been able to establish the participation of the fungus Saccharomyces mycoderma (?) in the carious process.

"I do not, however, wish to be understood as saying that acids or pathogenic bacteria, or putrefactive bacteria, or all together, are the sole and only cause of decay of the human teeth. What I am prepared to say is this, that in my opinion, there is not a single case of caries in which microorganisms do not play some part, and that in the most cases they play a very important part."

Miller (1883b) indicated that little was known of caries in enamel, saying, "Destruction of the enamel may take place quite independently of microorganisms." He also said, "I am a believer in acids, a believer in microorganisms, and a believer in an unknown cause; that is, I believe that there are agents active in the production of caries which are yet to be discovered."

Miller (1883c) succeeded in preparing about 150 sections of enamel from 125 different teeth to show caries in that tissue. He found "in the beginning of the carious process" a slight depression in the enamel surface containing "*Leptothrix buccalis* in considerable masses." He noted that beyond this with staining reagents "the enamel will almost always be very perceptibly discolored, as though acted upon by some agent producing effects undistinguishable from those of acids."

Miller (1883d) placed bread and saliva in a hole bored into a whole tooth and found that fermentation occurred while the mixture was in the hole but that on removal putrefaction ensued. Teeth bound together so as to leave a space of about 2 mm. between were decalcified at this point of lodgment of a saliva and bread mixture. By chemical analysis Miller showed loss of mineral matter in decayed dentin from various sources.

Miller (1883e) wrote 18 propositions following his two-year study of an estimated

8000 teeth. These propositions were, in brief:

1. Amylaceous or saccharine food and saliva generate acid.

2. There is constant formation of acid in the mouth because of retention of food in cracks, pits and fissures of teeth and between teeth.

3. The degree of acidity depends on length of time of exposure.

4. A cavity of decay containing amylaceous or saccharine food always has an acid reaction.

5. "The extent to which any tooth suffers from the action of the acid depends upon its density and structure, but more particularly upon the perfection of the enamel and the protection of the neck of the tooth by healthy gums. What we might call the perfect tooth would resist indefinitely the same acid to which a tooth of opposite character would succumb in a few weeks."

6. An occasional neutral or alkaline reaction in a cavity does not mean that acid did not produce the cavity.

7. A systemic condition which withdraws lime salts from a tooth or lowers its density or weakens the union of organic and inorganic matter renders it more liable to decay.

8. "Strong acid and corroding substances brought but momentarily into the human mouth may give rise to lesions of the enamel at points where the ordinary agents alone could never have begun."

9. *In vitro*, caries simulating natural caries in macro details can be produced with acid mixtures such as are found in the mouth.

10. Carious dentin is decalcified roughly in inverse proportion to depth.

11. Destruction of organic material follows, not precedes, decalcification.

12. Mouth fungi do not act directly in decalcification. Further study of their acid-producing activities is needed.

13. Fungi produce great changes in decalcified dentin and finally reduce it to a mass of debris and fungi.

14. "The invasion of the micro-organisms is always preceded by the extraction of the lime salts."

15. Destruction of organic material is accomplished by fungi.

16. "Inflammation can hardly be looked upon as a very important factor in caries of the teeth."

17. "Caries of the enamel is purely chemical, the decalcification resulting at once in the complete dissolution of the tissue."

18. Caries of cementum runs a course similar to that in the dentin.

Of the above, Nos. 1, 2, 3, 4, 6, 8, 9, 10, 11, 13, 14, 15, 17, and 18 may be regarded as conclusions reached by Miller as a result of direct observation on extracted teeth as well as those *in situ*. However, 5, 7 and 16 may be regarded as opinions. No. 12 is offered tentatively.

Miller's experiments in regard to structure of teeth and caries in support of his proposition No. 5 were as follows: He "broke up several teeth, perfectly free from caries, but of different density, into fragments of various sizes, and put the pieces in a mixture of saliva and bread." He kept the mixture at 37°C for three months, with renewal of the bread and saliva five times. The attack, producing typical caries, was variable. "In two cases, evidently owing to a defect in the structure, the teeth were attacked on the cusps and reduced to a powder; where the acid had penetrated through the enamel, its effect was seen to spread out in all directions in the dentine; where the enamel was very hard and dense, without crack or blemish of any kind, it had not even lost its lustre."

"This experiment shows clearly enough what a vast difference the structure (density) of the tooth makes in its resistance to decalcification, and offers an explanation of the question why all teeth do not decay alike. A dense tooth, covered with enamel, intact at every point, would probably

resist for years the action of an acid saliva to which a soft, defective tooth would succumb in a few weeks.

"It also shows that the process of decalcification may go on in cracks and fissures too fine for food to penetrate, in fact anywhere where saliva holding starch or sugar in solution may find access, and be retained even by capillary attraction, there to undergo fermentation."

The facts of varied attack of caries on enamel under artificial conditions are clearly stated by Miller, but he did not demonstrate any enamel structures associated with either freedom from or susceptibility to decay. His inclination to believe in enamel structure as a factor in caries is clearly evident.

It may be said that Miller was influenced by the then current belief that soft teeth are more susceptible to decay than hard teeth. The same may be said of his proposition No. 7, as he offers no experimental work to support systemic decalcification of teeth. As to No. 16, he simply found no evidence of inflammation; that is, his findings were purely negative.

Miller (1884) in a paper continued in five numbers of Independent Practitioner isolated *Bacterium acidi lactici* from carious teeth. He isolated lactic acid as zinc lactate from his bread and saliva mixtures. He indicated pleomorphism by showing change of thread forms to bacilli and bacilli to cocci and distinguished alpha and beta forms of certain of his "fungi". Later he described gamma, delta and epsilon forms and said of these organisms, since they were acid formers, "They may consequently all be looked upon as factors in the decay of the teeth." Miller summed up his work to date as follows:

1. He showed microorganisms present in dentin and stated there was no claim for priority, since Leber and Rottenstein (1867) had earlier found the same.

2. He found an advanced zone of softened dentin containing no microorganisms.

3. By chemical analysis, he showed decalcification in carious dentin.

4. He maintained, without direct proof except from *in vitro* experiments, that acids generated in the mouth produce decalcification of dentin.

5. He proved the presence in the mouth and in carious dentin of acid-producing fungi and showed in one case that the product was lactic acid.

6. He produced artificial caries in dentin.

7. He showed the effects of antiseptics on oral acid-producing fungi.

8. He described the characteristics of the acid-producing fungi.

Miller (1885) described methods for culturing mouth organisms and stated that he had isolated 22 different fungi from the human mouth. He concluded: "A great majority of the fungi found in the human mouth are capable of producing acid from cane or grape sugar, and it is probable that, *with very few exceptions*, all can do this when the proper conditions are presented to them. In nearly all cases which have been examined with special reference to this question, the acid has appeared to be lactic. The acetic acid fermentation, which cannot go on at temperatures above 35°C (Fluegge), is out of the question in the human mouth, nor is there, as yet, any proof of the presence of more than minute traces of butyric acid."

"The possession of peptonizing action by large numbers of these fungi readily accounts for the solution of the decalcified dentine.

"Any one of these fungi which can produce acid by fermentation of carbohydrates, or can dissolve the decalcified dentine, may aid in the production of caries, while any one which combines both these properties, as many of them do, may alone bring about the phenomenon of dental caries."

Miller in 1889 published the results of his researches in German, with an English edition in 1890. It would be difficult to find

anywhere in his work a statement of the "Miller theory" and certainly not in the form understood today. He did not, for example, believe that any one organism could by acid formation decalcify dentin but believed rather that any acid-former, if supplied with carbohydrate available to its metabolic mechanism, would demineralize dentin. It is not clear that he believed that bacterial acids *initiated* caries or that he considered a distinction between initiation and promotion of caries, but it is overwhelmingly evident that he believed that such acids were the immediate cause of decalcification once initiation had been effected. But those who have followed Miller have freely gone beyond the established facts. For example, Allan (1889) said, "The actual presence of an acid as a commencement or initiative step in the process of decay being acknowledged, Miller shows us whence it comes, names it and points out the little organism at work manufacturing it."

Allan's statement asks that *initiation of caries by acids be acknowledged without proof.* Miller and many others produced abundant evidence that bacteria enlarge cavities but there is only hypothesis that bacteria are involved in bringing about the condition in enamel that permits acid decalcification to proceed.

SPECIFIC ORGANISMS OF DENTAL CARIES

Lactobacillus acidophilus

Goadby (1900) described a gram-positive short bacillus isolated with great frequency from carious dentin and named it *B. necrodentalis.* He also observed *Streptococcus brevis* in many cases. He considered both organisms as concerned in decalcification of dentin prior to liquefaction of the organic matrix by other organisms. He demonstrated that liquefaction of gelatin was not necessarily an index of liquefaction of dentin matrix.

Moro (1900a, b) first used the name "*Bacillus acidophilus*" to designate a gram-positive bacillus isolated from the stools of infants. Tissier (1900) found a similar organism in the stools of infants and used the same name.

Rodella (1900, 1901) pointed out that the so-called acidophilus intestinal organisms did not require an acid medium for development. Cipollina (1902) similarly objected to the name "acidophilus."

Kendall (1910) objected to the name "acidophilus" as being a misnomer, since he too had found the organisms developed better in slightly alkaline media. He proposed "the name 'aciduric' in the place of 'acidophilic' as the type name for these bacteria, this term being more appropriate and at the same time indicating the most striking characteristics of the organisms of this group, namely, their ability to 'endure' acid." Kendall employed a dextrose broth containing acetic acid, equivalent to N/20 alkali, to isolate intestinal aciduric organisms.

Gies (1915a) introduced a series of papers by himself and collaborators, with a number of questions indicating the scope of their studies. Gies and Kligler (1915) in discussing the Miller theory said, "Immunity to caries, localization of caries, sudden halt in the process and eventual repair are questions that remain open and are not accounted for by this theory." They reviewed the literature of mouth organisms and especially papers dealing with dental caries. They found in general that the numbers of bacteria in scrapings from teeth, as estimated by direct counting and cultural methods, paralleled the condition of the mouth. The numbers were low for healthy mouths, high for "dirty" mouths, high in caries, reduced by cleaning, and increased by foods. In healthy mouths the proportions of different forms did not change with fluctuating numbers. In carious mouths the investigators noted "not only a complete change in the character of the flora and the relative prevalence of types from that of the healthy teeth; but there is also a distinct

difference between the types of bacteria in the early stages and those in later periods of decay." Streptococci were found more frequently than any other form, but, since their relative numbers diminished in caries, Gies and Kligler did not consider them to be important in caries etiology. Non-spore-bearing bacilli "were found irregularly on normal teeth, and were present in all cases in which decay had set in." These organisms were "capable of producing and resisting an acidity of 8 per cent *N* acid." They observed a "pleomorphic short thread-forming organism" capable of attaching itself to smooth surfaces and enclosing colonies of other bacteria. The aciduric bacillus was considered to be the *B. acidophilus* of Moro. They divided 58 strains into two groups on the basis of fermentation of sucrose, 20 being found positive. They found that 100 cc. of culture medium of *B. acidophilus* required 5.4–5.8 cc. of N/20 NaOH for neutralization if it contained fructose, maltose or lactose but only 0.9 cc. if sucrose were the sugar. Streptococcus medium required 4.0–4.2 cc. of N/20 alkali if glucose, sucrose or lactose were the sugar. (Data from Gies, Hull and Mullikan, 1915.) Of the thread forms, they studied 58 strains of *Cladothrix placoides* and 8 of *Leptothrix buccalis*, which were identified with *L. buccalis* of Miller and *L. racemos* of Williams and Vincentini. *C. placoides* and *B. acidophilus* were found to dissolve 17 to 24 mg. of calcium per 100 cc. of medium exposed to powdered teeth during 15 days of incubation; streptococci dissolved 9 mg.; and other forms less.

Gies, Hull and Mullikan (1915) found, in addition to the data discussed in the preceding abstract of the paper of Gies and Kligler (1915), that *C. placoides* formed acid equivalent to 4.3 cc. of alkali from sucrose, compared with 3.2 and 3.7 cc. from glucose and maltose, respectively. They wrote: "The comparative high acid-potentials of sucrose (cane sugar) and of maltose (sugar produced from starch in salivary digestion), in the presence of *Cladothrix placoides*, may be particularly significant, in the light of facts involved in the initiation of dental caries that are briefly summarized in the succeeding paper (Gies, 1915b). In this connection, also, the marked fermentative action on glucose, maltose and lactose by *B. acidophilus*, which accompanies *C. placoides* in the material in enamel cavities, is a matter of special interest. Between them, these two bacterial forms, which may grow actively in each other's presence, ferment the four common sugars with particular avidity."

Gies (1915b) discussed the significance of the preceding papers by Gies and collaborators. He said, "Mixed cultures from the teeth of Miss D., a case of perfect immunity (against all dental diseases), extracted practically as much calcium as any culture from decay cases." He said that coccus forms were not excluded from caries etiology: "It is by no means impossible that coccus forms *initiate* the solution of calcium and phosphate from enamel, and that *C. placoides* and *B. acidophilus* are stimulated to specially destructive focalization . . ."

Howe and Hatch (1917) studied the flora of cavities in first and second molars of children from 10 to 14 years old. They (a) filled the cavities without disturbing the decay and left them in that condition for 6 weeks to 3 months; (b) filled cavities with slightly antiseptic materials, with the fillings left for a shorter period; and (c) examined the cavities directly. They said: "The organisms constantly found in dental caries, and belonging as we believe to the Moro-Tissier group because of their high aciduric character, we have called: *Bacillus acidophilus* (Moro), *Bacillus X*, *Bacillus M*, *Bacillus Y*, *B. bifidus*." They discussed the pleomorphism of these organisms. In their conclusions they wrote: "Their high acid-forming properties limit the character of the flora found in carious teeth. They possess in a greater degree than do any other organ-

isms, the attributes that are considered necessary for at least inaugurating the process of dental caries."

Rodriguez (1922) cultured caries organisms and observed the terminal acidity. He indicated his belief that the organisms producing the highest acidity are responsible for dental caries. He described especially *Lactobacillus odontolyticus* Types I, II and III, isolated from deep layers of carious dentin and capable of producing terminal pH ranging from 3.9 to 2.9, as active in cavity production.

McIntosh, James and Lazarus-Barlow (1922) isolated organisms from carious teeth in broths approaching pH 3.5. They distinguished Types I and II of aciduric organisms which they called *B. acidophilus odontolyticus*. Type I was isolated from 88 per cent of their cases and Type II from 42 per cent. Their material was the deeper layers of dentin.

In a study of 49 cases of enamel caries made under the direction of Colyer (Anonymous, 1923b) *B. acidophilus odontolyticus*, as grown initially on agar plates at pH 3.5, was found in 6 cases. The lesions in all positive cases were listed as blackish. It was concluded that the organism "does not appear to be present in the early stages, i.e., in decay limited to the enamel." *B. acidophilus odontolyticus* was found in the dentin of 14 of 20 cases of carious deciduous teeth.

McIntosh, James and Lazarus-Barlow (1924) admitted the pleomorphism of their Type II bacillus with coccal and diplococcal forms. Strains freshly isolated from various sources most frequently failed to ferment sucrose but gained the power on culturing on sucrose-containing media. Cross-agglutination was found between the Types I and II, especially in those isolated from milk and human sources.

In an addendum it was recorded that E. C. Dodds examined *old* broth cultures from *B. acidophilus odontolyticus* and found in six separate analyses 0.6 to 0.7 per cent malic acid. In three of these 0.01 lactic acid was found. "From these analyses it appears that practically all the acidity is due to malic acid." The malic acid was isolated and identified by specific tests and especially by a mixed melting point. No direct statement was made of the original composition of the broth, such as its lactic and malic acid content, or of the carbohydrates.

Leigh (1924) found the enamel cuticle served as a nidus for bacterial growth, but no organisms could be seen beyond the apex of the V-shaped etchings in the enamel. "The predominant morphology of the organisms stained in most intimate relation with the enamel appears to be a short bacillary form." But as to the flora of carious dentin, he found that "following the invasion by a small group of anaerobes the infection becomes mixed."

Bunting and Palmerlee (1925a, b) pointed out in review that Miller believed "that a certain group of organisms, namely the acid formers, and not a specific species, is responsible for caries." (Miller isolated at least 10 different acid-forming organisms from the mouth.) They said Goadby's *B. necrodentalis* appears identical with *B. acidophilus*. They reviewed work on *B. acidophilus* and accepted the *aciduric* character of a specific organism as essential to initiation of decay. They isolated *B. acidophilus* in broth at pH 5.0 in every one of 38 cultures from beginning cervical caries, i.e. with white areas of decalcification; in 15 of 16 cases of deep caries; in 3 of 16 cases with history of complete freedom from caries. They also isolated an oidium-like organism from 22 of 46 carious mouths.

Bunting and Palmerlee found the *B. acidophilus* to be highly pleomorphic, thus accounting for many forms described by others. They believed Clarke (1924) to have dealt with *B. acidophilus* grown under more alkaline conditions and not with the new species he named *Streptococcus mutans*.

They did not believe classification on morphological grounds was satisfactory. Study of fermentation tests led Bunting and Palmerlee to conclude that no satisfactory classification of acidophilus forms could be effected. They found acid foods and alkaline mouth washes did not keep mouths free of acidophilus. Intensive treatment with the bactericidal dyes, brilliant green and crystal violet, in combination, effected a significant elimination of *B. acidophilus*.

Bunting, Nickerson and Hard (1926) scraped teeth and passed dental floss between teeth to sample mouths for *B. acidophilus*. The scrapings and floss were transferred to glucose infusion broth at pH 5.2. If palisaded gram-positive rods appeared in 48 hours, the finding was positive for *B. acidophilus*. If doubt existed, a transfer was made to glucose infusion agar plates. Small white colonies were examined for the characteristic palisaded arrangement and proof of acidogenic power obtained by seeding in glucose broth, "reaction unadjusted." If a pH of 4.0 to 4.2 or below was attained in one week, the specimen was judged to be a strain of *B. acidophilus*.

Only one positive culture was obtained in 184 cases of immunes ranging from 2 to 40 years old. Of 243 cases of caries susceptibles, 3 to 45 years old, *B. acidophilus* was obtained in 237.

These authors found *B. acidophilus* localized at the site of caries. That is, if only pit and fissure caries existed, caries-free interproximal areas would show no *B. acidophilus*. "It is very possible that many of the mouth organisms which are capable of producing mass overgrowths, such as the leptothrici and the cladothrici, play an important part in the localization of *B. acidophilus* and incipient caries."

The incidence of *B. acidophilus* diminished with age, being found in 42 per cent of 40 students between 20 and 25 but in only 15 per cent of 26 students between 25 and 35. Practically all had had caries. "This then suggests the existence of a metabolic control of *B. acidophilus* overgrowths and that the degree of activity of this organism is dependent on general metabolic changes related to age and bodily health."

They reported some data on caries incidence in various orphanages and schools in Michigan and Ohio. In a Toledo orphanage they found that only 35 per cent of children of age groups 3 to 6 and 7 to 13 had caries. They noted, "There were many children in whom there was severe dental hypoplasia but no evidence of caries."

Bunting, Nickerson and Hard attached small gold cups to teeth *in situ*, with *B. acidophilus* cultures and bread, renewed each week. Decalcification ensued in perceptible degree in a week to 10 days in 17 subjects. The teeth so treated became sensitive. Sections showed broad rather than true carious penetration.

It was found that Metaphen applied repeatedly, *with thorough prophylactic treatment*, would in some cases completely eliminate *B. acidophilus* from the mouths of caries-susceptible subjects.

Bunting, Nickerson and Hard (1927) summarized the above results.

Jay and Voorhees (1927) reported studies on a series of 22 children ranging from 5 to 13 years of age. Caries was generally associated with positive findings of *B. acidophilus* and *vice versa*. Positive *B. acidophilus* with no visible cavities in some cases preceded the finding of cavities 3 to 6 months later. Jay (1929) reported further on some of the cases of Jay and Voorhees (1927). One caries-free subject with positive acidophilus developed cavities. Of 5 other "immunes", 4 remained caries- and acidophilus-free; one became positive for both. Jay used the Metaphen treatment on half of 26 caries- and acidophilus-free children in an attempt to prevent caries. "After seven months, six of the thirteen children so treated developed carious lesions, while of the control group who received no treatment, caries occurred in only three." Cooperation was maintained from only a

minority of the patients of a group of practicing physicians who attempted to carry out the Bunting Metaphen treatments.

Okumura and Nakai (1927) isolated from carious dentin a gram-positive organism which they called *Bacillus P* and regarded it as identical with Goadby's *B. necrodentalis*. It was capable of decalcifying dentin. A gram-negative organism which caused only slight decalcification of dentin but caused cavity formation in artificially decalcified dentin, they called *Bacillus N*. They considered that streptococci are of little importance in caries and that staphylococci may play a part in dissolution of dentin matrix.

Morishita (1928) protested the designation *B. acidophilus* for acid-forming organisms isolated from carious lesions. He pointed out a number of differences from the true *B. acidophilus* of intestinal origin. Of particular importance among the ten differences listed was the terminal pH, being 3.8–3.0 for dental organisms and 4.4–3.8 for intestinal bacilli.

Morishita (1929) isolated aciduric organisms from saliva and teeth of caries subjects. The teeth were those with caries still limited to the enamel. The initial medium had a pH 4.8 to 4.2. He found aciduric organisms present in 56 carious teeth and in 59 of 60 salivas from caries subjects, and in all of 15 surfaces of decayed enamel. Aciduric organisms were present in the saliva of 1 of 6 cases of non-carious mouths. The strains isolated were not highly pleomorphic. Of 52 strains of aciduric organisms in the collection available to Morishita, all fermented glucose and levulose, 50 fermented maltose, and 47, lactose, but only 35 fermented sucrose. Magee, Drain and Boyd (1929) found no change in "the acid-producing power of the peridental flora" of 2 children during a dietary regimen of less than 3 months that caused arrest of caries. In a fourth child, with no dentinal caries but with decalcification of gingival enamel, no change in acid character of the flora occurred in 1 week on the diet. They said, "The data are offered as further evidence (Roskin, 1928) that dental caries is not primarily dependent upon the production of acid in the mouth." None fermented starch.

Bunting, Crowley, Hard and Keller (1928) gave 12 caries- and *B. acidophilus*-free children dental acidophilous milk each day for a period of a week. Seven of the children failed to show acidophilus implantation, one was positive and four were variable. None of seven adults similarly treated showed positive results. The authors postulated (1) an active immunity in the mouth or (2) an antibiotic relationship with other organisms. However, Metaphen treatment of mouths, designed to reduce all oral flora, did not permit subsequent implantation of *B. acidophilus*, suggesting the antibiotic postulate was invalid. Bunting, *et al.*, made a preliminary statement of the reduction by dietary means of caries activity as indicated by *B. acidophilus*, in two children's homes (106 and 228 children, respectively). The changes involved improvement of the quality of the food and reduction of sugar and white flour. The poorer response of the larger group was explained on the basis of less adequate dental services. There were more unfilled cavities in this group.

The investigators recorded that by use of 1:1000 hexylresorcinol 3 times daily and with no other treatment, *B. acidophilus* was eliminated from the mouths of 24 of 36 subjects in from 2 to 8 weeks. They recommended for elimination of *B. acidophilus*, topical applications of Metaphen during prophylactic treatment for 5 consecutive days, followed by daily use of hexylresorcinol by the subject. This regimen was to be interspersed with an occasional Metaphen treatment by the dentist.

Bunting, Nickerson, Hard and Crowley (1928a) considered that "dental caries has been definitely proved to be a truly infective disease and that *B. acidophilus* may be considered to be the specific etiologic factor responsible for the initial stages at least. . . ."

They found a high degree of association of *B. acidophilus* with active caries in the examination of 1335 children in public and private schools and in orphanages. They found differences in activity of caries in different groups and associated these differences with (1) more intensive dental care and (2) "plain and substantial food" in improving oral conditions. They reported Metaphen with intensive prophylactic treatment as "the most effective means of treating cases of extreme overgrowths of *B. acidophilus* and rapid caries which has thus far been suggested." These authors (1928b) summarized the above studies.

Bunting (1928) wrote of the correlation of *B. acidophilus* with caries: "So definite is this correlation that, in the opinion of this group, the presence or absence of *B. acidophilus* in the mouth constitutes a definite criterion of the activity of dental caries that is more accurate than any clinical estimation can be. Furthermore, it was noted that there was a spontaneous cessation of caries coincident with the disappearance of *B. acidophilus* from the mouth, either from prophylactic, therapeutic, or dietetic control. Caries-free individuals who upon first examination had *B. acidophilus* overgrowths in the mouth, later developed active caries. It was found that the degree of dental caries, and the activity of *B. acidophilus* in the mouth, are apparently related to the type of diet received and the form of dental attention afforded."

Bunting, Crowley, Hard and Keller (1929) summarized the foregoing paper and said of *B. acidophilus*: "As a rule, it may be said that, during early childhood, and up to 8 years of age, the organism is present in about 35 per cent; at the ages of 8 to 20 years, from 85 to 95 per cent are affected, and at over 20 years, there is an apparent decrease in its occurrence, to about 50 per cent. The chronologic appearance of the organism corresponds closely to the periods of greatest caries activity in the average individual."

Jay and Voorhees (1929) commented on the relation of *B. acidophilus* to dental caries. They pointed out that streptococci, being small, may be present in more advanced positions in carious dentin, but that in early caries *B. acidophilus* "overwhelmingly predominates." They stated that infection by *B. acidophilus* may be found at least three months prior to detection of cavities. *B. acidophilus* may be found as a transient in mouths immune to caries. As the types vary from day to day, failure of establishment is suggested.

Rosebury, Linton and Buchbinder (1929) compared 21 strains of dental aciduric organisms with 9 strains of *L. acidophilus* "in their morphological, biochemical, and serological relationships." "No consistent difference could be discovered between the groups in any part of the morphological or biochemical study." "In their serological reactions, no clear distinction between the two groups could be found. Most of the strains of both groups showed marked cross-agglutination." They thus differed from Morishita (1928, 1929). They recorded that in fermentation studies "sucrose gave markedly variable results. Tests made soon after isolation, in the case of the dental strains, and soon after we had obtained them in the case of the intestinal strains, indicated that only four dental strains and one intestinal strain (no. I) were able to ferment this sugar. . . . However, after three months, during which time all the strains had been growing uniformly, all thirty strains were able to ferment sucrose."

Jay and Esser (1930) observed caries and *B. acidophilus* in the mouths of 220 children 15 and 16 years old for a period of 9 months. Weekly cultures were made from each. In 9 susceptible cases Metaphen treatments were given on 3 successive days of each week, an average of 48 applications. None remained free of *B. acidophilus*, though 3 were classed negative. One or 2 new cavities developed in each of seven subjects. Another group of 60 used hexylresorcinol as a mouth wash 2 or 3

times a day. Of these, 14 developed a negative test for *B. acidophilus* and 2 of them had 1 new cavity each. Forty of the 46 who remained positive for *B. acidophilus* developed 1 or more cavities. Of 40 controls with no treatment, 15 became negative to *B. acidophilus*, i.e., a higher rate of improvement than seen in the hexylresorcinol group.

Hadley, Bunting and Delves (1930) described the characteristics of three groups of oral aciduric organisms. They were able to dissociate pure smooth strains into pure rough strains and *vice versa*. They stressed the importance of the rough types because, having long leptothrix filaments, these may promote plaque formation.

Howitt and Fleming (1930), in reporting a study primarily concerned with pyorrhea, said, "Organisms of the *Lactobacillus* groups were isolated on one or more occasions from every one of the (14) men, regardless of whether they had caries or pyorrhea, or both these conditions." They found "the oral bacteria are more influenced by the local food remains than by any general systemic effect produced by dietary changes."

Howitt (1930) in a study of the aciduric rod forms isolated during the preceding study (Howitt and Fleming, 1930) concluded that as there was no uniformity in fermentation, type of colony or agglutination in 47 mouth strains, "there is no one group of organisms covered by the term *Lactobacillus acidophilus*."

Rosebury (1930) said: "Every one of forty healthy young albino rats had aciduric bacteria of the *Lactobacillus acidophilus* type as a normal mouth organism" and "It is apparent that these organisms may be present in the mouth without giving rise to the condition" (of caries).

Bunting, Hadley, Jay and Hard (1930) gave some preliminary data on observations of response to dietary and therapeutic practices in 5 groups of children. In 156 and 118 children in Maumee, Ohio, and Lapeer, Michigan, homes, respectively, about 80 per cent of the children had no caries after a year on a balanced diet with a minimum of sugar. No cod liver oil or viosterol was given. Hexylresorcinol was used daily as a mouth wash. In a school with no dietary control but with the hexylresorcinol used twice daily, only 25 per cent of 104 children were caries-inactive. In a control school 18 per cent of 74 children were free of caries activity. In a fifth school with diet control alone, 75 per cent of 159 children showed inactivity of caries. The authors concluded the major part of caries control was because of the dietary procedures. They pointed out that restriction of sugar was common to control of oral conditions and to provision of a better balanced ration.

Rodriguez (1930) devised a horse serum-agar medium with initial pH 7.2 with anaerobic conditions and 10 per cent carbon dioxide as a specific medium for quantitative estimation of *Lactobacillus acidophilus odontolyticus*. The characteristic colony was surrounded by an opacity shown by Rodriguez to be due to precipitation at pH below 4.2. The growth of the organisms was particularly luxuriant, and other forms were present.

Rodriguez (1931) determined the numbers of *L. acidophilus odontolyticus* in the saliva of subjects after various procedures which were expected to reduce the counts. The original counts were roughly related to the number and size of the cavities. After the large cavities were filled the counts were diminished. After all cavities detected were filled there was a further decrease in the number of lactobacilli. Other procedures which then reduced these organisms were restriction of carbohydrates ("no candy, pastries or sweets of any kind allowed, and but a very small amount of bread"), prophylactic treatment ("scaling, polishing of tooth surfaces, etc.") and antiseptic agents. A teaspoonful of cod liver oil after each meal was not effective in reducing counts after cavities were filled and pro-

phylaxis effected. Orange juice was possibly of some value.

It is worthy of note that Rodriguez did not consider that *L. acidophilus* was proved to be "the specific bacterial factor of tooth decay" but regarded the count of this organism closely related to the existence of tooth decay even though "undiscoverable by our present means of clinical examination."

Rodriguez found a decrease in numbers of lactobacilli to a minimum at 10 years of age, an increase then to 14 years, decrease to a plateau at 17 to 30 years, and then a continuous decline.

Thompson (1931) found gram-positive, non-sporing, pleomorphic rods in 21 of 24 cultures obtained by swabbing the tooth surfaces in caries cases. These forms were present in all of 7 cases of arrested caries but in only 6 of 19 caries-free individuals. Thompson wrote, "Unless it can be shown that cavity formation offers a milieu without which aciduric bacilli of this type cannot survive (that is, in which they occupy the role of secondary invaders) the aciduric organisms must be included in any consideration of the etiology of dental caries."

Hadley and Bunting (1932) studied the *B. acidophilus* on the tooth surfaces, in the paraffin-stimulated saliva and in the feces of nine caries-susceptible and seven caries-immune subjects by repeated sampling. The organisms were separated as to the four types described by Hadley, Bunting and Delves (1930). They found *B. acidophilus* in all samples of tooth scrapings, saliva and feces of nine caries-susceptible subjects, and in 45, 78 and 65 per cent, respectively, from the immune subjects. The rough type occurred to the extent of only 7 per cent of those with caries. A type not found in caries was isolated in 40 per cent of the tooth surface samples from the immune subjects. They ascribed the comparatively high incidence of aciduric forms in the caries-free individuals to the inclusion in this study of atypical forms and to the large number of examinations. They considered it possible that in subsequent examinations of the subjects caries might be discovered.

Jay, Crowley, Hadley and Bunting (1933) discussed one subject with a very low count of *B. acidophilus* in the saliva and no caries. In continued observation the count suddenly increased and three months later caries was observed. They failed to implant *B. acidophilus* in the mouths of five children caries- and acidophilus-free by daily feeding of acidophilous milk. Restriction of carbohydrate in one subject resulted in decrease of acidophilus count to around 500 per cc. of saliva. After resumption of high carbohydrate diet for several days, the count mounted to over 1,000,000. The count was reduced to less than 100 by withdrawal of sugar and starch over a period of about a month.

Hadley (1933) modified Kulp and White's (1932) tomato agar by increasing the agar content to 2 per cent and adjusting the pH to 5.0 with lactic acid. This medium suppressed the growth of all organisms except acidophilus strains, a few streptococci and some yeasts. *M. tetragenus* and *Staphylococcus albus* occasionally appeared but could be recognized. By spreading a measured amount of diluted saliva on such agar plates, Hadley was able to determine quantitatively the count of acidophilus in stimulated saliva. She found, for example, an average count of 60,000 *B. acidophilus* per cc. of saliva for 14 caries-susceptible children in 79 examinations. The average for 10 caries-free children in 69 examinations was 600. One caries-free subject was completely free of *B. acidophilus* in tests over a period of 18 months. A single caries-susceptible subject showed a 40,000 to 300,000 count with a mean of 150,000. By carbohydrate restriction the count was reduced to 900 in 4 weeks. Upon resumption of a rich carbohydrate diet the count rose to 1,800,000 in 5 days. By means of an

extremely low carbohydrate diet a count of 100 was obtained, which returned to 150,000 on resumption of a higher carbohydrate intake.

Jay, Crowley and Bunting (1932b) stated that 68 per cent of caries-susceptible children showed positive skin reaction to injection of filtrate from *B. acidophilus* but that almost 30 per cent of caries-free children also showed positive results. The highest titre of *B. acidophilus* agglutinins was 1–640, with most at 1–320 in caries-free individuals, but 1–80 was the highest in 25 caries-susceptible subjects.

Rosebury and Greenberg (1932) seeded various strains of lactobacilli into veal-broth media with 2 per cent glucose and with initial pH values ranging from 6.6 to 2.8. After 7 days they measured the final pH of the series of tubes to calculate an index of aciduric and acidogenic power. The acid-production tests failed to distinguish between "caries" and "caries-free" strains. Rat strains were generally "weak".

Enright, Friesell and Trescher (1932) examined 50 subjects for caries and lactobacilli. "Of these 50 cases, 35 were recorded as non-carious, of which 18 were positive for lactobacilli and 17 negative. Of the 15 carious cases, 9 were positive for lactobacilli and 6 negative. Upon making follow-up examinations three to nine months after the first, it was found that the 6 cases of incipient caries without lactobacilli had not advanced; of the 9 cases of incipient caries with lactobacilli present on the first examination, 5 had become advanced caries." This provides strong circumstantial evidence of the importance of lactobacilli in the carious process. On further consideration of the 35 non-carious cases, it was found that 13 of the 18 cases that were positive for lactobacilli on the first examination had developed caries by the time of the second examination 3 to 4 months later, whereas 16 of the 17 cases that were negative for lactobacilli on the first examination were still without caries on the second examination. From the evidence thus gathered, the inference was made in 1927 that the initial bacteriological findings with respect to the presence or absence of lactobacilli seemed to be a true prognostic indicator of the conditions that would be observed on clinical examination three to six months later. Jay came to this conclusion independently, as is evidenced in his report of 1929. (See also Rodriguez, 1931.)

Boyd, Zentmire and Drain (1933) cultured the organisms of the saliva of 45 children, 34 of whom had been for long periods on diets capable of arresting caries. Cultures were made in 1 per cent glucose broth of initial pH 5.7 and on acid agar plates, initial pH 5.0. The authors state: "Summarizing the observations pertaining to the children receiving diets capable of arresting caries: there is nothing to indicate that prolonged use of such diets led to any characteristic change in the oral flora, to disappearance of *B. acidophilus*, or to lessened acid-producing power of the flora."

Schoenthal and Brodsky (1933) compared the dental classification of 109 children with the incidence of gram-positive acidogenic bacilli. They found the highest incidence in carious mouths but said, "The fact that acid-producing organisms were present in all mouths suggests that their presence does not necessarily lead to the development of caries."

Johnston, Williams, Anderson, Drake, Tisdall and Kaake (1936) in a study of *L. acidophilus* in 39 children used the method of Hadley (1933) in monthly examinations. Their observations at the beginning and end of a year were confirmed in cooperation with Jay, Hard and Hadley and by Bunting, Jay and Hard, respectively. Their data showing the occurrence of *L. acidophilus* in relation to caries progress were as follows:

In 1 case with markedly progressive caries *L. acidophilus* was continuously present; in 5 cases with slightly progressive caries *L. acidophilus* was continuously present in 4

and sporadically absent in 1; in 3 cases with very slightly progressive caries *L. acidophilus* was continuously absent in 2 and sporadically absent in 1; in 30 cases with nonprogressive or doubtful caries *L. acidophilus* was continuously present in 1, absent in 20 and sporadically absent in 9.

Morse, Losch and Davenport (1936) concerning association of aciduric bacteria with dental caries stated: "At each visit of child, duplicate cultures were taken on pH 5.0 broth; smeared and also plated after 48-hour incubation. Bacteriological observations were recorded during 342 child-years. For 98 children, there was correlation between caries and aciduric bacteria in 52 per cent of observed years; 19 per cent of child-years showed complete reversal in this relationship, with no correlation; 29 per cent gave indecisive results, both positive and negative cultures being obtained during year. The 7-year-old group of 52 children (age of highest incidence) showed 87 per cent of caries-free negative for aciduric bacilli in all observations. In group having 6 or less cavities: 33 per cent positive; 67 per cent either negative or indecisive. In group having 6 to 11 cavities, from birth to age 7, 65 per cent showed positive cultures. In group with highest incidence, 100 per cent gave positive cultures."

Jay (1936) in a review stated: "Many do not agree that causal relationship exists between the lactobacilli and dental caries. Regardless of this controversial point, these organisms afford an excellent means of estimating caries activity and in this capacity are invaluable aids in research. If an arrest of caries is imminent, the acidophilus count is bound to drop off far in advance of any clinical sign. The reverse is also true, for in increased caries activity the bacteriologic count mounts at least four months before new lesions can be demonstrated. Long, costly caries prevention experiments can be cut short or modified if the desired bacteriologic change does not take place within a reasonable time. The total disregard of bacteriology in caries experimentation is sheer extravagance. This does not mean that the efficacy of a caries prevention program is to be judged solely by the change in the oral flora. Bacteriologic examinations are highly informative, but the final test must, of course, be clinical. Caries cannot be said to have been successfully checked unless no new lesions appear and existing cavities grow no larger for at least a year. This necessitates a most rigid comparative clinical check-up at the beginning and end of the period of observation."

Jay (1936) then discussed three objections to the diagnostic use of *L. acidophilus* which he alleged to be due to misunderstanding. "First, some have been led to ignore these organisms because they may be demonstrated in mouths of individuals who are free from caries." "Second, some investigators claim that they have been unable to isolate *L. acidophilus* from carious lesions." Such would not be expected with arrested caries though open cavities exist. The third argument has been that *L. acidophilus* cannot be implanted in "immune" mouths. It may be noted that these three objections do not relate to the existence of active caries.

Canby and Bernier (1936) seeded superficial and deep carious dentin from 44 teeth into glucose-brain broth at pH 7.2. Of these, 8 were negative but were classed as arrested caries. In the other 36 cases, *L. acidophilus* was present in 31 of the 33 positive cases in superficial carious material, and in pure culture in 8. In the deep carious dentin, 13 of the 36 were negative; of the 23 positives, the lactobacillus was found in 21 and in pure culture in 18. The description of the cavities suggests that they were generally open. The investigators excluded streptococci as etiologic agents on aciduric considerations. They said: "While streptococci were frequently present in the superficial cultures, the majority of the strains isolated failed to grow in glucose broth of

pH 5.0. When transferred to acid broth of pH 4.0, all strains were negative. The maximum acidity produced by these streptococcal strains was a final pH of 4.7, and then in only two instances." They indicated results of pH measurements of carious dentin, however, and that "acids liberated are partially neutralized as soon as the calcium salts of the tooth are dissolved, forming a buffered solution which apparently establishes a final acidity of approximately pH 5.0 in the deeper area of the decalcified mass. In the superficial carious dentin, the acidity is less marked. The acids present become diluted and further neutralized by the buffering substances present in the saliva, with formation of an area favorable for the colonization and growth of the ordinary mouth bacteria."

Waugh and Waugh (1936) studied 69 selected Eskimos of southwestern Alaska. They found that 85 per cent of caries-free individuals showed no lactobacilli in the saliva.

Rosebury and Waugh (1939) found caries as it existed among 124 Eskimos of southwestern Alaska to be identical in type with that of American children, that is, consisting of occlusal and proximal lesions. *L. acidophilus* was found correlated with caries, there being positive cultures from 80.6 per cent of those with caries, from 13.6 per cent of those free of caries and from 42.3 per cent with a doubtful status. Yeasts also were somewhat correlated with caries, but the appearance of cocci on agar at pH 5.0 initially was random.

Snyder (1939) cultured the salivas of 17 caries-free children from an institution in Maumee, Ohio, and 21 caries-free children of similar ages from Ann Arbor, Michigan. Of the Maumee salivas, "only 11 of 159 were positive for lactobacilli, whereas of 178 specimens of saliva from 21 Ann Arbor public school children 85 were positive for lactobacilli" over a period of 18 months. One small "extension" developed in a Maumee child, but 6 Ann Arbor children were definitely subject to caries at the end of the period. "Other aciduric organisms were isolated, but of the yeasts, streptococci, and staphylococci only the yeasts seemed to have any relation to the caries observed and this role was apparently secondary to that played by the lactobacilli." It may be noted as significant that Snyder showed only *correlation* between lactobacilli and caries *activity* and his resumé of the findings of others whom he deemed to have made adequate dental observations was that the relation between lactobacilli and caries is one of correlation.

Speidel, Boyd and Drain (1939) determined the counts of *B. acidophilus* by Hadley's (1933) method in the salivas of 65 diabetic children on controlled diets which generally arrested caries. Clinical examination showed 39 with inactive or no caries and 26 with active caries. "In general the highest incidence of *Bacillus acidophilus* was found in cultures from children with active caries. However, since 83 per cent of the determinations on children with active caries and 60 per cent of the determinations on children free from active caries showed *Bacillus acidophilus* in some amount, correlation between the incidence of *B. acidophilus* and dental caries does not appear marked." They also found no relation between acid production of oral bacteria and activity or inactivity of caries.

Box (1939) found the pH of 31 samples of carious human dentin from extracted teeth to range from 6.5 to 4.0, 8 samples showing the latter value. In two other samples judged to be arrested caries the reaction was 7.0.

Curotto Devoto (1940) studied the saliva volume, tartar and *L. acidophilus* of 15 caries-free young adults, of 20 with medium degree of caries, and of 65 with extensive decay.

Arnold and McClure (1941c) compared clinical observation of activity of caries and lactobacilli of the saliva of 127 children of average age of 12.9 years. Counts were

made 4 different times in a period of 1 year. They disclosed "a close relationship between dental caries activity and the number of lactobacilli present in the mouth."

Warner and Arnold (1941) studied 32 strains of aciduric bacilli isolated from the saliva of subjects with caries and one strain each of *L. acidophilus* isolated from a breast-fed infant and from commercial acidophilus milk, respectively. They could not make a distinction between the oral and non-oral strains by either fermentation or agglutinin absorption methods. The fermentation tests indicated heterogeneity, the agglutinin absorptions, homogeneity within the three groupings (Hadley, Bunting and Delves (1930)) applied to the dental strains. Most of the strains formed acid from sucrose, though more were acid formers from glucose and maltose.

Snyder and Teachout (1941, 1942) isolated 30 strains each of oral yeasts, smooth lactobacilli, streptococci and staphylococci. By plating at pH 4.5, 4.7, 5.0, 5.3 and 5.5 they demonstrated that the lactobacilli were most aciduric, staphylococci intermediate, and only two streptococcic strains could grow in a medium as acid as pH 5.3. Viability was tested in 2 per cent glucose broth. Only two strains of streptococci survived for 10 days; all 30 strains of yeasts, lactobacilli and staphylococci survived. Seeded into glucose agar at pH 4.75, only lactobacilli uniformly reduced the pH further, and no evidence was found of symbiotic relation between any of the forms. They expressed an opinion: "It is extremely doubtful if the rate of acid formation, . . . is anywhere near as important in decalcification as the ability to live or reproduce in surroundings inimical to their survival."

Fosdick and Starke (1941) found that "*L. acidophilus* is able to catalyze the various steps in carbohydrate degradation" but at different rates than yeasts. Phosphorylation was comparatively slow for *L. acidophilus* but reduction of pyruvic acid to lactic acid was rapid.

Fancher and Fosdick (1941b) analyzed carious material removed from single unextracted teeth for lactic acid by the method of Miller and Muntz (1938). They found a mean of 0.367 mg. of lactic acid per 100 mg. of carious material in 68 cases, and 0.432 mg. in 29 cases after the ingestion of candy.

Taylor (1942) found that the *L. acidophilus* count in saliva increased with the number of cavities and concluded: "The incidence of dental caries is directly proportionate to the count of *Lactobacillus acidophilus* in the saliva".

Becks, Wainwright and Young (1941) found 48 (77.4 per cent) of 62 caries-free individuals had zero counts of *L. acidophilus* and only 3 had high counts (1,000 to 10,000 colonies per cc. of saliva). Of 66 subjects with caries, "only 4 had zero counts and 2 had insignificant findings; the remainder had high counts, most of which were extremely high, rising as far as 300,000 colonies."

Collins, Jensen and Becks (1942) found the following percentages of University of California students with "high" as compared with "zero" *L. acidophilus* indexes in three groups numbering 122 each: Caries-free group 18.9 per cent, active or inactive group 40.9 per cent, rampant caries group 87.7 per cent. The basis of the caries-free class was freedom from caries by clinical examination and roentgenogram. As 122 such cases were found, that number served as the basis of selection of the other groups. The acidophilus counts were subsequent to the clinical findings and were made on two sucessive days.

Becks, Jensen and Millarr (1944) reported a high correlation between *L. acidophilus* counts and dental caries activity, with 87.7 per cent of 1250 subjects with rampant caries having indexes over 1000 but 82.3 per cent of 265 caries-free individuals having indexes below 1000.

Becks, Jensen and Millarr (1944) reported a reduction of *L. acidophilus* counts in 81.7 per cent of 1228 rampant caries

cases "within a period of a few weeks by reducing the intake of refined carbohydrates and replacing the calories derived from these foods by increasing the intake of meat, eggs, milk and milk products." "The reduction of the L. a. index resulted in a drastic decrease in dental caries frequency. In a group of 790 rampant caries cases the prevention of new cavities was achieved along with a reduction of the L. a. index to the extent of 80 per cent. Of these, 62.3 per cent were arrested completely, while an additional 17.7 per cent developed only 1 or 2 cavities during the following year. In 665 cases with favorable L. a. response, no new cavities developed in 71.7 per cent and within the limits of 1 or 2 cavities in 88.4 per cent. This instance of favorable response establishes the definite relationship between the reduction of the L. a. index and the reduction of dental caries activity."

King and Croll (1939) fed 2 ounces of sticky candy daily to 8 children, 2 being caries-free at the start and the others having 1 to 3 carious cavities, very small or questionable. After 16 weeks more cavities were found. There was no control group. The lactobacillus counts in no case reached 10,000 per cc. of saliva, and there was no relation to the candy feeding. They could not find a relationship between caries activity and counts of lactobacilli in the 8 children and in 30 children from the Island of Lewis, 20 of whom were caries-free and 10 caries-active.

Whyte (1943a) isolated strains of aciduric organisms from 500 saliva samples from children. He concluded that "*Bacillus* or *Lactobacillus acidophilus* is not one single organism, but one of a group, the various members of which show a close relationship to each other in colonial appearances, cell morphology, biochemical reactions, serology and acidogenic properties."

Harrison and Opal (1944) cultured from the saliva and feces of 18 children for lactobacilli. The organisms were found in 66 per cent of the fecal specimens and 93 per cent of the salivas of the 11 children who had a definite dental caries activity. The cultures were maintained for a period of 16 to 20 months to stabilize their characteristics. The authors concluded: "Paired, oral and intestinal cultures from 8 individuals, studied comparatively, were found to be identical in morphology, fermentative action and immunological reaction.

"It is concluded that the cultures isolated from the intestine had their origin in the mouth and that the supply of these organisms is continually replenished by the swallowing of lactobacillus-contaminated saliva."

Becks and Jensen (1948) reported that only 10 per cent of 752 subjects with rampant caries failed to show inactive caries as measured by *L. acidophilus* counts and clinically when the fermentable carbohydrates of the dietary were restricted. In a control group of 347 subjects similarly treated as to repair of cavities but without restriction of carbohydrates, 91 per cent showed new cavities and continued high counts of *L. acidophilus* in a period of a year.

Variation of Counts of Lactobacilli

Smolelis (1945) found that the *L. acidophilus* count of saliva was doubled by incubation at 37°C for 24 hours before plating. Samples held for 96 hours at room temperature showed a 27-fold increase in count. When the holding temperature was 8°C for 48 hours there was a decrease in the counts.

Davies (1950) examined the salivas of 367 6- to 8-year-old children of Evanston and Oak Park, Illinois in 1946 and 1947, respectively, and in Evanston in 1948 after fluoride had been added to the water supply for 1 year to a level of 1 p.p.m. He found negative counts of lactobacilli in 25.5, 28.4, and 34.0 per cent. There was an increase in counts with increase of untreated carious surfaces, though the average number of carious surfaces per mouth were 4.0, 2.9 and 3.4 respectively. There was possibly an

increase in rate of flow of saliva with increased count of lactobacilli, but there was no relation to type of organism.

Davies plated salivas of 6- to 8-year-old children immediately, and after the salivas were kept at 25–35°C and 5–10°C for 24 to 96 hours. He wrote: "It will be noted that there is a definite decrease in the average count per milliliter of saliva after it had stood under either condition for 24 hours. After 48 hours the average count for both is nearly the same as that of those plated immediately. At 72 hours there is another decrease for the specimens at room temperature, but not so great a decrease for those at refrigerator temperatures. The two conditions seem to have about the same effect on the count after 96 hours. This data should be qualified by stating that 100 specimens were carried for the first 24 hours; 50 for 48 hours; 30 for 72 hours; and only 16 were carried for 96 hours after the routine immediate plating had been done."

Permar, Kitchin and Robinson (1946) concluded from a study of variability of counting of oral lactobacilli: "Series of plates, each made from single specimens of saliva, with extreme care in measurement, gave colony counts which varied over wide ranges. Different methods of mixing the original specimens were employed in an effort to secure better distribution of the organisms. Hand shaking, an amalgam mixer, a Kahn tube shaker, and 3 types of beaters were used. None of these increased the uniformity of counts. More consistent results were obtained when 5 cc. of the original specimen were placed in an equal amount of fluid agar and beaten before a second dilution was made and the specimen plated. All methods used gave some series showing wide variations.

"The assemblage of the data from each cooperator emphasized the wide range of counts obtainable from a single specimen. It is obvious that, while trends are apparent in the lactobacillus counts of individuals, conclusions as to hourly or daily fluctuations are unjustified when based on single counts or series of counts such as used in this study."

Boyd, Wessels and Cheyne (1948a, b; 1949a, b) observed the development of new carious surfaces in teen-aged girls over a period of 30 months with from 4 to 8 counts of *L. acidophilus* during the last 9 months of the study. They found no significant difference in the median counts between extremes in development of carious surfaces. They (1949a) said: "Generally speaking there was a slight trend toward parallelism of the Lactobacillus counts and rates of progression of caries when massed data were used. However, when the group as a whole was subdivided according to the rate of caries progression, there was little difference between the range of Lactobacillus counts observed among those with the least and those with the greatest progression of tooth decay." A correlation coefficient of 0.1959 was found between lactobacilli counts and caries increment but the value was not statistically significant (1949b).

The entire statement of Marshall-Day, Shourie, Hein, Leung and Simmons (1949) relative to correlation of *L. acidophilus* and caries is as follows: "Samples of saliva collected from 564 14-year-old boys from six Puerto Rican localities were air mailed to the bacteriological laboratory of the New York State Department of Health for *Lactobacillus acidophilus*, cocci, and yeast counts. In 312 of these cases duplicate samples were sent to the Michigan Caries Control Laboratory for lactobacillus counts only. The average period between collection and analyses was 3 days, the maximum being 4 days. *L. acidophilus* counts of the duplicate samples showed a high coefficient of correlation between the two laboratories (0.74). The standard product moment correlations between the lactobacillus counts and the D.M.F. teeth, number of open cavities (excluding roots), and D.M.F. areas were only 0.30, 0.33, and 0.33, respectively. Similar correlation between cocci and

D.M.F. teeth gave a coefficient of 0.27. The correlation coefficients between lactobacillus counts and the amounts of gingival involvement and the amount of debris around the teeth were much lower. The data show no evidence that the L. a. count is closely correlated with either the cumulative caries experience or the gingival condition of the children."

Sullivan and Storvick (1950a) found a statistically significant positive correlation coefficient of 0.245 between DMF teeth and lactobacillus counts in 574 college students. Other correlations between lactobacillus counts and other salivary constituents found by Sullivan and Storvick (1950a, b) are shown in the following table.

	Starch hydrolyzing time	Buffer capacity	Ammonia nitrogen	Lactobacillus counts	Snyder test
pH	+0.063 +0.023	+0.252* +0.279*	+0.098* +0.019	+0.032 −0.034	— −0.085
Starch hydrolyzing time		+0.102* −0.067	−0.028 −0.041	−0.082 −0.006	— +0.014
Buffer capacity			−0.085 −0.018	−0.109* −0.056	— −0.037
Ammonia nitrogen				−0.020 −0.130*	— −0.107*
Lactobacillus counts					— +0.349*

* Statistically significant correlation.

Streptococci

Sieberth (1900) found streptococci as the only organisms present in pulpitis and believed they originated in carious dentin, as he showed them to be present in such tissues.

Baumgartner (1913) described the beginning of caries on the enamel surface as, first, a development of a bacterial film 0.1 mm. thick, followed by a change in refraction of light by the enamel. Then followed irregular lines and discontinuities of the surface. The attack proceeded to the cement substance and then to the prisms. He said the organisms which caused these changes were streptococci, though other forms appeared as secondary invaders. The streptococci continued to lead the advance into the dentin. He considered the idea that acids are the agents of tooth destruction as only a vague surmise.

Niedergesäss (1915) found cocci, generally *S. pyogenes*, in the deepest layers of carious dentin. The deeper the layer, the less there was of admixture with other organisms. The cocci formed acid, but the maximum concentration was independent of the available glucose. The maximum acid production, 0.006 to 0.05 N, was attained in 24 hours, and in 72 hours the cocci were no longer viable. He regarded these organisms as the cause of caries. If $CaCO_3$ were added to the culture medium the cocci remained viable up to 12 days. He showed that calcium passed into solution. He assumed that most of the acid formed was lactic acid. Mixed cultures of organisms from carious material formed no more acid than his pure cultures. He confirmed Sieberth in finding streptococci in pulpitis. The cocci were only slightly pathogenic to guinea pigs.

Hartzell and Henrici (1917) concluded from review of the literature and their general studies on mouth organisms that

"streptococci are the microbic agents that cause dental caries, whatever other factors may enter into the etiology. The fact that they are the sole organisms found constantly in the advancing border of the carious process indicates that they possess an *invasive power* not exhibited by other bacteria found in cavities. It may be that the solution of the lime salts is accomplished by acids formed from other bacteria, altho mouth streptococci are powerful acid formers; it is quite certain that the digestion of the organic matrix is accomplished by the proteolytic activities of such saprophytes as *B. proteus*, *B. mesentericus*, and certain of the anaerobic bacilli. But these processes are secondary. The penetration and necrosis of the dental tissue, seen in the advancing border of the process, is very evidently due to the streptococci, and the streptococci alone." They also indicated belief that the same organisms, *S. salivarius*, caused pulpitis, alveolar abscess and pyorrhea alveolaris.

Henrici and Hartzell (1919) found *Streptococcus viridans* the most frequent invader of vital pulps of carious teeth and considered the route of invasion was "clearly along the dentinal tubules."

Sperling (1922) isolated *Streptococcus lacticus* (Kruse) from the dentin of 56 of 60 carious teeth and concluded it was the chief cause of caries. The borings from dentin were obtained from (a) teeth in the mouth (b) freshly extracted teeth and (c) teeth 2 to 21 days after extraction. Sperling found the organism produced *l*-lactic acid in cultures by isolation of the zinc salt.

Clarke (1924) pointed out that "the teeth examined by Howe and Hatch (1917) and McIntosh, James and Lazarus-Barlow (1922) were in a comparatively advanced stage of caries, with cavity formation, and the conditions in such cases are very different from those which obtain at the initiation of the disease. The cavities, open to the mouth, retain considerable quantities of food material which rapidly ferments and becomes acid, and the resulting acidity encourages the growth of *B. acidophilus* while inhibiting that of other bacteria." Clarke used carious dentin of fissure caries in which the enamel was practically intact and approximal surface lesions in which the carious foci were confined to the enamel.

In glucose agar plates he found "a streptococcus of very distinctive characteristics and, although not acidophile, an extremely active producer of acid." He named it *Streptococcus mutans*. It was isolated from 27 of 40 fissure cases, with 4 plates sterile. Of the remainder, typical colonies were seen but the organism was not proved by isolation. It was found in 9 of 10 approximal cases and typical colonies were seen in the other. "*B. acidophilus* was present in 9 fissure and 5 approximal cases", all being comparatively open. Only 2 sugars of importance in the mouth were discussed in the biochemical reactions of *S. mutans*, glucose and lactose being fermented with acid production. Seeded into a glucose broth at pH 7, *S. mutans* produced a reaction of pH 4.2 in 24 hours. The organism, which would not grow below pH 5.6, died out in 24 to 48 hours in the broth cultures which reached pH 4.2.

Maclean (1927) confirmed Clarke in finding *Streptococcus mutans* present in carious teeth. He distinguished *S. mutans* and *B. acidophilus* by fermentation reactions, colony formation and serological tests and stated that they are different organisms. Of 38 teeth examined for bacteria in carious dentin, 18 were sterile. *S. mutans* was found in 10 teeth and in pure culture in 7; *B. acidophilus* was found in 7 teeth but never in pure culture. He found *B. acidophilus* in the saliva of caries and non-caries subjects; *S. mutans* was found with much less frequency and only in caries cases.

Howitt, Fleming and Simonton (1928), in a study of mouth flora in a single caries-free man on successive diets, said, "streptococci were the outstanding forms, both relatively and absolutely, from both the aërobic and

the anaërobic plates having a reaction of pH 7.4, while the aciduric rods predominated in the acid (pH 5.0) agar mediums." "There is a tendency for the aciduric bacteria, especially the rods, to increase in the presence of carbohydrate food. This increase was probably due to the food remaining in the mouth, as the numbers decreased when the tooth-brush was used."

DeVries (1930a, b) rejected streptococci as having a primary role in the etiology of dental caries because they occurred in relatively small numbers in the deep layers of carious dentin compared with their occurrence in the superficial layers of the same tooth, and there was no relation between the number of organisms in carious dentin and the condition of the tooth. DeVries (1930a, c) rejected *B. necrodentalis* of Goadby as having a primary role in caries etiology because he did not find it constantly present in carious tissues, it was present in deep layers of carious dentin in only small numbers, if at all, and its presence or absence was not related to the condition of the tooth.

Tucker (1932) seeded scrapings of the surfaces of teeth of 422 children into broth at pH 5.0. "Streptococci of various types were isolated most frequently, but *L. acidophilus*, *Staphylococcus albus* and yeasts also developed in the acid medium." His attempt "to correlate the incidence of dental caries with the occurrence of some one type of aciduric micro-organism" was unsuccessful. "Aciduric streptococci were found in the mouths of practically all of the children irrespective of the incidence of dental caries . . ." and "*L. acidophilus* was found most frequently and most consistently in the mouths of those children whose teeth contained three or more cavities." "*L. acidophilus* occurred rather frequently and consistently in the mouths of children who had never had any dental caries, or who had developed no new carious lesions for two years. From this it appears that *L. acidophilus* is not an obligate producer of dental caries." Tucker showed that his failure to find *L. acidophilus* in many cases was not because it was crowded out by streptococci. He succeeded in recovering it from *in vitro* mixtures with streptococci in an initial ratio of only 1 to 10,000.

Fish and Maclean (1934) cemented crowns of carious human teeth onto prepared roots of teeth in the mouths of 2 dogs and 1 monkey. *Streptococcus mutans* could not be recovered from the carious dentin after 2 to 4 days in the case of the dogs but was recoverable after 11 days from 4 such teeth in the mouth of the monkey which itself had carious teeth. From observations on *S. mutans* in 7 human carious teeth immersed in saliva, they said, "Immune male human saliva kills off *Streptococcus mutans* and other organisms in a carious tooth *in vitro*" in 2 to 4 days. They concluded, "It also appears that arrest of caries is due to a change in the environment of the tooth and not to any vital resistance on the part of the dentin."

Berenson (1935) concluded from direct counts of oral flora that there was no difference in the percentages of cocci and bacilli in the mouths of 58 caries-free children, 317 with 1 to 3 cavities, and 125 with 3 to 9. However, in children 6 to 10 years of age there were lower percentages of both acidogenic cocci and bacilli than in 10- to 14-year-old caries-free children. In dentinal caries cocci predominated slightly in percentage over bacilli. In the outer layers of caries he found the bacilli, but in the deeper layers cocci predominated or were in pure culture. The bacilli, various types, gave terminal pH values 4.2 to 3.6; the cocci, 5.0 to 4.5.

Anderson and Rettger (1937) found lactobacilli in 12 of 65 individuals with varying degrees of dental caries, but acidogenic streptococci in 55 cases. They said, "The media and methods used in our investigation were conducive in every instance to the growth of the lactobacilli when these were present in viable form." They used media at both pH 6.8–7.0 and at 5.0. They ob-

served gram-negative cocci and leptothrix- and yeast-like forms that were acidogenic, and they were not inclined to deny the significance of these in caries.

Tunnicliff and Hammond (1938) isolated rough colony types of *Streptococcus viridans* from the pulps and dentin of carious teeth. Rough colonies could be stabilized from smooth colonies and vice versa by cultural methods. "Both rough and smooth strains grow in 1 per cent dextrose, pH 4.4–5.0, and may live in this acid medium one week or more." These authors suggested that some of the various forms in dentinal tubules as illustrated by Miller (1890) may have been bacillary, filamentous and pleomorphic coccus forms of streptococci. They compared acid formation by streptococci and lactobacilli and said, "Only after from three to six days' incubation in 1 per cent dextrose broth was as much acid produced by lactobacilli as was formed in twenty-four hours by smooth strains of greening and anhemolytic streptococci (pH 4.4–4.8)."

Bibby, Hine and Clough (1938) found streptococci less inhibited in growth by saliva than lactobacilli.

Bibby (1938a) devised and tested a method of differential counting of direct smears from different areas of the mouth. If 250 or more organisms were counted, the experimental error was satisfactorily small. He found (1938b) greater variation in the percentage of organisms of one type in different parts of a single mouth than between the same parts of two different mouths. High percentages of gram-negative cocci were found and a relatively low percentage of gram-positive bacilli. Bibby (1938c) recorded an average of 40.2 per cent gram-negative cocci and 5.1 per cent gram-positive bacilli in smears from 68 cavities. These latter "represent certain bacillary elements which in no circumstances could be considered lactobacilli."

Bibby and Hine (1938) examined "forty-four smears from different types of carious cavities in forty-two mouths," finding that considerable variation occurred. In a single cavity there was daily variation, but the order of occurrence remained about the same. They said: "The demonstration of complex bacterial flora in the depth of all cavities, with no distinct morphologic group predominating, indicates that this location favors no particular group of organisms. It also suggests that no specific bacterial species can be considered the cause of dental caries. This idea is supported by the absence of significant differences between the flora of rapid and those of slow caries. The high percentages of cocci, especially gram-negative, in all cavities suggests the possibility that these organisms are causative agents. The relatively high percentages of gram-negative bacilli, filaments and fusiform organisms as compared with the gram-positive bacilli raised doubt concerning the importance in caries of lactobacilli which are included in the last named group."

Strean (1939) assigned to lactobacilli an early role in dental caries associated with plaques. He showed streptococci to be present in dentinal tubules and indicated their action to be of a dual nature of acid decalcification and proteolysis of the dentin.

Hammond (1939) reported direct observation of change of smooth colony forms of dental streptococci to rough forms, and the reverse. The transformations were through intermediate bacilli forms, with coccus organisms representing smooth colonies and filaments being most characteristic of the rough forms.

Belding and Belding (1940) asserted that there is an 80 per cent correlation between speed of acid formation by saliva with a wide variety of media and caries activity. They stated that an organism which they designated as *Streptococcus odontolyticus* "must be regarded as the principal agent" in caries. Also "Lactobacilli, like the streptococci, are universally present in all mouths and are numerically more prominent in the

caries-susceptible than in the immunes." They considered the cereals as the dietary ingredients most conducive to caries.

Canby and Bernier (1942) in considering streptococci as organisms having to do with caries have said, "Further, the fact that they are seldom isolated from deep carious dentin suggests that, in the light of present-day knowledge, caution must be exercised in placing emphasis on their role in the production or initiation of the carious lesion."

Bibby, Volker and Van Kesteren (1942) estimated the numbers of organisms in saliva by plate counts on three different media. They concluded "that in carious mouths lactobacilli make up little more than 1/2000 of the total organisms and less than 1/1300 of the acidogenic aerobic organisms. Yeast represents only 1/25000 of the total. Although more than 20 times more numerous than the lactobacilli, the streptococcal types growing on oatmeal bread also seem to be a numerically unimportant group. It seems likely, therefore, that the organisms principally concerned in acid production in the mouth are the acidogenic types (mainly streptococci) which have not been identified in this study." They found that oral streptococci formed acid more rapidly than lactobacilli, and said: "Therefore, since the streptococci are approximately 1,000 times more numerous than the lactobacilli and since it is likely that activity in the mouth parallels that in test tubes, it can be estimated that lactobacilli give rise to no more than .025 per cent of the acid formed by the action of salivary organisms on carbohydrates." They found that most oral bacteria formed increased amounts of acid in the presence of powdered dentin or enamel. The amount of tooth substance dissolved was proportional to the acid formed. As the acid was determined by titration with phenolphthalein as the indicator, it obviously represents acid which is free as well as that which is neutralized by tooth substance.

Bibby and Maurer (1942) measured total acid formation in 6 and 24 hours in salivas to which 1 per cent of glucose was added. The salivas from 22 subjects with active caries formed the most acid, those from 13 moderate caries subjects an intermediate amount, and from 19 with no caries, the least. "Hemocytometer counts, total of viable organisms, percentage of viable organisms and numbers of lactobacilli and yeast paralleled acid formation in different groups, but count for acid-forming streptococci was highest in caries-free group." They considered that in the production of different amounts of acid "total bacterial count is more important than any other single factor." Bibby and MacKinnon (1943), in a similar confirming study, noted that "the total bacterial count and the lactobacillus counts showed, especially in the low caries tests, accelerated growth after 8 hours, whereas the streptococcal population reached its high point at 4 hours and decreased thereafter." Also, "the only relationship between rate of bacterial growth and acid production appeared to be between the rapid growth of streptococci and rapid acid production during the initial hours of the tests."

Florestano (1942) cultured the organisms of the salivas of 136 carious subjects and 101 non-carious (absolute-immunes and no active caries). The initial plating was on acid-agar at pH 5.0. The acidogenic power of the organisms was then determined. The S forms of lactobacilli gave pH values from 4.00–3.60; 39 of 68 strains of streptococci showed pH 4.00–3.4; 10 of 25 staphylococci, 4.00–3.73; 4 yeasts, 5.50–5.17. Other strains of the above were acidogenic in lesser degree. Florestano said in conclusion: "Aciduric streptococci and staphylococci were isolated consistently from both carious and non-carious individuals. Their high acidogenic powers and presence in relatively large numbers in saliva suggests just as significant a role in caries as lactobacilli."

He also concluded, "No correlation could be made between the absence or presence of dental caries and the amount of acid formed by microorganisms from which individuals they were respectively isolated."

Possible special metabolism of streptococci: Owen (1923) showed that certain organisms that form gums from sucrose do it directly from sucrose and not from its constituents.

Krasnow and Rosenberg (1928, 1929) in studying the availability of carbon compounds for streptococci in various media, as indicated by continued viability, concluded that sucrose was more available for growth than the other sugars. Their series was sucrose, lactose, maltose, raffinose, mannite and dextrose. They suggested that dextrose was not utilized by streptococci, rather than that growth was inhibited by acid formation. "Glycerol and the organic acids (citric, lactic, malic, tartaric) were often more beneficial than carbohydrates."

Hucker (1928) said of *Streptococcus thermophilus:* "One of the striking characteristics of the organism is its preference for sucrose. Even tho it ferments other carbon compounds, the growth produced when sucrose is present is much more luxuriant than under any other condition." All of 56 cultures, however, produced acid from glucose and levulose as well as from sucrose and lactose.

Wright (1936a, b; 1937) has observed that certain strains of *Streptococcus thermophilus* isolated from milk did not ferment or grow on glucose, fructose, galactose or maltose but fermented and grew well on sucrose and lactose. He concluded (1937) that these carbohydrates were fermented directly without hydrolysis, since yeast, inhibited by fluoride or iodoacetate, produced reducing sugar but no acid, whereas the streptococci did not split sucrose to its constituent monosaccharides. The minimum inhibitive concentration of sodium fluoride was 20 p.p.m.

The foregoing evidence of inhibition of streptococci by glucose may be compared with the Hammond and Tunnicliff (1940) demonstration of cavitation of teeth by *S. viridans* in 1 per cent dextrose broth.

Various Forms of Organisms Associated with Caries

Miller (1883b) said: "I look upon the *Leptothrix buccalis* as the chief agent in the production of caries. I hope at another time to enter fully into the morphology, etc., of this fungus, so I will now only state that it produces not alone threads, but bacilli, bacteria, micrococci, and most likely screw forms, and that it is the coccus form which is most destructive to tooth tissue."

Miller (1885) described culture methods for microorganisms and stated that he had isolated 22 different fungi from the human mouth.

Gysi (1887) observed "fungi" in carious dentinal tubules in both ground and decalcified sections. He showed drawings of the "Fungus A of Miller, occurring in cocci, diplococci, and in chains" and "Fungus B of Miller, occurring in cocci, diplococci, bacteria, and often in leptothrix" and indicated his agreement with Miller on the constant presence of these acid-forming organisms in early carious dentin.

Galippe and Vignal (1889) isolated 6 species of bacteria from carious dentin, 4 being found in all of 18 teeth examined. Three of the organisms found constantly were acidogenic bacilli and 1 was a coccus. They found another large acidogenic bacillus in 8 cases and a large coccus form in 5. They expressed the opinion that caries was the result of lactic acid solution of the mineral matter of the teeth, followed by proteolytic action. They considered that teeth most resistant to caries had the higher mineral content.

Goadby (1910) recorded his observations that the salivas of caries-free human subjects and monkeys were similar in flora and in their mild acid reactions. He recorded cocci, sarcinae, bacilli of lactic acid type, necrodentalis and yeasts as present in carious

mouths. He stated, "As a general thing it takes about two and a half times as long to ferment a given quantity of cane sugar by means of mouth bacteria as it does the same amount of glucose or maltose." He pointed out that "yeasts, which are constantly associated with acute caries, are able to withstand a considerable concentration of lactic acid, and at the same time are able to go on fermenting fresh supplies of carbohydrate, provided the excess is neutralized locally, as it invariably is in dental caries, by the adjacent lime salt of the teeth." Goadby spoke of organisms of the *Staphylococcus viscosus* type "not in themselves fermenting carbohydrates", which form "carious gelatinous colonies, almost impossible to remove from the surface of the culture medium", which easily live "symbiotically with many carbohydrate fermenters." He implied plaque formation with "the dissolved lime salt diffusing out and the fresh acid diffusing in." He proposed displacement of acidogenic organisms of the mouth by other types, specifically mesentericus and subtilis bacilli and claimed excellent results by use of such organisms in pure culture in treatment of acute caries.

Howard Mummery, in discussion of Goadby's (1910) paper, pointed out that yeasts are too large to enter dentinal tubules. Sidney Spokes, in commenting on Goadby's (1910) observation that mild acid solutions (citric acid) were very efficacious in cleaning teeth, pointed out that in his experience some people taking sour milk treatment had developed caries. F. J. Bennett intimated that mouth organisms may draw on teeth for phosphate in order to ferment sugars through hexose phosphate. J. Sim Wallace inquired whether impure cultures would form acid as rapidly from sucrose as from glucose and cited Miller as showing no difference in amount of acid.

Pickerill and Champtaloup (1913) found cocci and bacilli in the salivas of Maori children and remarked that there appeared to be no difference from the flora of European children. They considered they had "quite sufficient evidence to show that immunity to caries and oral infections is not in such children due to the absence of those organisms which are usually regarded as causal factors in such conditions."

Okumura and Nakai (1927) conducted *in vitro* studies of the organisms from saliva and carious dentin which were capable of decalcifying enamel and dentin. They used mixed and pure cultures in sterilized bread or glucose media. They said the decay observed was "identical in every respect with the natural decay" but their brief discussion was limited to carious dentin. They concluded their *Bacillus P*, identical with the *Bacillus necrodentalis* of Goadby, was most important in decalcification but that *Bacillus N*, a proteolytic form, produced the structural changes associated with cavitation in the dentin. They said: "Streptococci have a certain decalcifying effect but do not produce any structural changes of the dentin; hence, they may be regarded as an unimportant factor in the etiology of dental caries."

Jay (1927) isolated 12 strains of Leptothrix by anaerobic culture from carious lesions. He said, "Though they utilize carbohydrates to produce acid, their status as an etiological factor in dental caries is extremely doubtful." He also remarked, "The pleomorphism of this organism is very likely responsible for the differences of opinion advanced by early workers who were not able to observe the organism in pure culture."

Knighton (1939a, b) cultured yeast-like fungi from different oral sources, including 50 carious teeth from 26 mouths. The organisms found were mainly *Monilia albicans*, though *Monilia mortifera* and *Saccharomyces cerevisae* were seen in one case each. "In the same mouth yeast-like organisms might be isolated from one tooth and not from the others." He said: "The mere finding of yeast-like fungi in oral lesions is not sufficient evidence to attach an etiological importance to the organisms. The fact that the or-

ganisms are found in many normal mouths does not license the oral pathologist and bacteriologist to disregard them if found in lesions," and (1939a) "No correlation could be found between the presence of yeast-like organisms and dental caries."

Bibby and Berry (1939) examined filamentous bacteria from human mouths and cultured 83 strains, classifiable in 7 groups. They said: "The failure to find a significant difference between the occurrence of filaments in healthy and diseased mouths suggests that these organisms are not important in oral disease. The high concentration of acid formed by some of the strains in carbohydrate-containing media, however, supports the possibility that these filaments may have an important part in the causation of dental caries." Also, "The characteristic arrangement of filaments on the surfaces of the teeth suggests that they may exert a determining influence on the flora there. Thus the possibility of their playing an indirect but significant role in the mouth, by determining the basic nature of the oral flora as a whole, cannot be disregarded."

Hine and Bibby (1939) made direct counts of microorganisms in daily and fortnightly smears from teeth and found that "considerable variations occur from time to time" but that some groups of organisms "maintained a relatively constant level in some mouths."

Bibby and Maurer (1941) in comparing *cultural* and *direct counting* methods of estimating total bacterial populations used horse serum in salivas to prevent agglutination. Though they found 6 times as many bacteria as previously reported by others, the total in cultures still represented only 1/6 that of direct counting.

The Aciduric Hypothesis: One of the main claims made for the lactobacillus as the specific organism of dental caries is its demonstrated ability to survive in higher acid concentration than any other common mouth organisms. That the aciduric property might be considered one of the essentials of a caries-producing organism was suggested by Gies and Kligler (1915) and by Howe and Hatch (1917) but Rodriguez (1922) and McIntosh, James and Lazarus-Barlow (1922) very definitely advanced this property as the essential characteristic of the specific organism of dental caries. McIntosh, James and Lazarus-Barlow set forth, presumably as a hypothesis: "It is reasonable to suppose that the bacteria which produce large quantities of acid will be able to live in relatively high concentrations of acid. Therefore, bacteria which are able to decalcify enamel must be able to live in a very acid medium. This is briefly the thesis on which we have based this research and the basis of our technique to discover the bacterium." The premise of the foregoing is faulty in logic and in fact. There is no reason why acid producers must be able to live in acids as the production of the acid is a process separate from that of survival. Many strongly acidogenic bacteria are feebly aciduric. The thesis could have been stated without deduction from the premise, but it requires proof rather than axiomatic acceptance.

McIntosh, *et al.*, found teeth were whitened in acid broths at pH 4 or lower. They therefore used media of pH from 4.5 to 3.0 to isolate organisms from carious dentin and stated that their most successful method "was to emulsify the carious material in pH 3.5 broth in the first case, as in this the vast majority of tooth organisms fail to grow."

Sierakowski and Zajdel (1924) confirmed the observations of McIntosh, James and Lazarus-Barlow with respect to the occurrence of two types of aciduric bacilli in carious teeth. They used a bouillon at pH 3.4 to isolate the organisms.

It may be noted that the aciduric hypothesis of McIntosh, James and Lazarus-Barlow was used as a fact and was not subjected to test. Its adoption as a fact led others to the use of acid media in order to exclude less aciduric organisms regardless of their acidogenic power.

Bibby, Volker and Van Kesteren (1942) said: "The ability of organisms to multiply at acidities below those at which teeth de-

calcify rapidly,—variously fixed at pH 4.5 (Hoff and Köseg, 1937), 4.0 (MacIntosh, James and Lazarus-Barlow, 1922) and 5.2 (Klinger, 1938)—has doubtful significance because the buffering action of the dental tissues themselves will prevent the maintenance of such acidities, and even if a pH below the critical point should be reached, it could not persist in proximity to dental tissues for a long enough period to affect bacterial activity." It will be shown that organisms not regarded as highly aciduric are nevertheless capable of decalcifying enamel and dentin by acid solution of the inorganic salts.

Interaction of Oral Microorganisms

Stephan and Hemmens (1947c) found that mixtures of yeasts, streptococci and sarcina with lactobacilli in high cell concentration limited the ability of the latter microorganisms to produce and maintain a low pH by fermentation of glucose.

Scrivener, Myers, Moore and Warner (1949) found that *B. brevis* and the dialysate from *B. brevis* cultures inhibited the growth of *L. acidophilus*. "In the proper concentrations, sodium fluoride was found to stimulate *B. brevis* growth."

Hill, White, Matt and Pearlman (1949a, b) fractionated saliva to obtain extracts which produced prolonged hyperglycemia when injected into animals, suggesting inhibition of carbohydrate metabolism. The extracts suppressed the growth of *L. acidophilus*.

White and Hill (1949) tested the antagonistic action of various types of oral flora by means of "wells" in agar plates. They found no organisms that would inhibit growth of *L. acidophilus*. Lactobacilli, however, inhibited *Aerobacter aerogenes*, *Escherichia coli* and some strains of sarcina. The inhibiting agent seemed to be lactic acid through lowering of the pH.

Proteolytic Microorganisms

Nuckolls (1946) observed cocci in deep enamel and dentinal carious lesions but rod and thread-like forms in the more superficial areas.

Pincus (1949a, b) found amino acids, hexosamine and hexuronic acid present in residues of enamel after dissolution in 2 per cent hydrochloric acid. The protein was estimated as 1 per cent of the enamel. The sulfur content of the protein was 0.4 per cent or 0.004 per cent of the enamel on the basis of 1 per cent protein. Hexosamine was estimated as 0.09 per cent of the enamel. He (1949a) offered a concept of caries as dissolution of enamel by sulfuric and lactic acid produced by enzymatic action on enamel protein.

Matthews, Atkinson, Saunsbury and Klegg (1949) found no carious attack on 11 sound human teeth, sterilized by boiling and incubated for periods of from 15 days to 4 months with cultures of *Staphylococcus pyogenes*. They isolated this organism from only 1 of 11 carious teeth. In the mouthwashings from 194 children they found *Staphylococcus pyogenes* absent in 36 with no caries, present in 42 with no caries, absent in 52 with caries and present in 64 with caries. No staphylococci were found in probings from 17 early carious lesions. The authors state: "These investigations do not support the view that *Staphylococcus pyogenes* causes dental caries or that its presence in the mouth is an indicator of active or incipient caries."

Burnett and Scherp (1949) isolated 250 strains of proteolytic bacteria from saliva and carious cavities in enamel and dentin of 144 subjects. The organisms were aerobic gram-positive sporulating bacilli, gram-positive cocci and some gram-negative bacilli and cocci. Their ability to utilize gelatin, casein, blood serum and coagulated egg was closely correlated with ability to digest decalcified enamel and dentin. However, intact enamel and dentin were not affected by these organisms in broth cultures in periods extending to 5 months and no caries-causative action could be assigned to them.

Dannenberg and Bibby (1950) treated

coagulated egg white in Mett tubes, undecalcified human dentin and the organic residue of dentin decalcified with normal hydrochloric acid, with zinc chloride and potassium ferrocyanide. These preparations were found to be no different in digestibility by trypsin than untreated similar proteins.

Conclusions

1. There has been no conclusive demonstration that a single bacterial species as an excavating agent is associated with dental caries, but rather it is likely that cavity formation is due to the activities of any organisms that form acids and of those that can destroy the organic substance, particularly of dentin.

2. The *rate of formation of acid* is the most important characteristic of an organism in the solution of the inorganic portion of tooth substance, provided the organism can flourish in the mouth.

3. The *aciduric* ability of mouth organisms is of secondary importance to acidogenic power in the formation of cavities because, since tooth substance neutralizes the acids formed, bacteriostatic acidities are not maintained.

4. Oral streptococci probably account for more destruction of tooth substance in caries than any other forms, as they are rapid acid formers, are constantly present and can penetrate into the deepest parts of a lesion in the dentin.

5. Lactobacilli under precise conditions of culture may serve as an index of caries activity.

6. There is some evidence from cultural studies that the type of carbohydrate ingested may alter oral flora, that is, that sucrose would tend to favor and glucose to suppress streptococci as compared to lactobacilli.

7. Some evidence has been reviewed to show that bacteria cannot be eliminated from the mouth by antiseptic agents tried to date.

8. Of the other clearly recognizable forms of mouth organisms, the yeast-like types probably are not direct cavity forming agents, being too large to enter into the deeper parts of the hard tissues.

9. Different forms of mouth organisms may act in symbiotic relationship to advance cavitation more rapidly because of increased rate of acid formation.

PLAQUES

Because acid normally present in saliva and that formed by bacteria but unconfined bacteria are of too low a concentration to attack enamel, and also because such acid does not account for the localization of caries, considerable attention has been given to plaques as a localizing and concentrating feature of bacterial metabolism.

Black (1886b) announced isolation of oral cocci which caused a peptonized broth, with 2 per cent sugar, to gel rapidly, and considered the gel-formation as a possible mechanism of the origin of plaques.

Williams (1897) observed dense, felt-like films of microorganisms on both carious and non-carious surfaces. He pointed out many V-shaped spaces between the rods which he regarded as due to the action of acids from the microorganisms. His figure 55 on page 288 shows a shallow depression in enamel, of which he says on page 293, "This is the most interesting and instructive balsam-mounted section of the commencement of caries that I have ever seen." As the volume of this depression is approximately 40,000 times that of *L. acidophilus* or comparable organisms, it could scarcely be regarded as "beginning caries." Williams concluded: "From the facts now in our possession we may, I believe, sum the whole question up by saying that if the environing conditions of the teeth are such as to favor the development and activity of acid producing bacteria, and if those bacteria are permitted to become attached to the surface of the enamel, it is doomed, although it may be the most perfect that was ever formed. On the other hand, if

those environing conditions are not present the worst enamel will not decay."

Black (1898) expressed the opinion that "the one thing necessary to the beginning of caries is the formation of such a gelatinous microbic plaque in a secluded position where its acids may act without too frequent disturbance, as in pits, fissures, approximal surfaces, about the margins of the gums, etc., and there give rise to caries."

Miller (1902) said of plaques: "They are not characteristic of carious, but of dirty teeth, or rather of teeth whose surfaces are not kept perfectly cleansed either by natural or artificial friction." He said as a rule films accompany decay but that there are "abundant cases where caries is present without a trace of film."

Miller (1902) did not accept plaques as an essential to his chemico-parasitic theory of caries. He found plaques frequently on lingual surfaces where carious attack seldom appears. He also found incipient caries with no plaque cover. By staining with eosin he found plaque to be very prevalent. He said of plaques and caries: "The presence of a film on a carious surface cannot, however, be taken as evidence in favor of the supposition that this film gave rise to the caries. Caries occurs at points which cannot be kept free from accumulations of food, *i.e.* at points which are not kept mechanically cleansed. Films are likewise found under exactly the same conditions, and consequently films and caries *must* occur together, but we are hardly justified by this fact in making one of them dependent upon the other. If there is any interdependence at all, it is just as natural to suppose that the softening of the surface of the tooth produced by a beginning decalcification furnished a more ready opportunity for the attachment of the film."

Lothrop and Gies (1910) said of mucin in plaques, "Any influences of mucin as an adhesive medium, and of bacteria and fungi as corrosive and *enamel-puncturing* agents, in the initiation of carious processes, are doubtless exercised to their greatest degrees during periods of sleep." They recommended removal of plaques by acids such as occur in foods because mucin is precipitated by acids and loses its mucilaginous characteristic.

Bibby (1931) in studies in New Zealand described a pigmented plaque which "appears on the lower third of the teeth as a brown line, sometimes fine and mottled, at other times strong and bold." Chemical examination revealed "an organic protein element soluble in alkali, evidently a form of mucin, as well as small quantities of calcium carbonates and phosphates." Seven thread-forming organisms were isolated, four being anaerobic. "The incidence of caries in 100 cases having brown plaques was little over one-half of the average in 1000 patients of corresponding ages, attending the same clinic, but having no brown plaques." Bibby considered that the freedom from caries may have been associated with diet but indicated his opinion that "a sweeping condemnation of all deposits on the surface of the teeth is unjustified."

Hewat (1932) in observations on children of 14 New Zealand orphanages and in a school group of 79 children (total of 338 individuals, average age 11 years) recorded incidence of pigmented plaques (Bibby, 1931). "No relationship was found amongst the orphanage groups between the incidence of caries and the presence of the plaque." The school group had the highest percentage of decayed teeth of the 15 groups. Hewat said, "No connection between the amount of carbohydrates in the diet and the presence of the plaque was found amongst orphanage groups, but it is interesting to note that the school group, who alone consume any quantity of confectionery, shows no evidence of the pigmented plaque."

Dobbs (1932a) removed plaques from teeth by means of 5 per cent HCl with $KAl(SO_4)_2$. He found KOH and lactic acid readily diffused through such plaques, but Na_2HPO_4 did not pass through. His method of removal of the plaques may have altered

the properties of these films. He said: "Dental caries was not found beneath all plaques. Whitened areas of decalcification were found beneath many plaques, suggesting a self-limiting process. This was particularly true beneath thick dense plaques, and might be due to the absence of carbohydrates for acid production. . . ." He produced artificial plaques on teeth by immersing them in bread and saliva mixtures for 8 hours and then drying for 16 hours. The plaques were attained in 12 days of this procedure. He likened the procedure to the *in vivo* condition of relatively dry mouth during sleep. Dobbs wrote in discussion, "Whether plaque is essential for the progress of decay has not been established, but it is apparently essential for the initial lesion of decay."

Etherington and Trimble (1934) determined the pH of plaque material as suspended in distilled water. The values ranged from 4.6 to 6.8 in 18 individuals and in every case the plaque was more acid than the saliva. "In eleven cases where caries was active, acidities of plaques averaged substantially the same as those of six cases where caries was inactive."

Blayney, Kesel and Wach (1935) observed plaques in ground sections, some associated with caries and others not. "This showed some bacterial plaques do not produce caries, and suggested hypothesis that types of contained organisms may be different." They associated caries with plaques containing *L. acidophilus* and found 83 per cent agreement between direct observation of plaques and cultures for acidophilus in 30 subjects. Noyes (1935) correlated the preceding direct observation method with the clinical condition of the surface from which the plaque was taken. The smears were positive for 73.1 per cent of the surfaces clinically found to be carious; they were positive for 13.3 per cent that were found negative. Bradel and Blayney (1940), continuing the preceding studies of Blayney, Kesel and Wach (1935) and Noyes (1935) found in 30 cases agreement of cultural methods from plaques with the direct observation of smears out of 56 or 53.56 per cent. They reported: "A total of 5,418 samples were obtained from 477 patients giving evidence of caries activity during the period of observation. A total of 3,920 samples were obtained during periods when macroscopic lesions were present. The remainder were collected at periods when gross lesions could not be demonstrated. Of the 3,920 cultures, 3,002, or 77.6 per cent, were positive for lactobacilli." In 333 specimens from 56 subjects who had shown no caries in 5 years, 25.2 per cent were positive for lactobacilli. In 1091 specimens "from patients wearing dentures, 899 or 82.4 per cent were positive for lactobacilli." During the edentulous period between extractions and wearing of dentures only 20 of 185 samples were positive with very low counts.

Beust (1936) examined 287 extracted molars and bicuspids with interproximal caries. Of these, 231 showed facets because of interproximal wear. Caries occurred both inside and outside 15 of the facets but only inside the facet in the remaining 216 teeth. He concluded: "Interproximal caries of enamel begins, in practically all cases, at the actual point of contact of a tooth with its neighbor.

"Presence of an acid-secreting plaque or colony at this point is environmentally and mechanically impossible."

Stephan (1938) measured the pH of plaques suspended in water by a colorimetric method. He found the pH to range from 4.6 to 7.0 in 211 samples. The mean value obtained from the numerical expressions of pH was 5.9 for the series.

Stephan (1940) adapted the antimony electrode to the measurement of the pH of plaques *in situ* after the rinsing of the mouth with sugar solutions. Use of a 10 per cent solution of glucose or of sucrose caused a drop of about 2 pH units in a plaque within 2 to 5 minutes to values of 4.5 to 5.0. A 1 per cent lactose solution lowered the pH by

0.3 units compared with 1.5 for 1 per cent glucose. A solution of 1 per cent of boiled starch showed a reduction of 1.5 pH units in 51 minutes. The pH in all cases tended to return to the initial values in about 2 hours. The pH of five proximal cavities opened by operative procedures and without added carbohydrates ranged from pH 4.6 to 4.1.

Stephan (1944) studied the change in pH of the plaques, *in situ*, on the anterior teeth of 65 persons. He found the average to range from 7.0 for the caries-free group to 6.4 for those with extreme caries activity. After rinsing the mouth for 2 minutes with a 10 per cent glucose solution, the caries-free showed none with pH below 5.0; the caries-active groups showed more than half the measurements were below 5.0. The pH of the floor of the mouth was slightly affected by the glucose, the cheek surfaces showed irregular change but the dorsum of the tongue had a sharp drop resembling that on tooth surfaces. Stephan concluded that bacteria capable of rapid acid production occur on tooth surfaces of both caries-free and caries-active individuals, that carbohydrate is necessary for the acid production for a degree that decalcifies teeth and that accessibility of saliva influences the intensity of acidity since lower anterior teeth showed less effect of the glucose rinse than did the upper teeth.

Stephan (1945) determined the pH of carious dentin in 22 lesions characterized by undermined enamel and with only a small opening to the surface. The average pH was 4.77 *in situ* and 4.82 in suspension of some of the carious dentin in distilled water immediately on removal from the teeth. The range was 4.4 to 5.3 in each type of measurement.

Stephan and Hemmens (1946, 1947a) investigated the rates of production and consumption of acid, as measured by pH of the media, by 17 microorganisms isolated from dental plaque material and used in cell volume concentrations from 1 to 33 per cent. They found that only when the cell concentration was high were "the pH changes as rapid as those which occur in the microbic plaques on teeth" (Stephan, 1938). Lactobacilli produced a rapid drop of pH but a slow subsequent rise, indicating that this organism is a rapid producer of acid from glucose and a slow consumer of the acid. A strain of neisseria, on the other hand, was relatively a slow producer but rapid consumer of acid. They pointed out that cell concentrations are high in dental plaques and that the conditions existing there may produce rapid acid formation by some organisms and rapid acid consumption by others.

Stephan and Hemmens (1947b) tested the pH lowering ability of 40 strains of oral microorganisms in 33 per cent cell concentration from glucose, fructose, galactose, maltose, sucrose, lactose and starch. In general, acid was produced more rapidly from the monosaccharides, less so from maltose and sucrose, and slowly or not at all from lactose and starch. With high concentrations of glucose there was rapid acid production in general by those which formed acid, but at low concentrations sarcina and streptococci did not maintain low pH because of their rapid consumption of acid. It was shown that lactic acid added to the medium in contact with high cell concentration of sarcina showed the same rate of change to higher pH as from an equal concentration of glucose. Staphylococci and sarcina showed a very rapid rise of pH when urea was the substrate, gamma streptococcus an intermediate rate and alpha streptococcus, neisseria, lactobacilli, monilia, yeasts and actinomyces no utilization of urea. Diphtheroid apparently produced a urease as an adaptive enzyme, since the rise in pH produced by this organism began after about 3 to 4 hours of incubation. They wrote, "The differences in the pH curves produced by different microorganisms suggest that, in addition to the ability of microorganisms to produce acid, the inability to consume acid and the inability to

produce alkaline substances are also of importance in regard to etiology of dental caries."

Miller, Muntz and Bradel (1940) collected composite samples of plaque and tested them for ability to form lactic acid from glucose. Collections were made from 20 caries-free individuals, so judged by clinical and radiographic findings and from low counts of lactobacilli and yeasts. Acid production was compared with that of plaques from 32 caries-active individuals. This statement was made: "The capacity to decompose glucose to lactic acid appears to be the same in both groups. The production of acid varied with the quantity of plaque substance."

Hemmens, Blayney and Harrison (1941) reported preliminary data on an attempt to make a complete study of the flora of plaques. The percentage incidences of certain aciduric microorganisms in 39 bacterial plaques from carious areas and in 41 plaques from noncarious areas were: for streptococci 21 and 44 per cent, for lactobacilli 41 and 12 per cent, for micrococci 15 and 7 per cent and for yeast 10 and 7 per cent. While diptheroids were most frequent of the nonaciduric organisms, "3 colony types of alpha-hemolytic streptococci (so-called green streptococci)" as a group were far more prevalent than any other bacterial group. "Although not isolated from a few samples, it seems probable that streptococci were present in all the plaques, failure to culture them being due to over-crowding, overgrowth by other bacteria or some other technical mishap." Also, "Of all the nonaciduric types recovered, the cocci, and more especially the streptococci, were the most active acid producers. Many of these strains lowered the reaction of glucose broth to pH 4.0 within 24 or 36 hours. Some of the diphtheroids and fusiform bacilli were able to reduce the reaction to a point as low as pH 5.0. Despite the marked ability of these organisms to produce acid, none of the types isolated on blood agar was able to survive long after the culture medium had attained the final acidity indicated above, and none of them grew on the media used for the isolation and cultivation of the aciduric forms." Of the anaerobic fusiform organisms they said: "The frequent isolation of fusiform bacilli from the bacterial plaque is of considerable theoretical interest. These organisms require anaerobic conditions for propagation in pure culture in the laboratory. Their presence in considerable numbers in bacterial masses of microscopic thickness on exposed, well aerated surfaces of the teeth indicates the action of some protective mechanism in the plaque, probably exerted by aerobic organisms. This observation is not presented as a suggestion that these organisms may play some role in the etiology of dental caries. It does serve, however, to emphasize the point of view that anaerobic microorganisms cannot be eliminated from consideration merely on the basis that caries begins in an aerobic environment."

Blayney, Bradel, Harrison and Hemmens (1941) in a preliminary report of long-term study of the flora of plaques associated with dental caries said: "Of the 42 carious areas, cultures from 6 were always negative for lactobacilli even after demonstration of caries; cultures from another 6 regions were all positive following the demonstration of caries, while cultures from 30 areas fluctuated from positive to negative. From 63 negative areas, cultures from 32 were all negative for lactobacilli; 3 areas were positive in more than 50 per cent of cultures; 24 areas were negative in more than 50 per cent of cultures, while 4 areas gave equal number of positive and negative cultures. Comparison of clinical and bacteriological findings showed 15 areas with positive cultures in advance of positive clinical findings; whereas in 14 areas, caries was demonstrated before a positive culture was obtained."

Blayney, Bradel, Harrison and Hemmens (1942) observed the frequency of occurrence of lactobacilli in plaques removed at regular

6-week intervals "from the proximal surfaces of premolar teeth, from the time the tooth erupts into occlusion and makes contact with the proximating tooth until a readily demonstrable lesion develops." Bitewing roentgenograms and clinical examinations were used to detect caries. The percentage frequencies of finding of lactobacilli from 10½ months before to 9 months after discovery of a positive lesion were: Before: 7-27-24-20-38-34-36-43; After: 57-43-53-63-67-77.

Hemmens, Blayney, Harrison and Bradel (1943) in a study of the flora of plaques taken every 6 weeks from the time of eruption until caries appeared, said: "It is not possible from the results to state that any of the cultivable microorganisms is the cause of caries, but a number of types showed an increase in incidence with the appearance of the lesion. The most marked increase was in the lactobacilli, while beta hemolytic streptococci, small gram-positive rods, rough colony type streptococci and aciduric streptococci showed increases of lesser degrees. A number of microorganisms, including fusiform bacilli, smooth colony, alpha type streptococci and filamentous rods decreased in incidence with the appearance of caries."

Hemmens, Blayney and Harrison (1943) in a study of the flora of saliva and plaques taken simultaneously from 60 subjects reported: "No qualitative differences were found in the floras of the 2 sources" (saliva and plaques) "but some quantitative differences were noted. Diptheroids, gamma streptococci, anaerobic gram-negative cocci, one type of fusiform bacillus and filamentous forms were more common in plaque cultures than in the saliva, and the reverse was true of yeasts, micrococci and, to a slight degree, of lactobacilli."

Muntz (1943) incubated plaque material with 0.1 per cent glucose and found lactic acid was formed in less than theoretical amount for the glucose consumed. Volatile and other non-volatile acids were formed, the volatile resembling acetic and propionic acids in steam distillation characteristics. Lactic acid was formed by the plaque material most rapidly at pH 7.0 and at about ⅓ rate at pH 4.5. It was destroyed most rapidly at pH 7.0. Lactobacilli produced lactic acid from glucose but did not metabolize it.

Day (1944c) found plaques on the teeth of 309 of 314 children of villages near Hissar, India, where 74 per cent had mottled enamel and there was an incidence of dental caries of only 0.62 cavities per child. On the basis of both permanent and deciduous teeth, 74 per cent of the group were caries-free. The diet was severely deficient in vitamins A, D and C and in calcium and animal proteins. There was a "comparative absence from the diet of sugar and other fermentable carbohydrates." Day said, "Under the conditions reported, the presence of these plaques would appear to have no bearing upon the caries process, especially in view of the fact that both upper and lower anterior teeth were in all cases entirely caries-free."

Hemmens, Blayney, Bradel and Harrison (1946) described the frequency of occurrence of 27 forms of microorganisms in plaques taken from 87 areas of premolars of 44 children. In a total of 939 plaques, 611 were obtained before clinical or X-ray evidence of caries and 328 after carious lesions were detected. The investigators arbitrarily assigned a 36-week "transitional" period as preceding the first evidence of caries and presented their data of frequency as precarious, transitional and carious. Those showing an increase of 10 per cent or more were: beta hemolytic streptococci, 10 per cent; lactobacilli, 34 per cent. Among those showing decreased frequency were streptococci, fusiforms and actinomyces.

Hill and White (1948) found in *in vitro* experiments of 90 minutes' duration that rate of formation of acid in saliva was not increased by increased concentration of *L. acidophilus* but was increased by the residual matter of saliva obtained by filtration or centrifugation.

Strålfors (1948a) determined the minimum pH of plaques on the teeth of 110 individuals following rinsing of the mouth with a 10 per cent glucose solution and examined the relation to the number of lactobacilli in the salivas. He concluded: "There is a significant statistical relation between pH minimum and number of lactobacilli. Individuals with a higher pH minimum have fewer lactobacilli, that is, lower caries activity than those with lower pH minimum."

Hill and White (1949) found that lowering of pH in incubated saliva to which sucrose was added was not correlated with the count of lactobacilli but was apparently associated with substances present in sediments in the saliva.

Discussion

It seems evident that plaques vary widely in their characteristics and especially in their constituent flora. They may or may not be associated with carious lesions and some types may be protective against caries. (Bibby (1931), Dobbs (1932b.)

The frequent occurrence of lactobacilli in plaques is possibly associated in high degree with the existence of a carious lesion, but the relationship has not been shown to be other than association. The existence of the carious lesion may favor the growth of the lactobacilli, which in turn by their metabolism may promote development of the cavity, but that lactobacilli initiate the process has not been shown. Likewise a causal role cannot be assigned to any other microorganism present in plaques or to plaques themselves.

The demonstration that the conditions within plaques may be anaerobic considerably extends the biochemical reactions that may be expected to take place in close proximity to the tooth surface. The rapid production of acid after rinsing of the mouth with sugar solutions is an indication of the nature and speed of the reactions that may occur in plaques. The pH within the ranges in which enamel is attacked is significant as to the part plaques may play in development of cavities. That plaques may not be seen on certain incipient lesions does not mean that they were never present or that they will not later be established.

The high incidence of plaques on mottled enamel indicates that certain microorganisms can flourish in intimate contact with enamel with a high fluorine content.

ARTIFICIAL CARIES

The production of caries-like lesions in human teeth in extra-oral environment has played a large part in the study of the factors in oral environment suspected of a role in dental caries. As narrated earlier, artificial caries produced by microorganisms in a mixture of bread and saliva furnished some of the strongest evidence produced by Miller to sustain his chemico-parasitic theory of dental caries.

Related to artificial caries produced by microorganisms are caries-like lesions produced by various acids and, through this link, the solution of enamel powder by acids. The action of acid on enamel powder is of importance because the rate of solution has been invoked by Volker (see the section on Fluorine and Dental Caries) to explain the mechanism of fluorine in protecting teeth from caries, and Fosdick and coworkers have devised a test for caries susceptibility based on solution rate of enamel.

In general the action of acids on enamel in any form is reviewed below as related to caries, and interpretations are suggested in the "conclusions" section.

Artificial Caries by Acids

Mummery (1910) exposed the crowns of various teeth to 0.075 per cent lactic acid until chalkiness appeared. They were tested daily by scratching with a needle. He found that there was wide variability in acid attacks on enamels; that cusps and incisal edges decalcified first; and that hypoplastic enamel showed no difference in decalcification from other enamels.

Hartzell and Larson (1924) stated they could find no destruction of enamel other than a slight opacity after immersion of whole teeth in 0.3 per cent lactic acid, said to have been at pH 2.0, for 4 months.

Friesell and Vogt (1926) tested solution of enamel of whole teeth in aqueous solutions flowing over the teeth at a rate of a liter per day. Water, fully carbonated at atmospheric pressure, produced opaque spots in enamel in 2 days and in 3 days the entire surface was opaque. Lactic acid at pH 6.6 produced detectable decalcification of enamel in 6 weeks, and carbon-dioxide-free distilled water in 8 weeks. They stressed the fact that saliva would not be so effective because of its content of calcium and phosphorus.

McClelland (1926) found no loss of weight of enamel pieces immersed in buffered solutions of pH 6.5 to 11.0. "Pieces of enamel in solutions having a hydrogen ion concentration of pH 6.0 and below are decalcified and the rate of speed at which decalcification progresses is in proportion to the degree of acidity."

Thurlow and Bunzell (1927) established that teeth kept for several years in salt solutions with preservatives did not differ in solubility from freshly extracted teeth. The enamel of hard teeth did not differ in solubility from that of soft teeth. There was a difference in the rate of solution of enamel by organic acids.

Dobbs (1932a), in studies of decalcification of enamel with 0.75 per cent lactic acid, found mechanically cleaned intact surfaces of teeth of adults more resistant to acid than areas from which the surface layers were ground away. Tryptic digestion of intact teeth removed the surface-resisting property of enamel. Unerupted teeth had no superior acid resistance at the enamel surface. He concluded that "the protective membranes on unworn surfaces are organic in nature, and acquired after the tooth erupts."

Dobbs (1932b) exposed teeth to lactic, butyric, acetic, succinic and malic acids of about 1 per cent concentration and to 0.1 per cent hydrochloric acid. He found: "The decalcification was not uniform; some teeth had softened enamel; others were unchanged. Areas on the same teeth varied in time and degree of decalcification, *i.e.*, the worn surfaces were first to show softened enamel. These areas, free from visible plaques, were kept clean by attrition from mastication and by excursion of food, and are not usually susceptible to dental caries." There was great variation between teeth from different subjects. "Areas on enamel that showed resistance to weak acids, in *in vitro* experiments, were found to be covered with plaques of sufficient size and density to be distinguished without the aid of a stain."

Enright, Friesell and Trescher (1932) "exposed small areas on the labial surfaces of extracted central incisors, through windows in asphaltum coverings, to wide range of acid concentrations of both lactic and citric acids for different periods of time extending up to sixty days." Their sections showed decalcification as confined to a small area, in that way resembling natural caries. In advanced stages the decalcification showed a caries-like spread along the dentino-enamel junction.

Enright, Friesell and Trescher (1932) also exposed teeth through windows in asphaltum coating to citrate and lactate buffers and found decalcification in the range pH 4.0 to 8.0. They said, "Certain ground sections of artificial caries were found which so closely simulated natural caries that any dental histologist would experience difficulty in deciding whether they were sections of artificial or natural caries." Though decalcification was generally proportional to hydrogen ion concentration, "there were sufficient exceptions to this rule to indicate that different enamels vary in their susceptibility to the action of acids." Lower incisors were as susceptible to decalcification as upper incisors or even more so.

Enright, Friesell and Trescher (1932) saturated citrate and lactate buffers with calcium and phosphate ions by a three-day

contact with shaking. These buffers decalcified teeth in 17 days if the pH were 5.0 or lower. However, the pH of the original buffer was restored by means of the pertinent acid after the saturation procedure and, since "the pH values had shifted to the alkaline side generally about 0.4 of a pH unit," it is evident that their buffers were not actually saturated with calcium and phosphate ions. The writers concluded from this experiment, however, that "organisms incapable of producing or tolerating a pH of less than 5.0 in the mouth could be excluded as potential causes of caries of the enamel."

Hoff and Köszeg (1937) studied enamel solubility by rate of penetration by a micrometer needle into an area of enamel previously flattened by grinding and with the surface for attack restricted by wax. They determined the penetration of the needle following exposure to buffers at pH 5.00 and found an accelerated depth of penetration as pH was reduced to about 3.18. They found variation in rate of solution of enamel from different specimens.

Mizuma (1937) studied solubility of pieces of enamel approximately 50 mg. in weight. He found no appreciable solubility above pH 6.77. Solubility was found related to pH, lemon juice at pH 2.4 dissolving 77 per cent of the enamel compared to 1.5 per cent in grape juice at 3.5. Deciduous enamel was more soluble than permanent enamel, and the solubility of the latter diminished with advancing age.

Klinger (1938) studied the solubility of uniform pieces of enamel in various acids and buffered solutions. He found no difference in loss of weight of such pieces on aging for 0, 1, 2, 4 and 8 days after extraction. He found that there was resistance to solution at the outer layer of enamel and stated: "The enamel, which is put into solution, may become chalky on both sides, but mostly on the surface that was bordered by dentin. Sometimes the outer surface remains unchanged, but chips off as if it were a layer of shellac and underneath were a mass of chalk. This outside layer cannot be the vestige of the Nasmyth's membrane, which is more impervious to acid, because this same phenomenon was also noticed on such enamel dissolved, which had the outer surface somewhat ground off. It seems more plausible that there is a difference of resistance against the action of acids on the inner and outer layers. This possibility is substantiated by the inspection of the enamel with the Roentgen-spectroscope, which shows differences in crystalline structure between the outer and inner layers." Klinger found no difference in solubility between 20 samples of enamel from caries-free teeth.

West and Judy (1938) exposed the enamel of teeth to solutions of candies acidified with citric acid. The enamel was decalcified as shown by the amount of calcium and phosphorus in solution and a chalky layer was formed on the enamel, which they interpreted as replacement of phosphate by citrate. If saliva were used as a solvent for 20 per cent candy content, the pH was about 5.0, compared with 2.6 for water solution, and little or no destruction of enamel occurred. With 40 per cent candy in saliva, the pH was about 3.6 and enamel was dissolved. "Strong solutions of sucrose, levulose and non-acidified candy did not dissolve tooth enamel."

Kirkpatrick (1939) by *in vitro* studies of decalcification of enamel of human teeth by orange juice (pH 3.3) and diluted lemon juice (pH 2.6) estimated daily drinking of the diluted lemon juice would completely erode enamel in about 3 years. The orange juice would require 4 times as long. Partially neutralized diluted lemon juice at pH 4.6 showed negligible erosion. Kirkpatrick suggested that plaques, *in vivo*, would protect against these fruit drinks but that abrasions would accelerate.

Trask, Ziegler and Maloof (1940) determined the loss of weight of pieces of cracked whole teeth when exposed to solutions of various acids and sugars. In general the loss of weight was proportional to hydrogen ion

concentration. Sugars were without effect. Malic acid, though mentioned in the text, was not studied although its isolation from broth cultures of oral organisms has been reported.

McClure (1943) observed very marked destruction of the molar teeth of rats that received for periods of 42 to 85 days various dilute acids, "soft" drinks and fruit juices substituted for water. The pH of the beverages were: ginger ale 2.8–3.2; Cola drink 2.6–2.7; grape fruit juice 3.2–3.2; cranberry juice cocktail, 2.5–2.6. The last was the most destructive.

Solubility of Enamel Powders

Benedict and Kanthak (1932) determined the rate of solution of powdered human enamel in various buffered solutions. They found in general that the lower the pH, the greater the rate of solution. The rate was rapid at first and they considered that it was slowed down by impedance of diffusion of the solvents by the organic matter of the enamel. They found that enamel was soluble in distilled water but that solution of enamel by saliva in its usual pH ranges was prevented by the normal saturation of saliva by calcium and phosphate ions.

Kanthak (1934) in a continuation of the preceding study concluded that rate of solution of enamel powder bore "no relationship to age or state of the teeth."

Forbes (1933) studied the influence of calcium and phosphate ions on the solution of enamel powder by carbonic acid. Concentrations of CO_2 in water equivalent to those expected from saturation with exhaled air had a definite solvent action but this was prevented by 3 mg. of Ca and 14 mg. of P per 100 cc. of solution at pH above 6.4. Slight decalcification occurred with pH 5.7 and calcium at 6.6 mg. per 100 cc.

Karshan and Rosebury (1934) "studied effects on powdered enamel, of buffers of lactic, succinic, and malic acids, without added Ca or P, to determine relationships at equilibrium of pH to dissolved Ca and P. Systems containing different amounts of titratable acid, all with enamel in excess, were shaken and determinations made on aliquot parts removed at intervals up to the point of equilibrium. The results indicate that (1) in lactate buffers, pH change at each time-level is strictly proportional to dissolved P at that level; (2) enamel is soluble over the entire acid range of pH; (3) the probable curve of solubility—dissolved P plotted against pH, both at equilibrium—changes direction between pH 5.0 and pH 4.0, solubility increasing slowly down to pH 5.0, rapidly below pH 4.0." Also, "Interpolations on the curve plotted with these data suggest that lactobacilli may alone be capable of causing solution of enamel under oral conditions."

Volker (1940) determined the solubility of enamel from various kinds of teeth "dried in air and size graded 100–200 mesh" by weighing the weight loss of 50 mg. samples after stirring in 20 cc. of 0.2 M acetic acid/sodium acetate buffer at pH 4.0 for 1 hour at 38°C. Volker studied the effect of mesh size on the amount of enamel dissolved, and reported, "it is evident that the velocity of solution of enamel particles is proportional to their surface areas." The source of the enamel studied for the effect of size of particle was not stated nor were the conditions of the experimentation presumably different from those yielding the further data.

From the other tests, Volker concluded that deciduous enamel was more soluble than permanent and offered a possible explanation on the basis of the greater content of magnesium salts. Another possible explanation is that the fluoride content of deciduous teeth may be generally lower than that of permanent teeth. (See section on Fluorine and Dental Caries.)

Volker said: "The absence of a significant difference between the solubility of enamel from carious and non-carious teeth indicates that the resistance or susceptibility of a tooth to caries is not dependent on the

relative acid solubility of the enamel. This conclusion is not at variance with the chemo-bacterial theory of dental caries which postulates that the carious process is initiated by the decalcification of the dental hard tissues. This finding merely points out that the enamel of all teeth would be decalcified by acid solutions at approximately the same rate, unless the acid solution was removed or neutralized by some external agency. The explanation of the slightly lowered solubility of carious enamel is not clear. One possibility is that the percentage of organic material in the carious samples is of a greater order than that of the non-carious samples because of the large amount of unsupported enamel included in the former. Enamel of this type may previously have been partially decalcified, resulting in the deposition of an increased percentage of organic material which would resist destruction by acids."

Artificial Caries by Microorganisms

The experiments of Miller in producing artificial caries in human teeth by immersing them in fermenting mixtures of bread and saliva have been described earlier in this section.

Seitz (1921) exposed sound human teeth to mixtures of bread and saliva. The teeth were coated with wax except for a small selected area. The mixture was renewed weekly by discarding half and adding fresh saliva and bread to the original volume. The earliest decalcification occurred in 33 days in a hypoplastic incisor, and in 124 days caries was found in the exposed site in all teeth. He said the decay showed no difference from natural caries, though his description began with caries of the dentin. Addition of marmalade to the bread-saliva mixture did not accelerate caries development; in fact no caries was found in 61 days. The number of teeth used in this and the other experiments was not stated.

The author pointed out that the mouth organisms formed *l*-lactic acid normally, but *d*-lactic acid if the fermenting power was unbalanced. The degree of acidity attained was not dependent so much on growth as upon the ability of organisms to withstand acid. Seitz isolated pure cultures of staphylococci, *B. aerogenes* types and the *Streptococcus lacticus* (Kruse) and used them in glucose broth to produce extensive caries in teeth in 32–62 days. He claimed this as the first demonstration of the ability of a single strain of bacteria to produce caries. He said the decay produced varied with the strain and with the amount of *d*-lactic acid produced.

Howe (1922) said: "Histologically Miller studied only the carious mass. He ignored the condition of the tooth substance immediately in advance of the decay. Bacteriologically his work was limited and he found no specific organism which he could regard as the etiological factor in caries."

Howe repeated Miller's experiments by placing "teeth in fermenting aqueous mixtures of dextrose, maltose, lactose and saccharose, of dextrin and white flour, and of bread. In some of the tubes we used saliva from individuals that had extensive tooth decay, in others saliva from cases free from decay, and in others saliva from unselected cases. After six months some of the teeth showed an etched appearance, some a decalcified effect, and in others no change was discernible. In general the effects resembled those on teeth subjected to the action of a weak decalcifying agent. The most pronounced thing brought about was the great difference *in structure* of the various teeth. Miller himself noticed this."

Rodriguez (1922) suspended non-carious teeth in mixed cultures of 3 types of *Lactobacillus odontolyticus* so that the crowns were immersed. Decalcified areas developed and the bacteria were found in the dentin in 12 weeks. Teeth suspended in cultures of cocci, sarcinae and yeasts showed little if any changes.

McIntosh, James and Lazarus-Barlow (1922) using their Types I and II of

B. acidophilus odontolyticus found that decalcification of enamel occurred after periods as long as 17½ weeks, but that natural caries was not precisely simulated. Liquefaction foci were produced in the dentin, *after entry from the pulp chamber*, though the organisms were non-liquefiers of gelatin and failed to form indole. It is worthy of note that attack on enamel surfaces was not uniform, though in their technic there were no apparent stagnation areas.

Hartzell and Larson (1924) found decalcification of enamel in a single test with *Aspergillis niger* but not with four other species of molds. They considered that enzyme decalcification at pH 7.5 could occur.

McIntosh, James and Lazarus-Barlow (1924) asserted that in 1922 they had produced " 'artificial caries' which was indistinguishable from the 'natural' condition." *In vitro* preparations of artificial caries, with the tooth covered with a celluloid varnish except for a small area of enamel, was said to resemble natural caries in all respects. The illustrations were of caries in the dentin and hence throw no light on initiation in the enamel. The investigators failed to produce caries in rabbits by fixing cultures of *B. acidophilus odontolyticus* to their teeth by means of gold bands. Also cultures fed to a monkey with a mixed diet produced no caries in 3 months. Holes were then drilled to the dentin in two molars and a paste of the organisms with biscuit introduced. There was no evidence of caries in a month. Cultures applied to the upper incisors by bands and with acid sodium phosphate added to the ration produced no caries in 7 weeks nor in an additional 16 weeks in which the bands were not in place.

Clarke (1924) exposed three sterilized teeth to glucose broth cultures at pH 7.0 seeded with *S. mutans*. The teeth were transferred to fresh media daily for 7, 9½ and 13 weeks, respectively. At the end all showed enamel decalcification and the latter two definite invasions of the dentinal tubules by bacteria. Clarke noted that "the growth adhered markedly to the surface of the enamel . . ." A fourth tooth under similar conditions but with Type I *B. acidophilus* obtained from Lazarus-Barlow showed "at the end of 13 weeks only a superficial decalcification of the enamel." He said: "In McIntosh, James and Lazarus-Barlow's experiments the medium was changed once a week, and the teeth therefore remained in a concentration of acid sufficiently high to produce decalcification of the enamel for fully 6 out of every 7 days. The fact that the colonies of *S. mutans* adhere closely to the surface of the teeth appears to be of great importance, as a local concentration of acid in contact with the enamel is thereby produced, and this could obviously give rise to rapid decalcification independently of the acidity of the medium as a whole."

Bunting and Palmerlee (1925a, b) found lactic acid at pH 5.0 would whiten tooth surfaces in 4½ weeks. The teeth were covered with shellac except for a small area of enamel. Teeth exposed to broth cultures of *B. acidophilus* showed superficial whitening in the course of a month with pH at 3.4 to 4.2. This was not regarded as caries. Teeth, shellacked to leave a small exposure of enamel, were decalcified in that area when exposed to broth cultures of acidophilus, but it was a general effect and not the penetration of enamel observed in natural caries. A dog tooth was similarly treated and underwent decalcification.

Maclean (1927) found that neither *S. mutans* nor *L. acidophilus* decalcified teeth if the culture medium was restricted to 0.1 per cent glucose and the medium changed frequently, with the pH always higher than 5.5. With 1.0 per cent glucose, both organisms penetrated enamel and dentin.

Anderson and Rettger (1937) exposed small areas of enamel to broth cultures of a single strain of oral streptococcus, one

strain of oral lactobacillus, and a mixture of these two organisms. The teeth were transferred daily to fresh broth and the pH was never lower than 4.2. "There was no marked difference in the degree of enamel dissolution brought about by the 2 organisms."

King (1937) wrote: "Recently I have been studying the effects of mechanical injury of the enamel in so-called 'artificial caries.' The decalcifying action of a mixture of bread and saliva on human teeth is greatly accelerated by injuries to the enamel surface—either natural, such as attrition, or artificial. Insufficient attention appears to have been given to the earlier enamel changes in artificial caries by some of the previous workers on the subject."

The enamel lesions produced *in vitro* by Pincus (1937) by bacteria at pH 7.2 to 7.4, presumably by proteolytic action, were described as a type of erosion. However, in a continuation of the study (1939) lesions were found in 9 of 42 anterior human teeth incubated in a carbohydrate-free medium at pH 7.4 to 7.8 and it was intimated that they were caries-like.

Osborn, Noriskin and Staz (1937a) speaking of experiments with refined sucrose, cane juice and cooked cereals said, "In the later experiments a groove was ground in the enamel, because it was found decalcification took place sooner and could be observed more easily on a worn surface." Also, "The authors do not imagine that they are dealing with *in vitro* caries."

Hatton (1938) described decalcification in sheltered areas of the enamel of human teeth alternately exposed to and freed from moving saliva. Sugar was present in the saliva from chewing gum used in its collection.

Sullivan (1939) confined lactobacilli in "artificial plaques" of nutrient agar over small areas of teeth. The teeth "were then suspended in 1 per cent glucose broth (pH 7.0) and incubated at 37°C." The teeth were transferred twice daily and kept in glucose-free broth overnight. The exposure to this procedure was continued for 2 weeks. Three such teeth with no bacteria in the plaque showed no decalcification, those with nine strains weakly acidic organisms showed a dullness of the enamel but no definite decalcification, but nine teeth with strongly acidic bacteria were cream colored, soft and pitted.

Hammond and Tunnicliff (1940) incubated autoclave-sterilized caries-free teeth in 1 per cent dextrose broth culture with a pure strain of *S. viridans* in its coccus form. The broth was replaced weekly or biweekly and pH 4.2 was maintained as an average. The 16 teeth used were coated with wax except for small areas over enamel. Decalcification of enamel was observed in as few as 14 days. Organisms were found in dentinal tubules in 3 to 4 months. "After 4 months' incubation the tubules contained cocci, bacilli, crescents and filaments similar to those seen in more advanced natural caries." Similar results were obtained with *B. coli* but after a longer time.

Dietz (1943) devised an apparatus that permitted observation of plaque and cavity formation in the enamel periphery of thin sections of human teeth immersed in saliva renewed at short intervals. The saliva from caries-susceptible individuals was adjusted to a pH of 6.0. Glucose, 120 mg. per 50 cc., was added to the saliva to prevent it from becoming alkaline under putrefactive influences. A small area was covered with macerated bread. The saliva was in contact with the periphery of the enamel only part of the time as a simulation of conditions in the mouth. Dietz said "it had been found impossible to initiate caries if the section were constantly submerged in the saliva," indicating that a much lower pH than 6.0 may have occurred under the bread. In order to obtain decalcification Dietz found it "advisable to polish the surface of the tooth from which the section was to be made. The observation of a 'wet section' frequently revealed that the exteriormost 5–15 per cent

of the enamel is relatively amorphous in comparison with the well-defined structure of the remainder of the enamel." Also, "The section after completion is treated with a 5% lactic acid for a few seconds. . . ." Dietz observed a brownish decalcification in 24 hours with no change in surface continuity, but in 72 hours there were V-shaped etchings. A plaque formed centripetally, consisting essentially of "yeasts, gram-positive rods, leptotrichia, gram-negative diplococci, streptococci, gram-positive bacilli, and filamentous organisms." He gave the opinion that "the caries-like lesions produced are probably the results of the acidogenic participation of the total local flora." A definite cavity appeared and was illustrated, but it was not enlarged after 148 hours, in a period of observation of 1008 hours.

Weisberger (1950) produced artificial caries in an exposed area in unerupted, intact teeth covered by wax. The teeth were exposed to a synthetic medium inoculated with saliva sediment and changed weekly for 4 months. Caries-like lesions, characterized by a brown pigment, occurred if glucose were present. The pH fell rapidly to about 4 and amino acids were not deaminized. In the absence of glucose, the medium became alkaline "with increase of ammonia (plus urea) nitrogen" and amino acids decreased. No carious lesions were formed. If the solution was buffered at pH 4.0, without glucose, decalcification without pigmentation was produced.

Discussion

The evidence from artificial caries studies is that the enamel of any tooth will be decalcified by acids either independent of or formed *in situ* by bacteria. This has not been established for teeth from fluoride areas, either mottled or normal, but it is likely that such teeth would be dissolved by acid, since artificially fluorosed enamel powder shows reduced rate of solution though not absolute resistance. (See section on Fluorine and Dental Caries.) However, there is abundant evidence that intact teeth are highly variable in their resistance to acid decalcification *in vitro* and there is indication that the resistance is associated with the surface. Whatever may be the nature of this varied surface resistance to acid attack or to initiation of artificial caries by various bacteria, *the resistance plainly resides within or on the surface of the tooth.* Furthermore, since, in an *in vitro* experiment, (a) the teeth are non-vital, (b) are in many cases not in saliva medium and (c) if they are fully exposed to saliva there is no experimental restriction on distribution of bacteria exposed to various surfaces, it would appear that the resistance *in vitro* has nothing to do with metabolism or oral environment. *The only plausible explanation of failure of some teeth or areas of teeth to be attacked in an in vitro experiment is a difference in surface structure between different teeth and different parts of the same tooth.* However, if there are indeed real structures of dental enamel surfaces that confer resistance to caries, the origin is *in vivo*. The parts played by pre- and posteruptive conditions can only be surmised, but *surface* conditions of enamel are obviously subject to alteration in either phase of tooth history.

The above concept of variable resistance to initiation of caries because of difference in structure at the surface is not necessarily related to *progress* of caries after initiation. The latter may also, and is frequently asserted to, be related to structure of the enamel. The concept that initiation and progress of caries may be different in mechanism is sustained by the evidence that sugar did not initiate caries in rats but did promote enlargement of cavities initiated by coarse cereal particles.

The diminished rate of solution of enamel by solvents saturated with calcium and phosphate ions suggests that some of the protective action of saliva against caries is due to its inorganic ions.

Attack of acids on enamel appears to be simple acid solution of the inorganic com-

ponents and not a simulation of dental caries. Restriction of the acid action to a small area in no way changes the mechanism of the enamel destruction and hence would not be expected to simulate caries in the *enamel*.

SOME PHYSICAL AND CHEMICAL CHARACTERISTICS OF SALIVA

Acidity

Starr (1922a) examined the pH of human saliva colorimetrically, using mainly bromthymol blue. Collection of resting saliva under oil gave values about 0.20 pH units lower than without oil. Centrifugation raised the pH about 0.30 units and this was ascribed both to loss of CO_2 and to precipitation of more acid material. Stimulation of secretion by chewing paraffin for 5 minutes raised the pH in five subjects from 0.10 to 0.60 units and pH returned to original levels in 10 minutes.

Starr (1922b) found in 7 subjects a drop in pH of saliva 0.10 to 0.25 units after meals, the lowered value being inversely related to increased alveolar CO_2. Ingestion of $NaHCO_3$ in 10 experiments by 4 subjects resulted in a more alkaline urine but more acid saliva, the pH reduction being of the order of 0.70 units. In voluntary deep breathing for 10 minutes by 9 subjects the pH of saliva was increased by 0.15 to 0.60 units; in 1 subject who had ingested 15 gm. of $NaHCO_3$ before deep breathing the pH increase was from 5.80 to 6.95. Fatigue resulted in decreased salivary pH in 8 subjects. Excitement in 4 subjects caused increases of 0.60, 0.45, 0.50 and 0.20 pH units. The range of pH of 610 samples of unstimulated saliva from 228 normal individuals was from 5.75 to 7.05, with a mean of 6.6, median 6.6 and mode 6.6 to 6.7; "86 per cent of the specimens ranged from 6.35 to 6.80, inclusive." The mean pH of 58 sub-breathing stammerers was 5.8 and that of hyper-excitable psychopaths was 7.4.

Henderson and Millet (1927) found that the pH of saliva was low on rising, 6.0 to 6.6 in 11 subjects, but that it rose rapidly as activity increased. They found an increase in pH after meals and an increase on exercising jaws without chewing any foreign substance. The pH returned to normal after chewing but after meals it became, after the peak, lower than the normal.

Clark and Carter (1927) measured pH colorimetrically. They found the pH of cannulated saliva about 0.1 units lower than expectorated saliva. Paraffin-activated saliva was 0.15 to 1.00 pH units higher than resting saliva in 5 subjects. The pH increased on keeping in an open container for $1\frac{3}{4}$ hours from 7.1 to 7.7 but the CO_2 content remained unchanged.

Roskin (1928) analyzed the salivas of 17 diabetic and 13 non-diabetic children. Of the former, 15 had been under dietary control and dental caries among them was generally arrested. The incidence of salivary calculus was high in this group. Caries was very high in the non-diabetic group. She found pH ranged from 6.3 to 7.0 with a mean of 6.8 in the diabetic group, and from 6.3 to 7.3 with a mean of 6.9 in the non-diabetic group.

Starobinsky (1929) determined the pH of the saliva of 100 women at 3-, 6- and 9-month periods of pregnancy. The mean values found were 7.05, 7.1 and 7.09.

Karshan, Krasnow and Krejci (1931) found the average values for the pH of the resting salivas of subjects with no decay, arrested caries and active caries to be 7.03, 7.08 and 6.90; for activated saliva the corresponding values were 7.54, 7.40 and 7.39. The titratable alkalinity for resting saliva was 67, 68 and 67 and for activated saliva 126, 118 and 116. They concluded no differences existed between the different groups of subjects.

Forbes (1932), in experiments with 6 individuals, found a marked decrease in acid-neutralizing power and pH of saliva after a light meal but lesser fall after a heavier meal of meat, vegetables, bread, dessert and coffee. The change was not ascribed to mastication, as paraffin chewing increased neutralizing power and pH. There

was possibly a reciprocal relation between acid-neutralizing power and salivary phosphorus but a direct relation between acid-neutralizing power and CO_2-combining power.

Forbes and Gurley (1932) found "a high-cereal or a high-grain diet tends to decrease salivary acid-neutralizing power, while a high-meat, high-egg, and high-vegetable-and-fruit diet tends to increase it. No relation was found between the acid-neutralizing power and the phosphorus concentration of saliva." Six subjects were used and the dietary periods were a week to a month in duration. There were too few subjects for conclusions in regard to caries but "the subject immune to caries had a very high salivary acid-neutralizing action, while the very susceptible one had the lowest acid-neutralizing action. The values obtained for the others were in between."

Krasnow (1932) reported the pH of unstimulated salivas as 6.98 for caries-immune and 6.92 for caries-susceptible subjects and 7.43 and 7.35 for stimulated salivas. Corresponding titratable acidities were 181 and 192, and 333 and 294.

Eddy, Heft, Rosenstock and Ralston (1933) observed the variation of the pH of unstimulated saliva at half-hour intervals in 3 subjects. The values, by the quinhydrone electrode, ranged from 6.8 to 7.9. Two of the subjects, who ingested a quarter of a pound of candy in a 10-minute period, showed increase of pH in the next half hour to maxima of 8.0. Ingestion of starch as farina or as bananas caused no appreciable change in pH.

Hubbell (1933) found the pH of stimulated saliva of 15 caries-free children to average 7.3, with a range from 6.9 to 7.6, and of 17 caries-active children, 7.1 with a range 6.8 to 7.4. She concluded there was no consistent difference. She found the CO_2 capacity of the stimulated saliva of the caries-free children to be 31.0 volume per cent and ranging from 20.1 to 45.9; similar values for the caries-active children were 21.6 and 8.3 to 50.9. Titratable alkalinity was 114.0 ml. of 0.02 N HCl per 100 ml. of saliva and ranged from 94.7 to 146.8 for the caries-free, and 91.9 and 69.0 to 137.2 for the caries-actives. She concluded these buffer indexes were significant.

Koehne, Bunting and Morrell (1934) were unable to correlate variations of carbon dioxide capacity and calcium and phosphorus content of saliva "with significant variations in the intake of alkaline ash, of essential foods or of calcium or phosphorus or to significant variations in the absorption or retention of calcium or phosphorus."

Grove and Grove (1934, 1935) found the pH of salivas of caries-susceptible individuals to range from 6.8 to 7.4, and of caries-immune subjects from 7.1 to 7.6. The method of determining the pH was not described.

Skosovsky (1935) measured the pH of various oral sites with the antimony electrode. He found areas that were constantly different in reaction from other sites. He found the oral cavity more acid in caries and ascribed a causal relationship. No data were given.

Brawley (1935a) determined the pH of resting saliva of 3404 individuals by a colorimetric method. The average pH values were 6.76 for 1826 males and 6.73 for 1578 females. In studies on the same group Brawley (1935b) found no difference in morning and afternoon pH of saliva but observed that the value was high on rising (6.88 for 60 cases) and decreased after meals (6.8 and 6.83 before breakfast and lunch, respectively, and 6.73 and 6.68 after these meals).

Brawley (1935c) found no change in the pH of resting saliva of children given vitamin A and D concentrates for a period of 1 year. There were 133 children in the experimental and 164 in the control group, the terminal pH values being 6.79 and 6.78, respectively.

Krasnow (1936) has summarized in tabular form the data of investigations of sali-

vary constituents in relation to caries. She concluded: "Indeed, pH, calcium or phosphorus analyses have not yielded any absolute basis for the differentiation between caries-free and caries-susceptible individuals. Yet it is difficult to accept as accidental the regularity with which the tendency to lower or higher concentrations of these salivary components occurs. Satisfying definiteness cannot be realized without further work."

White and Bunting (1935), in their study of salivary ammonia, said: "It has not been possible to correlate the ammonia content and the pH of activated saliva with the occurrence of caries as suggested by Grove and Grove." They did not describe their method of measurement of pH but it was determined on the stimulated salivas of 21 caries-free and 16 carious individuals. Their values ranged from 6.8 to 7.6.

White and Bunting (1936) reported a mean value of pH for the resting saliva of 7 caries-free children as 6.6 and 7.17 for stimulated saliva. The corresponding values for 13 caries-susceptible children were 6.6 and 7.26.

Grossman and Brickman (1937) found the average of pH values of salivas of 22 students, 3 taken in duplicate, to be 6.7 during the day and 6.3 during sleep. In 11 ambulant hospital subjects the averages were 6.3 and 6.1. "Several of the subjects experienced some dryness of the mouth during the night, but although the collected specimen was scantier than during the day there was a sufficient supply for tests."

Ziskin and Hotelling (1937) gave the regression coefficients of an equation fitting the data of Bodecker index of caries as a function of age, pH, and number of pregnancies in 324 cases, 164 being first pregnancies, one the eighth, and the remainder intermediate numbers. The average duration of pregnancy at the time of examination was 5.76 months. The pH range was 6.30 to 7.10. "The mean value of the hydrogen ion concentration for the 31 non-pregnant cases is 6.720, which is significantly greater than the 6.617 for the pregnant cases." They concluded that "while the saliva is slightly more acid during pregnancy, the degree of acidity is not sufficient to produce tooth decay" and also said, "The truth seems to be that gastric hyperacidity associated with pregnancy may intensify mouth acidity somewhat."

Wills and Forbes (1939) concluded that "a diet low in carbohydrate and high in protein, and 5 per cent vegetables, increased the capacity of the saliva to neutralize acid." The effect was believed to be systemic.

Florestano, Faber and James (1941b) fed half of a grapefruit 3 times a day to 67 men and used a control group of 64. The experiment lasted 6 months. Counts of the microorganisms of saliva were made monthly on (a) plain nutrient, (b) tomato juice agar at pH 6.6 and (c) blood agar. A moderate and erratic reduction in counts on all three media occurred in the experimental group. They said, "It appears that reduction in bacterial numbers of the oral flora was due more to local influences of citrus fruit and fruit juices than to systemic involvement. . . ." An increase in buffering power of the saliva was observed. The pH of the urine was 0.5 pH units higher in the test group at the end. No dental examinations were recorded.

Florestano, Elliott and Faber (1941) determined the relative effects on mouth organisms of oral application of (a) water (b) orange juice (c) grapefruit juice and three commercial mouth washes containing, respectively, hexylresorcinol, sodium ricinoleate and a sodium alkyl sulfate. The last three were more effective in reducing bacterial counts but they did not prevent subsequent growth of bacteria in the mouth as well as did the citrus juices. The effect was ascribed to stimulation of the secretion of a more alkaline saliva, although acidity in possible removal of dental plaques was recognized. (Gies, 1921).

Swerdlove (1942) found a correlation coefficient of -0.06 between the pH of

resting saliva and the Bodecker index of caries in 351 dental students.

Whyte (1943b) determined the "average pH" of the salivas of 50 boys and concluded there was no correlation between pH and average caries figures. His "average pH" values were 6.920 and 7.008 for two groups of 25. He accepted the value of pH 4.0 as necessary to decalcify enamel.

Arnold and McClure (1941c) reported: "Three specimens of stimulated saliva collected at 2- and 3-month intervals from the same 12- to 14-year-old school children were tested for following properties: (a) Total solids, (b) loss on ignition (organic matter), (c) ash, (d) percentage ash in total solids, (e) total nitrogen, (f) percentage nitrogen in total solids, (g) ammonia nitrogen, (h) total nitrogen minus ammonia nitrogen (corrected nitrogen), (i) corrected nitrogen in organic matter, (j) pH of isoelectric zone, (k) p.p.m. oxygen consumed from permanganate, and (l) oxygen consumed from permanganate per mg. of organic matter in saliva.

"The data were studied in relation to numbers of *L. acidophilus* in the saliva and to the degree of caries activity as determined by clinical examination. No significant relationship was present.

"The pH of the isoelectric zone of saliva protein was found to vary among different salivas, but this variation was not correlated with caries activity or with numbers of *L. acidophilus* organisms in the saliva."

Eisenbrandt (1944) made 1552 electrometric determinations of pH of the saliva of 7 subjects in a year. He found the mean pH was 6.64 with standard deviation of the mean, 0.006. The extremes found were 5.28 and 7.48. He found seasonal variation with minima in January and May and maxima in March and October. There was a diurnal rise. The pH of individuals were characteristic in a long series of determinations but each showed the annual and diurnal variations.

Føyne and Hobaek, (1947a) observed more rapid acid formation from sucrose by saliva of persons with active caries than of caries-free. Precipitates of the saliva contained acid-forming substances and the authors postulated a non-bacterial acid-forming enzyme already present in the saliva.

Dewar (1949) measured the pH of resting saliva *in vivo* in 250 individuals by means of three indicator papers and a glass electrode dipped into a drop of saliva held on the tip of the tongue. The mean values were "found to be 7.1 plus or minus 0.3 and 6.83 plus or minus 0.32 respectively." The indicator papers gave higher values than the potentiometer readings in the low ranges. Readings were recorded as low as the range 5.6–5.9 by the potentiometer. All subjects "were either affected by caries or were edentulous or had some teeth missing."

Ericsson (1949) gave the pH of resting and stimulated salivas from various groups as follows: Adults with low caries rate 6.75 and 7.25; adults with high caries rate 6.47 and 7.03; children, low, 6.95 and 7.44, high, 6.75 and 7.41; adult vegetarians 6.65 and 7.28, vegetarian children 6.91 and 7.55.

Sullivan and Storvick (1950a) analyzed the salivas of 574 college students for various constituents possibly associated with dental caries and (1950b) the salivas of 555 14- to 16-year-old children from two regions of Oregon. In the college students a significant correlation of 0.196 between DMF teeth and salivary pH was found. The correlation coefficients are given in the table on page 267, the first number in each pair being from the college students and the second from the younger group.

Buffer Capacity of Saliva

Marshall (1915) expressed the neutralizing power of saliva as the sum of the amounts of acid and alkali to bring the reaction to the end-points of para-nitro-phenol and phenolphthalein, respectively. He found higher neutralizing power in the stimulated saliva of non-carious subjects than in the resting saliva. In caries-active subjects the neutralizing power of resting saliva fre-

quently was higher than that of activated saliva. He called the percentage of neutralizing power of resting to activated saliva the "salivary factor". He concluded, "In persons who are either absolutely immune or for the present immune from caries the magnitude of this factor (expressed in percentage) varies between 43 and 80, while in persons whose teeth are carious this factor varies between 80 and 132."

Clark and Carter (1927) found the CO_2 content of resting saliva of 8 subjects to be about 15 volume per cent; of stimulated saliva, 34 volume per cent. They found formation of CO_2 in saliva prevented by mercuric chloride but not by chloroform and therefore deduced that it was formed by enzymatic action of non-bacterial origin. They concluded, "There is no demonstrable relationship between the pH, the volume per cent of carbon dioxide, and the ammonia content."

White and Bunting (1936) found the carbon dioxide capacity of resting saliva of 12 caries-free children to range from 4.4 to 32.5 cc. with an average value, 10.3. The values for stimulated saliva were 13.6 to 61.1 and averaged 40.6. The corresponding values for 13 caries-susceptible children were 5.6 to 21.1 resting, with 9.8 average, and 24.3 to 49.2 and average of 35.6 for stimulated saliva.

Karshan (1936) found the following average values for the CO_2 capacity in ml/100 ml of stimulated saliva: caries-free 31.1, arrested caries 30.2, active caries 19.5, miscellaneous 20.8. He concluded the values were significantly lower for the active caries and miscellaneous groups. Karshan (1939) reported the average CO_2 capacity of resting salivas for the four classes of subjects as 13.4, 12.9, 9.5 and 10.8. He also analyzed the stimulated salivas, and his results, combined with those previously found (1936) were 30.5, 30.2, 19.5 and 23.9. The differences between the caries-active and the caries-free and arrested caries groups were statistically evaluated and found to be significant.

Karshan, Siegel and Waugh (1939) gave the following average values for CO_2 capacity of the salivas of Eskimos: stimulated, caries-free 43.3, caries-active 37.5; resting, caries-free 23.6, caries-active 21.1. The differences corresponded in direction with previous studies by Karshan.

Karshan, Rosebury and Waugh (1939) found the average CO_2 capacity of the salivas of 17 caries-active Eskimo subjects to be 24.5 ml. for 100 ml. of stimulated saliva, and that of 28 with no decay significantly high at 36.3.

Strålfors (1947b) measured the buffer capacity of plaque from human teeth by titration in a cup in a glass electrode and compared his results with the saliva of eight individuals. The buffer capacity of plaque was much higher than that of the saliva. The ratio was greatest between pH 5 to 4 and least from the pH found at the start to pH 6. The ratios ranged from a minimum of 1.12 to a maximum of 17.76.

Rae and Clegg (1949) measured the buffering capacity of the salivas of 73 individuals and the lactobacillus counts as an average of three successive days. They found no positive correlation.

Sellman (1949) measured the amount of acid necessary to lower the pH of salivas of 11 caries-resistant and 10 caries-active subjects to 6.0, 5.0, 4.0 and 3.0 successively. At all levels the caries-resistant salivas required significantly more acid.

Sullivan and Storvick (1950a) found a statistically significant negative correlation coefficient of 0.123 between DMF teeth and the buffer capacity of the salivas of 574 college students.

Calcium and Phosphorus and Dental Caries

Bunting and Rickert (1915) estimated the CaO content of salivas gravimetrically. They found that the salivas of students with no caries had in excess of 3.0 mg. of CaO per 25

cc. and those with caries less than that value. They correlated the calcium content of the saliva with the appearance of the teeth, the high values being associated with hard dense teeth free of caries and the low with "soft-appearing" teeth. Bunting and Wixon (1917) confirmed the findings of Bunting and Rickert (1915) though the values were all reduced somewhat. They failed to increase the calcium content of saliva by daily feeding of calcium salts over a period of 2 weeks. Spencer-Payne (1924) stated that he found diurnal variation in the calcium content of saliva but no relation to caries.

Pattison (1926) determined the calcium of the salivas of the tuberculous children who were the subjects of the study at Sheffield by Mellanby, Pattison and Proud (1924). He found the salivas of 20 children given Diet A (with cod liver oil) averaged 10.68 mg. of calcium per 100 cc. and of 19 children given Diet B (no cod liver oil) averaged 4.68 mg. of calcium.

Clark and Shell (1927) determined the *balances* of calcium, magnesium, sodium, potassium, chlorine, phosphorus, sulfur and nitrogen of five men on five successive diets over a period of 28 weeks. They stated in conclusion: "It has not been possible to demonstrate the existence of relationship between the amount of a given element ingested or retained and the amount appearing in the circulating blood or in the resting saliva." They observed that the inorganic phosphorus of saliva was 3 to 5 times as high as in blood plasma.

Roskin (1928) found no difference in the calcium and phosphorus of the salivas of 17 diabetic children with arrested caries, and of 13 non-diabetic children with active caries. "A high phosphorus value was frequently, but not invariably, accompanied by a high calcium value." The mean values for calcium were 5.7 and 5.5 mg. per 100 ml. of saliva for the caries-arrested and caries-active groups, respectively; the phosphorus averages were 12.1 and 11.5.

Entin and Geikin (1928) found a mean content of 12.25 mg. inorganic phosphorus per 100 cc. in the saliva of 138 men. The correlation coefficient with Entin's caries index was $+0.118 \pm 0.061$. The indicated positive correlation of salivary phosphate with sound teeth was not considered significant.

Entin (1929) gave the composition of the saliva of 12 caries-free men and 12 men with extensive caries as: calcium, 16.3 and 14.0 mg./100 cc. respectively; phosphorus, 11.3 and 11.4; potassium, 72.8 and 60.8; pH, 7.63 and 7.00.

Horton, Marrack and Price (1929) reported the calcium content of the salivas of 741 children aged 3 to 12 years, in relation to number of carious teeth. The saliva was stimulated by the sucking of "acid drops." They found a decrease of calcium in saliva associated with an increase in the number of carious teeth. For example, in 86 subjects with no caries only one had less than 7–8 mg. calcium per 100 cc. and that value was in the 6–7 mg. range; in 159 children with 5 or more carious teeth only 25 had more than 8 mg. of calcium and the lowest value was in the 2–3 range. In 166 cases, after the filling or extraction of carious teeth the calcium content of the saliva was increased, especially in those cases with over 4 carious teeth. They, therefore, considered that "the reduction of calcium in saliva is secondary to the caries."

Hubbell and Bunting (1932) stated in regard to calcium and phosphorus of saliva, "A total of 902 determinations on children with active caries has given an average calcium of 5.1 and phosphorus of 12.8 mg. per 100 cc., while 275 determinations on children who were free from caries have averaged 5.4 calcium and 13.3 mg. phosphorus." They found no difference in the calcium and phosphorus between children who developed caries in a period of over a year and those who showed no new cavities. They found calcium and phosphorus reduced

about 20 per cent in concentration with increased volume of flow of saliva, but the amount of each element secreted per minute was increased three-fold in the more copious flow.

Hubbell (1933) concluded there were no consistent differences in the calcium and total phosphorus of the stimulated salivas of 15 caries-free and 17 caries-active children.

Youngburg (1932) found the inorganic phosphorus of the salivas of 92 normal subjects ranging from 21 to 80 years of age to average 17.50 mg. per 100 cc.; that of 86 caries subjects, age 6 to 80, to average 18.13 mg.; and of 25 nephritics to be 17.54 mg. He concluded, "There is no reason to believe that the phosphorus of the saliva plays any role in dental caries." He found 96 per cent of the phosphorus was present as orthophosphate, the remainder being in protein combination.

Eddy, Heft, Rosenstock and Ralston (1933) found the phosphorus of unstimulated saliva of three subjects to vary at half-hour intervals during the day and from day to day, the range being from 6.9 to 19.8 mg. per 100 cc. After the ingestion of one-half pound of candy there was a sharp drop of salivary phosphorus in two subjects. A lesser lowering was observed in two subjects after consuming starch. A slight similar effect was noted from a protein meal but none after ingestion of cream.

The final report of the Committee for the Investigation of Dental Diseases (1936) on Mellanby's studies in three institutions at Birmingham, England, gave data on calcium content of the saliva as related to dietary supplements and caries and concluded that "there is no definite evidence of any real relationship between the prevalence of caries and the calcium content of the saliva in the children under review."

White and Bunting (1935) found 5.79 mg. of calcium per 100 ml. of resting saliva in 12 caries-free children and 4.99 mg. in the stimulated saliva. Corresponding values or 13 caries-susceptible children were 5.89 and 4.87. The phosphorus values were 16.0 and 13.3 for the caries-free group and 15.9 and 12.9 for the caries-susceptible children.

Wach (1936) found a mean calcium content in the resting salivas of 10 caries-free individuals of 6.9 mg./100 cc. and in 14 with caries of 6.3. Because of the variation, 3.66 to 10.6 and 3.0 to 8.3, respectively, he did not regard the differences as significant. The ratio of potassium to calcium was about 10 to 1, with no relation to caries. He found no relation of salivary calcium and potassium to the concentration of these elements in the serum of either caries-susceptible or caries-free subjects.

Jonsgar (1937) found in a single subject 5.0 to 6.4 mg. calcium per 100 cc. of unstimulated saliva and 3.3 to 5.4 in saliva stimulated by various means. In 9 children, 6 to 14 years old, the calcium content of resting saliva averaged 4.6 mg. per 100 cc. and 5.0 after taking a bone mineral preparation, Kalfocitt.

Brawley and Sedwick (1938) determined the calcium content of the resting and activated salivas of 547 male and 247 female children age 3 to 18 years. They found no significant differences as to age and sex. The calcium of resting saliva averaged 6.88 mg. per 100 cc. with a range of 3.9 to 10.2 mg.; the values for stimulated saliva were 6.23 and ranged from 3.2 to 10.1. The difference of the means was statistically significant.

Brown and Klotz (1934) divided 18 students into 3 groups of 6 each with low (55.0 cc.), medium (141.9 cc.) and high (251.7 cc.) secretion of paraffin-stimulated saliva per hour. The chloride content of the salivas increased with volume of secretion, being 39, 51 and 63 mg. per 100 cc., but phosphate content decreased, 50.7, 43.3 and 37.2 mg. per 100 cc. Total nitrogen also decreased, but solids and ash were relatively constant. Brown and Klotz (1937) determined sodium, potassium and calcium in the paraffin-stimulated saliva of 18 subjects, 15 being the same individuals as in the preceding study.

Brown, Wright and Limbacher (1938) found in saliva collected from a single subject in successive 15-minute periods that calcium tended to decline in concentration but phosphorus was relatively constant. In a series of resting and stimulated salivas from a single subject with volume varying from 28 to 213 cc. per hour, solids, ash, chloride and pH increased with production rate; calcium rose slightly; phosphorus remained constant. In hourly samples from a single subject chewing 4 gm. of paraffin continuously for 6 hours, the volume of secretion fell steadily from 155 to 75 cc. per hour, total solids, ash and chlorides declined. Calcium almost doubled in the third, fourth and sixth hours. Phosphorus was up about 50 per cent in the second, third and fourth hours and then returned to near the original level.

Krasnow (1932) found no significant difference in the calcium and phosphorus content of stimulated or unstimulated salivas of 11 caries-free and 14 caries-susceptible young adults. There was also no difference between the two groups in the change of these values in morning and evening samples.

Karshan, Krasnow and Krejci (1931) analyzed the blood and saliva of (a) 17 subjects with no caries experience (b) 6 with arrested caries, defined as persons with no active caries though having small fillings 5 to 15 years old and (c) 21 caries-active subjects, excluding those with large cavities with hardened dentin. They found no significant differences between the groups in CO_2 capacity, CO_2 content and pH of plasma; total protein, calcium and phosphorus of serum, though the latter was low; pH and titratable alkalinity of resting and stimulated saliva; and calcium of stimulated saliva. They found the phosphorus of stimulated saliva of the 16 caries subjects averaged 9.9 mg. per 100 cc.; of the 15 immune and 9 arrested cases, 11.3 and 11.6, respectively. They did not regard this or other slight differences as significant because they were within normal ranges and there was considerable overlapping of values.

Karshan (1936) analyzed stimulated saliva of 22 caries-free, 15 arrested-caries, 41 active-caries and 27 miscellaneous subjects, the last including "individuals who had cavities or fillings, but could not be placed in either the arrested- or active-caries groups." The data for each component were grouped for all classes and each class tested as differing significantly from the general mean. In this method of analysis the chances were 27 to 1 that the caries-free and arrested groups had a higher content of salivary calcium than the total group; inorganic phosphate was significantly lower for the caries-active group (odds 60 to 1); the amounts of calcium and phosphate removed by shaking 10 cc. of saliva with 5 gm. of tricalcium phosphate were significantly higher for the caries-free and arrested groups; the protein removed by the above procedure showed no relation to caries; the CO_2 capacity was significantly higher for the caries-free and caries-arrested groups; there was no significant difference in the ammonia nitrogen for any group, though the caries-free group showed the lower value.

Karshan (1939) extended his studies of saliva constituents and found no significant differences between the caries-free and caries-arrested groups; the differences of all components were significant between the caries-active and either the caries-free or caries-arrested groups except the total protein of the arrested and active groups. Karshan interpreted his findings as indicating that "the saliva of persons free from dental caries would protect enamel against solution by acids to a greater extent than would the saliva of persons with active decay."

Karshan, Rosebury and Waugh (1939) analyzed the salivas of 49 Eskimos, 17 having caries, 28 no caries, and 4 with doubtful caries. They found higher content of calcium and CO_2 capacity in the caries-free group, the difference approaching sig-

nificance. The difference between calcium removed by tricalcium phosphate and phosphate removed in the same process approached significance. The amount of calcium removed was lower for the caries group, but the phosphorus removed was higher. They found no correlation of salivary constituents with counts of *L. acidophilus*.

Karshan, Siegel and Waugh (1940) examined the salivas of 19 caries-free Eskimos and of 10 Eskimos with caries, only 3 of whom had active decay. "Higher mean values for total calcium, inorganic phosphate and carbon dioxide capacity were found in a caries-free group than in a group with caries."

Karshan, Pedersen, Siegel and Tenenbaum (1940) reported higher values of calcium, inorganic phosphate and CO_2 combining capacity in 28 caries-free, 9 arrested-caries and 21 miscellaneous Greenland natives than in 29 active-caries subjects.

Becks (1928) reported the mean calcium content of saliva as 12.13 mg. per 100 cc. and phosphate content as 13.16 mg. The latter was found to be variable during the day. He said, "A calcium compound which is negatively charged and readily absorbed by positively charged absorbents is present in the saliva."

Becks and Wainwright (1934) critically reviewed the literature on the calcium content of saliva and Wainwright (1934) discussed the analytical method.

Becks and Wainwright (1938) examined the literature of phosphorus in saliva to determine the normal values. Of five reports, they found only the data of Clark and Shell (1927) acceptable for total acid-soluble fraction, that is, the phosphorus of a trichloracetic acid filtrate; of 18 reports for total phosphorus only the data of White and Bunting (1936) were acceptable; and for inorganic phosphorus, only those of Clark and Shell (1927) and Becks (1928, 1929). Becks (1938) concluded, "The inadequacy of reported material and procedures makes it impossible to establish definite normal percentage ranges for the various" fractions of phosphorus in saliva. Wainwright (1938), after a critical study of the methods of the determination of phosphorus in saliva, considered a modified procedure of Bodansky was the best.

Becks (1939) and Wainwright (1939) studied the rate of secretion of resting (R. S.) and activated salivary (A. S.) flow. Becks and Wainwright (1939) said: "A comparison of the advantages and disadvantages of the use of R. S. and A. S. leads to the conclusion that R. S. is better for routine analytical purposes. The deciding factor in this choice is the observation that as a result of activation patients with originally low or high rates of flow of R. S. arrive at approximately the same rate of flow of A. S. thereby disguising the true secretory condition of the resting gland which differs greatly among individuals."

Becks and Wainwright (1941a, b) determined the variations of calcium and phosphorus in resting and activated saliva and found for these elements an inverse relationship to volume of secretion. These are exceptions to the Heidenhain law that "*with increased speed of salivary secretion. . . there is an increase in concentration of solids.*" The authors recommended that Ca and P values for saliva be given as mg./hr. together with rate of flow or, if mg. per cent values are given, that rate of flow be added so that mg./hr. rates can be derived.

Becks and Wainwright (1942) found pure chicle gum stimulated the secretion of saliva in four subjects to about half the volume of paraffin stimulation and ascribed the difference to the tougher and harder consistency of the chicle. However, three flavored chicle gums induced a rate of flow far higher than that for paraffin, and the calcium content was higher than in resting saliva in accordance with Heidenhain's law (see preceding paragraph). Phosphorus levels in all cases were lower than those of resting saliva.

Wainwright (1943) determined the in-

organic phosphorus of the resting saliva of 650 of the 661 individuals observed for rate of flow of saliva by Becks and Wainwright (1943), excluding 11 because the rate of flow was only 0.5 cc. per hour. A mean of 16.8 mg. per 100 cc. was found, with a range of 6.1 to 71.0 mg. A slight increase was observed with advancing age. There was no difference in rate between the sexes. The phosphorus per hour was found to average 3.06 mg., with a range of 0.21 to 16.72 mg. The rate was slightly lower for the 5- to 9-year class, but no other significant variation was found due to age or sex.

Becks and Wainwright (1943) found the rate of flow of resting saliva of 661 healthy individuals ranging in age from 5 to 95 years to vary from 0.5 to 111 cc. per hour, with a mean of 19 cc. They found no relation to sex. Rate of flow was 15 cc. for the 54 subjects 5 to 9 years old, 24 cc. for 32 subjects 25 to 29 years and 19 cc. for 40 of the 45- to 49-year class.

Becks (1943) determined the calcium content of the resting saliva of 650 of the 661 individuals observed for rate of flow by Becks and Wainwright (1943). He excluded from study 11 whose resting saliva rate of flow was 0.5 cc. per hour. He found a mean calcium content of 5.8 mg. per 100 cc. with a range of 2.2 to 11.3 mg. There was a trend to a maximum at middle age and males had a slightly higher mean than females, 5.9 compared with 5.6, but "the sex groups are considered to be alike." The calcium per hour was 1.06 mg. for the 650 individuals with a range from 0.12 to 6.28 mg. No age or sex relation was found except a slightly lower rate for the 5- to 9-year group.

Becks, Wainwright and Young (1941) determined the rate of flow, calcium and phosphorus of resting salivas of 90 caries-free individuals (designated CF) and 108 with active caries (CA). They found no significant differences between the groups with respect to calcium and phosphorus, either as to concentration or rate per hour. In 50 CF and 50 CA cases, selected to equalize age and rate of flow, the mean concentrations of calcium were 5.74 and 5.73 mg./100 cc., respectively; the values for inorganic phosphorus were 13.24 and 12.28 and not significantly different. The authors criticized Karshan (1936, 1939) as (a) neglecting rate of flow of saliva and (b) using such small groups of subjects that chance differences appeared. Karshan (1942) replied to the criticism of Becks, Wainwright and Young by showing no significant variation in rate of flow of saliva between caries-free, caries-arrested and caries-active groups, and pointed out that chance differences did not appear between his caries-free and caries-arrested groups. He suggested that the differences in experimental procedures made strict comparison of the results of the two studies difficult.

Becks, Wainwright and Young (1943) examined the after-breakfast saliva of 25 caries-free and 25 caries-active subjects of equal mean ages. They found the calcium of both resting and activated saliva lower in the caries-active group; the inorganic phosphorus was lower in the caries-active group in resting saliva but higher in the activated saliva. They wrote: "However, the important facts remain that the differences are not statistically significant and that CF individuals show values that are just as low as any of the CA group."

Deakins, Cheyne, Bibby and Van Kesteren (1941) concluded from a study of 18 different chemical and bacteriological features of the saliva of 20 subjects that there is too great a variability of saliva constituents to make effective interpretations on small numbers of subjects. Calcium and phosphorus determinations were not included.

Becks and Wainwright (1944a, 1946a) found: "Calcium and phosphorus mg. per cent levels were directly related in all age groups of 650 individuals examined ($r = +0.402 \pm 0.033$). The average ratio of calcium to phosphorus was 0.36 mg. per cent ($\sigma = 0.12$ mg. per cent) with an ob-

served range from 0.12 to 1.22 mg. per cent. The calcium-phosphorus ratio was highest between the ages of 20 and 29 years and lowest in younger and older groups. The calcium-phosphorus product mg. per cent was lowest in the youngest age groups, while the mg./hr. product varies greatly and without relation to age."

Wainwright and Becks (1944, 1946) stated: "Salivary analyses of 650 individuals, accumulated over a period of 3 years, sorted according to the month of examination, were studied for possible seasonal effects. They did not disclose any seasonal variation of rate of flow, calcium or phosphorus content of resting saliva."

Becks and Wainwright (1944b, 1946b) found low correlation coefficients of calcium and phosphorus content of saliva to rate of flow (-0.156 and -0.28 in 650 subjects). The correlations of mg. per hour of calcium and phosphorus with rate of flow was high ($+0.913$ and $+0.810$, respectively). By partial correlation studies the calcium and phosphorus output in saliva was shown to be only slightly related. (Partial correlation coefficient, $+0.154$.)

Davies and Rae (1948) showed no relation of total and of organic phosphate of the salivas of 35 subjects in relation to counts of lactobacilli.

Conclusions: The preponderance of the evidence is that there is no relation between the concentrations of calcium and phosphorus of saliva and the incidence or progress of caries. Those reports which show lowered levels of these components with increased carious attack present data which are statistically valid by only narrow margins.

Amino Acids

Updegraff and Lewis (1924) concluded, from analyses of saliva for non-protein nitrogen other than urea and ammonia nitrogen and by recovery experiments with added amino acids, that the amino acid nitrogen of normal saliva was of the order of 1 mg. per 100 ml.

Kirch, Kesel, O'Donnell and Wach (1947) analyzed the salivas of 18 individuals for 16 amino acids. They reported, "Variations in the concentration of these acids were independent of caries activity."

Of 17 amino acids tested by Calandra and Fosdick (1947) "only valine, norleucine, phenylalanine, and threonine had an appreciable enzyme inhibiting action, while hydroxyproline, tryptophane and isoleucine had a very mild effect." The effective concentrations of the amino acids in the saliva samples were much higher than those found for any amino acids in saliva.

Goldberg, Gilda and Tishkoff (1948) analyzed saliva for amino acids by paper partition chromatography. They identified glutamic acid, aspartic acid, glycine, alanine, methionine, arginine, leucine, lysine, phenylalanine, taurine, tyrosine, valine and proline, with slight indications of serine and histidine.

Fosdick and Blackwell (1949) found indications of higher concentrations of aspartic and glutamic acids in salivas of caries-active subjects compared with salivas from "caries-immune" and pyorrhea subjects.

Ammonia and Urea

Heyward (1881) was probably the first to analyze saliva for ammonia. His method was by the color developed on a strip of filter paper, moistened with Nessler's reagent, suspended in a test tube in which a measured amount of saliva was treated at 30°C with magnesium oxide. Comparison was made with colors developed from known concentrations of ammonium chloride. He found ammonia present in the saliva of all of 19 subjects. In the whole salivas of 10 of them the ammonia ranged from 4 to 10 mg. per 100 ml. In his own saliva it varied from 4 to 6 mg. in 7 successive days. With the saliva of the submaxillary glands excluded, Heyward found 1 mg., and with parotid gland saliva blocked, 3 mg.; the mixed saliva, on the day of that experiment, showed 4 mg. per 100 ml.

Schmitz (1922) found the urea plus am-

monia nitrogen in the salivas of 45 pathological subjects increased with the urea nitrogen of the blood and averaged 89.4 per cent of the blood urea nitrogen. Since the ammonia content of saliva passed through a Berkefeld filter remained unaltered on standing, he concluded that salivary urea is changed to ammonia by bacteria rather than by a salivary enzyme.

Hench and Aldrich (1922) found the combined urea and ammonia nitrogen of saliva to range from 6 to 13 mg. per 100 ml. and to increase with an increase of blood urea.

Morris and Jersey (1923) found ammonia nitrogen levels of 2.4 to 3.7 mg. per 100 ml. of saliva and commented that it increased on standing, probably at the expense of urea. Urea plus ammonia nitrogen increased with increased volume of flow due to mastication, and also, but not so much, under acetic acid stimulation.

Morris and Way (1924) examined the salivas of subjects suffering from chronic nephritis, mercuric poisoning, lysol poisoning and diabetes. They reported: "Salivary urea tended to increase with blood urea. However in terminal nephritis the amount of urea eliminated through the salivary glands did not continue to increase and in some cases was actually less than normal. This was apparently due to failure of the glands to function, possibly because of general failure of body function. In anuria following lysol poisoning, salivary urea percentage exceeded the blood percentage."

Updegraff and Lewis (1924) found the ammonia nitrogen of stimulated saliva of 34 normal persons to range from 2.1 to 13.2 mg. per 100 ml., with an average of 5.7. The urea nitrogen was 0.0 to 6.7 and averaged 4.7. They stated that "urea added to saliva is also converted to ammonia on standing without a preservative" and thus accounted for the origin of at least some of salivary ammonia. Chloroform checked the conversion of urea to ammonium carbonate, and precipitation of protein by the Folin-Wu method gave stabilized filtrates.

Clark and Carter (1927) found that chloroform partially inhibited the formation of ammonia in saliva, and mercuric chloride prevented any changes. They concluded that part of the formation of ammonia was due to bacterial action, prevented by chloroform, and part by enzymes in saliva.

Vladesco (1928) reported ammonia present in the saliva of the dog, cat, horse and man. The ammonia of human saliva was characteristically high, ranging from 0.030 to 0.252 mg. per 100 cc. Comparison was difficult, however, because in the animal experiments saliva secretion was stimulated by pilocarpine or arecoline. Vladesco and Popsco (1929) found the ammonia concentration of pilocarpine-stimulated saliva very much reduced. They considered the ammonia was formed by the salivary glands as part of the mechanism of the maintenance of the alkali reserve.

Grove and Grove (1934, 1935) found the ammonia nitrogen of saliva of caries-susceptible individuals to range from nil to 8.0 mg. per 100 cc. The range for caries-immune subjects was from 4.00 to 10 mg. Grove and Grove postulated protection against caries by solution of plaques by ammonia but demonstrated such solution on artificial plaques by ammonium hydroxide or carbonate, certainly at pH values never found in the mouth.

White and Bunting (1935) analyzed the salivas of 21 caries-free and 16 caries-susceptible children and young adults for ammonia by aeration. They found 3.64 to 26.32 mg. per 100 ml. the extreme amounts of ammonia nitrogen and no relation to dental caries. They found the permutit method, used by Grove and Grove (1934, 1945), unreliable.

Youngburg (1936) obtained values for ammonia nitrogen of the saliva ranging from 1.28 to 13.66 mg. per 100 cc. in normal individuals. In the salivas of 45 subjects with active dental caries the range was from 3.57 to 16.96 mg., and he concluded there was no relation of salivary ammonia to caries.

He found no solution of mucin by 10 mg. of ammonia per 100 cc.

Karshan (1936) found no relation between the ammonia content of saliva and dental caries.

Karshan (1936) found 3.9 mg. ammonia nitrogen per 100 ml. in the salivas of 13 caries-free subjects, 5.2 mg. in 27 active-caries cases, and 5.0 mg. in 14 subjects who had cavities and fillings but could not be classified as active or arrested cases. He concluded there were no significant differences and that the results tended to be opposite to those found by Grove and Grove (1934, 1935).

Stephan (1940b) reported that some organisms present in the mouth, such as *Staphylococcus albus* and *aureus* but not lactobacilli, contain urease. Thus these organisms tend to produce alkalinity in the mouth and especially in plaques. "The pH of bacterial material on tooth surfaces has been found to rise as high as 8.5 following rinsing of the mouth with a carbamide (synthetic urea) solution."

Stephan (1943) used urea solutions on homogenized and layered plaques and on plaques *in situ*, either with or without a mouth rinse of 10 per cent glucose solution. If the glucose solution contained 0.01 per cent urea, comparable to his estimate of 0.013 per cent urea in saliva, he found no effect on the course of pH drop and subsequent rise. However, 0.1 per cent urea retarded the fall of pH and 1, 10 and 40 per cent urea in the glucose rinse prevented the lowering of pH. Solutions of urea of 40 and 50 per cent concentrations protected plaques from the effects of glucose for 24 hours.

Stephan and Miller (1944) observed the development of new carious surfaces in 6 individuals who used a saturated solution of urea as a dentifrice for 16 to 26 months. The toothbrushing was done for 4 minutes twice a day. There were 8 surfaces that showed new carious lesions, compared with 152 in a preliminary period. A control group of 6 individuals had 71 new carious surfaces during the experimental period and 69 in the preliminary period.

Stephan (1949) reported: "Only slightly stimulatory or inhibitory effects were observed with either Na_2HPO_4 or $(NH_4)_2HPO_4$ for the 30 strains of lactobacilli studied, and no significant difference was observed in the effects produced by the sodium and ammonium ions."

Wach, O'Donnell and Hine (1942) observed the counts of lactobacilli in the salivas of 18 subjects before and 14 days after thrice daily use of a mouthwash containing 0.5 per cent quinine hydrochloride, 4 per cent urea and 25 per cent glycerin. The counts fell in all subjects but rose in 9 days after discontinuing the mouthwash in 11 subjects tested. The average pH of the salivas rose in 13, remained unchanged in 4 and fell in one case.

Kesel, O'Donnell, Kirch and Wach (1946) observed a reduction in the counts of lactobacilli in the salivas of 44 of 45 subjects who used a tooth powder containing 5 per cent dibasic ammonium phosphate and a 5 per cent solution of the salt as a mouth wash twice daily for 5 months. A control group of 10 subjects showed no such change in counts of lactobacilli.

Cary (1946) analyzed the salivas of 9 caries-active and 11 caries-free subjects for ammonia, urea, protein and non-protein nitrogen. The analyses were done on fresh mixed saliva, the same after incubation with and without glucose for various periods and on saliva obtained from the parotid and the submaxillary gland with the minimum bacterial contamination. Cary concluded: "The salivary glands have neither an excretory nor a secretory function in the formation of ammonia. Normal saliva owes its ammonia, to an overwhelming if not complete extent, to the presence of bacteria." "The source of salivary ammonia is mainly urea and protein. Urea is quickly hydrolyzed, but mucin may also be rapidly attacked." He found the average ammonia nitrogen content of saliva from the caries-active sub-

jects to be 3.81 mg. per 100 ml. and a range from 0.28 to 9.56; similar figures for caries-free subjects were 4.72 and 1.08 to 14.0. He concluded: "The evolution of free ammonia from saliva is not significantly greater in caries-free cases."

Fosdick and Ludwick (1948) used permutit for the double purpose of a dentifrice and base exchange collection of ammonia from plaques from the teeth of 1000 individuals. They found "the ammonia content of the brushings was correlated with the *L. acidophilus* count and the clinical estimation of caries activity."

Ludwick and Fosdick (1950) used a dentifrice containing permutit to collect the ammonia of both saliva and dental plaque removed from mouths during the brushing of teeth. They found no significant difference in the ammonia from 300 subjects clinically immune to caries and 700 clinically susceptible or, in the same group, 388 judged immune bacteriologically and 612 judged susceptible. In a group of 25 immune and 25 susceptible subjects the ammonia of prophylactic cleaning of the teeth showed no significant difference. They concluded that "the ammonium concentrations on the surface of the teeth do not differ between caries-active and caries-immune individuals."

Henschel and Lieber (1949) reported that in a group of 100 subjects using a cosmetic dentifrice for an average of 7.8 years the number of DMF surfaces increased at the rate of 2.39 per year. After use of a tooth powder containing 22.5 per cent urea and 5 per cent dibasic ammonium phosphate 2.9 years the DMF rate was 1.55. A control group on cosmetic dentifrices had a DMF rate of 2.31 per year for 12 years and 2.48 in the test period. No evidence was given that the reduction in new carious surfaces was statistically significant.

Sullivan and Storvick (1950a) failed to find a significant correlation coefficient between the salivary ammonia nitrogen and the DMF teeth of 574 college students.

Cobe (1950) reported as follows: "High urea, low urea, quaternary compound, and cosmetic pastes and powders were studied *in vitro* and *in vivo*. Three hundred children, 10 to 14 years old, were placed under observation for 18 months. Groups were given pastes and powders containing: (1) high percentage urea, (2) low percentage urea, (3) a quaternary ammonium compound, and (4) no ammonium compound. *Lactobacillus acidophilus* counts revealed an average decrease of 46 per cent in the first 3 groups and an increase in Group 4. The greatest decrease followed the use of quaternary ammonium compound; the next greatest was the high urea dentifrice group. Powders were 8 per cent better than pastes."

Douglas, Kirchheimer and Layton (1950) stated in a brief abstract that "several strains of lactobacilli are neither destroyed or greatly inhibited in their growth by the addition of high concentrations of ammonium ion to the culture media."

Volume of Secretion

Rigolet (1901) reported the case of a man 44 years old with complete absence of salivary secretion for at least three years, and extensive caries of all the teeth. Rigolet considered the case as a demonstration of the antiseptic power of saliva. Miller (1903b) reported a similar case of a woman in whom "the teeth began decaying in a frightful manner, especially at the necks and along the free margins of the fillings, where caries as a rule seldom occurs."

Hubbell and Bunting (1932) found no correlation of caries with the calcium and phosphorus content of stimulated salivas of children. In a study of 65 samples of saliva with a rate of flow of 0.5 cc. per minute, and 97 samples at 2.0 cc. per minute, they found both calcium and phosphorus significantly diminished in concentration in the more copious secretion, but the absolute quantities of these elements per minute were increased about three-fold. They expressed the opinion: "At present we can make no

satisfactory correlation between rate of flow and tooth preservation, although there appears to be a slight tendency for the caries-free individuals to secrete larger volumes than is the case of those with active caries."

White and Bunting (1936) found the rate of secretion of resting saliva to average 0.46 ml. per minute in 12 caries-free children and 0.40 in 13 caries-susceptible children. The corresponding volumes of stimulated saliva were 1.52 and 1.45 ml.

Trimble, Etherington and Losch (1938) in 244 experiments with 107 students found the average stimulated secretion of saliva was 34 cc. per 15 minutes, with a range from 9 to 68 cc. "Of the 57 students having less than the average rate of saliva secretion, 28 developed new smooth surface cavities" during one year. In the 50 with more than the average rate, 15 showed new cavities.

Gore (1938a) devised an appliance for collecting separately the secretion of the parotid glands and that of the sublingual and submaxillary glands taken together as the mandibular saliva. He found his own resting saliva to be 20 cc. per hour for the parotid glands and 19 cc. for the mandibular glands. Under stimulation the volumes were 120 cc. and 36.4 cc. per hour, respectively. The night volumes, in both cases, were diminished, especially from the parotid glands.

Gurley (1939) reported extensive caries in the teeth on the left side only of a woman 20 years of age. "Dysfunction of the parotid gland of this side was almost complete... The entire mouth was very dry, producing barely enough moisture to saturate a 2-inch piece of litmus paper in a minute."

Losch and Weisberger (1940) reported observation of a single case of xerostomia in a girl 12 years of age. The girl was under observation for 8 years. All deciduous teeth except the maxillary second molars were carious at 4. "All of the adult teeth were seriously involved with smooth surface caries before they were in service one year." The paraffin-stimulated salivary flow was 1 cc. per hour.

Cushman, Etherington and Thompson (1940) reported for a group of 21 13- to 16-year-old subjects re-examined after one year, secretion of 22.1 cc. of saliva in 10 minutes by those showing no new cavities; 16.3 cc. with 1 to 3 new cavities; 12.3 cc. with 4 or more new cavities.

Cushman, Etherington and Thompson (1941) reported an average stimulated saliva flow of 20.6 cc. in 10 minutes in 10 individuals with no new caries; 16.6 cc. in 7 with 1 or 2 new cavities; and 10.1 cc. in 9 having 4 or more low cavities. The increase of caries was accompanied by an increase in surface tension of the saliva.

Karshan (1942) reported the rate of flow in cc. per hour of unstimulated saliva with respect to caries as follows: Caries-free 24.4, arrested caries 24.6, active caries 27.1, and miscellaneous 22.1. The salivas were collected one to three hours after breakfast. The rates were not significantly different.

Bárány (1947) found 5.66 $\pm$ 0.35 ml. of saliva secreted in 5 minutes under stimulation of chewing a piece of unvulcanized rubber by 80 subjects with "much caries." The secretion rate was 6.80–0.42 by 77 subjects with "little caries." The difference 1.14 $\pm$ 0.55 ml. is statistically significant.

Amylase

Pickerill (1912) advanced the idea that ptyalin may be protective against caries by rapid digestion of starch accumulated on the teeth so that the soluble maltose would be rapidly swept away. He also reasoned that no ptyalin would be better than a scanty amount because the intermediate sticky dextrins would not be formed in the absence of starch digestion. He found acid fruits increased the ptyalin output about 10-fold over resting saliva. Biscuit and bread produced increases of about $\frac{1}{3}$ that amount.

Robb, Medes, McClendon, Graham and

Murphy (1921) said: "We are inclined to accept the view of Pickerill that the function of ptyalin is the cleansing of the teeth by the removal of starch. In the United States army there were found 43,000 upper carious teeth to 26,000 lower carious teeth. Since saliva gravitates to the lower teeth, one might suppose that it protected the teeth by neutralizing acids."

Hubbell (1933) determined the diastatic activity of the salivas of 15 caries-free children and 17 children with caries at 6-month intervals over a period of 18 months. The diastase in per cent of starch hydrolyzed to reducing sugar was 12.8 to 45.5 with a mean of 24.0 for the caries-free, and 11.1 to 45.2 with a mean of 27.6 for the caries children.

Day (1934) determined the amylolytic index (disappearance of iodine color) of the saliva of 55 children susceptible to caries and 55 relatively free of decay. The respective indexes were 600 and 529. A correlation coefficient of -0.511, eleven times its probable error, indicated true inverse relationship between ptyalin and caries, but Day did not regard diastatic power of saliva as "an important controlling factor in the incidence of dental caries."

Florestano, Faber and James (1941a) determined time of complete disappearance of starch-iodide color, using salivas of 76 men classed as caries-free and 90 with varying degrees of caries. They found a mean of 9.3 minutes of the caries-free group and 4.0 minutes for those with caries. For 28 men with all teeth intact the time was 9.3 minutes, compared with 3.6 for 28 with rampant caries. They believed that "the diastatic activity of saliva may serve as an index of caries susceptibility." Florestano, *et al.* suggested that Hubbell (1933) had too few subjects to show a significant difference in diastatic activity. It may be pointed out that they measured disappearance of starch, whereas Hubbell determined production of reducing sugar.

Turner and Crane (1944a, b) reported the time of salivary hydrolysis of starch to be inversely related to the number of cavities in 51 cases.

McClure (1939) has shown, with careful control of pH and other conditions during the enzymatic reaction, that there was no significant difference in the rate of formation of reducing sugar by salivas of children from Galesburg and Quincy, Illinois. Though the study related to the fluoride content of the water supplies, the dental caries rate of the children of Galesburg was $\frac{1}{3}$ that of Quincy children (see section on Fluorine and Dental Caries).

Fosdick and Rapp (1944a) found a correlation between the amylolytic index of saliva and the solubility of human dental enamel during fermentation. Addition of pancreatic amylase to saliva increased solution of the enamel.

Pratt and Eisenbrandt (1944) used the Klett-Summerson photoelectric colorimeter to measure by means of the iodine color, the starch remaining in a standard solution 1 minute after addition of 0.1 ml. of saliva. They found significant variations among 6 individuals but no significant bi-hourly variation for individuals. They made no studies on caries correlation.

Bergeim and Barnfield (1945) found no correlation between DMF in 46 students or the number of open carious lesions in 105 students and salivary amylases. Also no correlation was found in a group of 100 students classified only as to "high," "moderate" and "low" degrees of caries.

Bárány (1947) estimated ptyalin content of saliva by determining the reducing sugar after 4 minutes. He reported no significant difference in reducing sugar produced by 83 subjects with "much caries" and 77 with "little caries". The values were 1.29 ± 0.081 and 1.42 ± 0.087, respectively.

Hess and Smith (1948) measured amylase activity of saliva both by disappearance of the blue color given by iodine and starch

and by production of reducing sugar. They found no significant difference in either measurement between the salivas of 20 non-carious individuals and 36 with varying degrees of caries.

Sullivan and Storvick (1950a) found a significant negative correlation of 0.460 between DMF teeth and starch hydrolyzing time as measured by iodine color in 574 college students. Other relations of starch hydrolyzing capacity found by Sullivan and Storvick (1950a, b) have already been discussed.

Discussion

The pH of normal human saliva varies between 5.75 and 7.0, with a mean of about 6.70. It varies within ± 0.5 pH units under various normal circumstances such as in chewing, fatigue, change in breathing rate and by metabolic influences.

No relation has been shown between pH of saliva and dental caries, but a reduced potential alkalinity may accompany caries.

The carbon dioxide capacity of saliva is generally significantly higher for caries-free than for caries-active subjects.

There is no relation of the ammonia content of the saliva to dental caries.

Caries activity may be inversely related to volume of saliva secretion. Increase in incidence of caries in rats after removal of the salivary glands is in agreement with these observations on man.

In view of conflicting data no conclusions can be drawn concerning a relation between salivary amylase and caries activities. Dextrinizing and saccharifying activities of saliva may bear different relations to caries.

ANTIBACTERIAL ACTION OF SALIVA

Hugenschmidt (1896) concluded that "the bactericidal action of the saliva appears to be very *problematical*" after experiments with Chamberlain-filtered saliva, with and without heating, on torula, sarcina, staphylococci and cholera bacteria. He considered a relatively dry mouth as conducive to infection but related the effect to volume of secretion. He suggested that the buccal mucus secretion might have bactericidal properties but reported no tests.

Miller (1903a) concluded that human saliva, whether or not it is put through a Chamberlain filter, does not retard fermentation or putrefaction. While he found a little less acid formed by the salivas of persons immune to caries than by salivas from caries-susceptible subjects, he did not believe the difference sufficiently marked to account for the difference in caries attack.

Clough (1934), with a modification of the "well" technic (see below for details) of Fleming and Allison (1924), found: "Of forty-one different salivas tested against *Lactobac, acidophilus,*. . . all but one inhibited growth, the zone of inhibition varying from 0.5 mm. to 6 or 8 mm. Filtered saliva showed no inhibitory effect."

Taylor and Bibby (1935) with the "well" technic found the bactericidal component of saliva against *L. acidophilus* was filterable but that against *M. lysodeikticus* was removed by a Berkefeld filter.

Bibby, Hine and Clough (1938) adapted the method of Fleming and Allison (1924) to testing saliva for anti-bacterial effects, which consisted of placing saliva in "wells" of standard shape and regulated depth in inoculated plates. Culture media were varied for different organisms. A positive test was a colony-free zone of varying widths around the well. The method was sufficiently sensitive that a difference of 1 mm. in width of the zone was judged significant. They found that growth of air- and water-borne organisms was much more inhibited than that of bacteria isolated from the mouth and suggested that "the normal flora of the mouth is composed of organisms which, although originating from extraneous sources, have become so modified by the action of the saliva that they now constitute separate and sometimes distinct species." Saliva from different individuals varied in bacterial growth inhibition and there was an hour-

to-hour variation for a single individual. The active agent appeared to be a component of the saliva rather than of any cellular elements, though two agents were indicated by action against different bacteria. Van Kesteren, Bibby and Berry (1942) concluded from studies on 15 species of bacteria that "saliva contains at least two antibacterial principles. One of these resembles lysozyme, the other is apparently distinct from it."

Clough, Bibby and Berry (1938) found zones free of *L. acidophilus* about wells containing 250 of 260 salivas tested. The bactericidal agent was destroyed by heating to 75°C for 5 minutes, by ultraviolet light and by alternate freezing and thawing.

Hill (1939a) reported studies on a *L. acidophilus*-inhibiting factor in saliva that was present in higher degree in the saliva of caries-free individuals than in that of caries-susceptibles. Centrifuged saliva to which glucose had been added was dosed with *L. acidophilus* in standard dilution and plates were poured at pH 5.1 at intervals. Generally no colonies developed if "immune" salivas were used. The active principle was found to be non-dialysable, resistant to 80°C for 1 hour and adsorbable on the dead bodies of *L. acidophilus*.

Hill (1939b) has reviewed the literature on the ways in which saliva can affect microorganisms; namely, by (a) the amount of saliva secreted, (b) the presence of bacteriolytic substances, (c) the interaction of bacteria, and (d) the presence of substances which destroy, inhibit growth, or reduce pathogenicity of organisms.

Hill and Kniesner (1941a) reported effects of various manipulations of saliva on controlled inocula of lactobacilli. They found no effects by refrigeration, centrifuging or adding more than 2 per cent glucose. Filtration and heating of saliva resulted in less growth of lactobacilli. Buffering index of the saliva was not related to caries susceptibility, presumably measured by *L. acidophilus* counts. In four samples of saliva, cocarboxylase content was two and one-half times as concentrated as in two susceptible salivas.

Curotto Devoto (1940) confirmed Hill's findings (1939a) that the saliva of caries-free individuals and those with medium caries contained a substance which inhibited the growth of *L. acidophilus*.

Discussion

Saliva contains substances which are inhibitory of the development of microorganisms, particularly of those which are not normal inhabitants of the mouth. Whether the inhibitive action is sufficient to modify the course of dental caries remains to be established, but the evidence is indicative that some reduction of caries activity may be due to antibacterial substances of saliva.

CARBOHYDRATE DEGRADATION BY ORAL BACTERIA

Fosdick and Hansen (1936) speculated on the mechanism of formation of acid in the mouth by microorganisms at the expense of fermentable sugars. They suggested that carbohydrate degradation follows the mechanism of Embden for formation of lactic acid in muscle and of Meyerhof for the action of yeast on carbohydrates. They stated that in agreement with such a mechanism, fluoride, in unstated concentration, inhibited acid formation in saliva containing glucose. They found that yeasts formed acid to dissolve enamel powder in glucose-saliva but that *L. acidophilus* was without effect. Yeast and acidophilus was more potent than yeast alone, indicating symbiosis of these two organisms.

Hansen, Fosdick and Epple (1937b) studied acid and reducing sugar formation from starch and its degradation products, soluble by eight mouth organisms and acid formation from maltose. The production of acid was not as much as expected from the reducing sugars found. A combination of *L. acidophilus* and *S. cerevisiae* on a maltose-saliva medium produced more solution of

calcium from enamel than either of the organisms alone.

Fosdick, Hansen and Wessinger (1937) examined the reduction of pyruvic acid to lactic acid by dried mouth organisms. The conversion was of the order of about 3.3 per cent of the pyruvic acid present for *S. cerevisiae* and 2.3 per cent for *L. acidophilus*. They postulated degradation of glucose by mouth organisms as described by Meyerhof in fermentation by yeast, decalcification of enamel by pyruvic acid and reduction of calcium pyruvate to calcium lactate.

Fosdick (1939) incubated a mixture of 1 liter of saliva, 50 gm. of glucose and 10 gm. of Ca_3PO_4 for two weeks and isolated a phosphorus compound that may have been a hexose phosphate, and a precipitate "identified as a mixture of calcium lactate and calcium pyruvate." In a second trial, "No phosphoglyceric acid was obtained, but lactic, acetic and pyruvic acids were identified." In a third test "it was thought necessary to prevent the hydrolysis of the phosphoric ester by the addition of fluoride ions" and after 3 days of fermentation, 0.5 gm. of sodium fluoride (about 225 p.p.m.) was added. No precipitate was obtained at first but on concentration to 200 cc. "a precipitate was obtained that had the characteristics of phosphoglyceric acid." Fosdick concluded: The products of fermentation of mouth organisms in a saliva-glucose-tricalcium phosphate medium correspond with the products formed in tissue and during alcoholic fermentation. It is quite probable that the degradation of carbohydrate to lactic acid by means of bacteria, yeast and tissue enzymes follows similar paths.

"If this is true, several acids, such as phosphoglyceric, pyruvic, acetic, butyric and lactic acids, take part in dental caries."

Fosdick and Campaigne (1939) found a "correlation" of 75 per cent between clinical caries and production of a mixture of lactic and pyruvic acid in saliva incubated with enamel powder for 4 hours. These results were from 10 immune and 10 susceptible subjects. They considered this favorable agreement with the 92 per cent association of enamel solution with clinical state in 850 cases.

Fosdick and Wessinger (1940) used a dry powdered yeast (originally isolated from human mouths but not identified) to study the formation of various possible degradation products of glucose. They succeeded under various circumstances in showing (a) the phosphorylation of glucose, (b) production of phosphoglyceric acid (with fluoride inhibition at about 200–225 p.p.m.), (c) the degradation to pyruvic acid and (d) the reduction of the latter to lactic acid in the presence of hexose diphosphate. They suggested that all these acids may play a part in dental caries. "The primary ionization constant for phosphoglyceric acid is 3.8×10^{-3} and for pyruvic acid is 5.9×10^{-3}, as compared to the value of 1.38×10^{-4} for lactic acid. Since the former acids are formed first and are stronger than lactic acid, it would appear that they are the compounds that actually decalcify the teeth. The reason lactic acid, rather than phosphoglyceric and pyruvic, is isolated from carious material is the ephemeral existence of the latter. As soon as they are formed, the action of enzymes soon converts them into the final product, lactic acid." They stressed the possibility that mouth yeasts and *L. acidophilus* acted in symbiotic relation to decalcify enamel, the latter organism especially providing the reductase to convert pyruvic to lactic acid.

Fosdick and Rapp (1943) found that *Staphylococcus albus* could promote all the reactions which they considered necessary in the degradation of glucose to decalcifying acids, but the very rapid rate of destruction of pyruvic acid, with lactic acid being formed only under anaerobic conditions, led them to exclude this organism from a causative role in caries and to suggest "it is probable that it may have a pronounced inhibiting effect."

Neuwirth and Klosterman (1940a, b)

determined the lactic acid content of saliva 10 minutes after 1.5 gm. of carbohydrates were allowed to dissolve in the mouth. The method of Miller and Muntz (1938) was used for determining lactic acid. In five subjects glucose and sucrose caused comparable rises of from about 12 to 50 mg. of lactic acid per 100 cc. of saliva from control levels of 1.0 to 3.8 mg. Similar results were obtained *in vitro* but there were no effects if the saliva were sterilized by passage through a Seitz filter. Inulin, acacia, arabinose, xylose and gelatin were without effect.

Summerson and Neuwirth (1941) studied the nature of the acids produced from glucose in saliva in 15- and 30-minute incubation periods by use of the Warburg apparatus. They found that "lactic acid production accounted for only 50 per cent or less of the total acid produced, with no significant differences in this respect between carious and non-carious individuals. Pyruvic acid was occasionally found in small amounts, but lactic and pyruvic acid together in no way account for the total acid produced." Neuwirth and Summerson (1942) found that "oral microorganisms can metabolize both lactic and pyruvic acids almost as rapidly as they are formed. The metabolism of lactic acid leads to an intermediate production of significant amounts of pyruvic acid, but the further metabolism of pyruvic acid produces no lactic acid."

Discussion

Various acidic products can be isolated from incubated mixtures of mouth organisms and fermentable carbohydrates, namely, phosphoglyceric acid, pyruvic acid and lactic acid. Also, malic acid has been identified as an end product of *B. acidophilus odontolyticus* These acids may decalcify enamel *in vitro* in proportion to their acidity and period of existence. At least phosphoglyceric, pyruvic and lactic acids undergo metabolism by oral organisms.

Whether lactic acid arises from pyruvic acid or vice versa may depend upon conditions of the reaction mixture.

TESTS FOR CARIES ACTIVITY

Price has used a test for susceptibility to caries which consists of shaking saliva with bone chip and comparing the analysis of the saliva for calcium and phosphorus before and after the shaking. If the calcium and phosphorus content is increased in the saliva, the subject is judged susceptible; if decreased, immune. The test is described by Price (1932b).

Counts of *L. acidophilus* in the saliva, especially by the method of Hadley (1933) have been used as an index of caries activity, as discussed earlier. Below are discussed some other indexes which depend upon acid production as revealed by pH, titration or solution of enamel.

Fosdick, Hansen and Epple (1937) found a significant difference in the rate of solution of powdered human enamel by the salivas of individuals with and without caries on incubation in the presence of sugar. They proposed to use the increased rate of solution of enamel as a test for caries susceptibility and stated that there was an 85 per cent correlation with clinical condition. The solution rate was found to be correlated with *L. acidophilus* and yeasts. These organisms together in sterilized saliva containing glucose were more effective in dissolving enamel than either alone.

Hatton (1938) found powdered enamel was dissolved by incubation with sugar-containing saliva of caries-susceptible persons but not by that of caries-free individuals. *L. acidophilus* "when inoculated into sterilized saliva containing enamel particles and sugar, failed completely to dissolve enamel." However, when yeast was added, solution of enamel readily occurred.

Fosdick and Starke (1939) determined the amount of calcium and phosphorus in saliva in equilibrium with mixed human enamel made acid with lactic acid to various pH values ranging as low as 4.0. This pH

value was apparently asymptotic for lactic acid. The "apparent solubility product" calculated at various levels of pH was widely variable. The critical pH at which enamel would dissolve in saliva, dependent upon the calcium and phosphate ion concentrations of the saliva, was calculated to be 6.2 for 6.7 mg. of calcium and 11.8 mg. of phosphorus, per 100 cc. but for the same level of phosphorus with only 4.0 mg. of calcium it would be 6.5.

Snyder (1940) correlated counts of lactobacilli in saliva with change of color of brom-cresol-green in a glucose infusion agar, initial pH 5.0. The indicator change occurred at about pH 4.4–4.2, and was observed at different times, preferably in 48 hours. Although the test was developed on the assumption of the correlation of lactobacillus counts with caries activity, Snyder entertained the speculation, in view of other organisms and symbiotic possibilities, "whether the amount of acid produced by the oral flora might not be a more accurate index of caries activity than numbers of lactobacilli."

Snyder (1941) applied his colorimetric method of diagnosis of caries activity to a group of 63 children of average age between 6 and 7 years. He found "the colorimetric method separated the children into distinct groups more easily than lactobacillus counts." He recognized that acid production reflected the activities of a number of species of microorganisms.

Wach, Kesel, Hine and O'Donnell (1943) tested for caries activity by titrating the acid formed in a 4-hour incubation time in 3 cc. of saliva to which was added 1 cc. of 0.4 per cent glucose. They and Kesel (1943) compared the method with the enamel dissolution test of Fosdick, Hansen and Epple (1937a) and the *L. acidophilus* counts of Hadley (1933) on 50 subjects. Agreement of the fermentation test and *L. acidophilus* counts was obtained in 34 cases, and with the enamel solubility test in 28. In 818 saliva fermentation tests on 50 subjects, 86 per cent coincided with presence or absence of caries. "However, the amount of acid formed in the test was not indicative, necessarily, of the extent of clinical caries activity."

Kesel (1943) has discussed the value of caries activity tests. Such tests, if reliable, would show response to therapy in a short time instead of the long periods necessary to determine caries directly by clinical study. Activity tests would aid in diagnosis and planning of treatment. Thus, "If the test indicated that caries was inactive, it would not be necessary to intervene" in areas of doubtful caries.

Coolidge (1947) determined the increment of phosphate content of materia alba over a 10-minute period after a glucose rinse as a measure of caries activity in contact with enamel. The results from five carious and five non-carious areas showed an increase in phosphate with caries and decrease in non-carious materia alba.

SYSTEMIC IMMUNITY TO ORAL BACTERIA

McIntosh, James and Lazarus-Barlow (1925) found agglutinins for *B. acidophilus odontolyticus* in the blood of 7 of 10 subjects in a maximum titre of 1–160. They also found 1–160 titre for a single monkey. There was no bactericidal action demonstrable in saliva.

Ross, Krasnow and Samet (1927) inoculated 18 rabbits with vaccines prepared from two strains of *L. acidophilus*, one being from a commercial milk preparation and the other isolated from carious human dentin. "Sera from fourteen . . . showed agglutination to a greater or less degree. Saliva from these rabbits bore agglutinins in 7 of 14 cases. The titre was much weaker than that of sera, and the observed agglutination was very slight. Cross agglutinins for two different strains were present." Uninoculated rabbits were negative throughout.

In a study of "three human non-susceptibles and three normal dogs . . . in relation to five strains of aciduric rods isolated from

teeth having caries," Rosebury (1930) found "Tests for agglutinins and for growth-inhibiting factors in the saliva . . . resulted negatively in virtually all cases."

Jay, Crowley and Bunting (1932a) prepared a combined sterile filtrate from 40 strains of oral lactobacilli. A fraction was prepared by precipitation with alcohol. A skin reaction was obtained on injection of the filtrate but it was not antigenic. A combined vaccine prepared by heating the same 40 strains of *L. acidophilus* at 57.5°C caused severe local abscesses in two subjects. Injection was discontinued. The two subjects who had the severe reaction no longer reacted to the filtrate and showed *B. acidophilus* agglutination in dilutions of 1:160 and 1:320. A subject who was positive to the filtrate showed faint agglutination at 1:20 and 1:40 dilutions.

Jay, Crowley, Hadley and Bunting (1933) were not able to produce agglutinins in rats by vaccines of mixed types. They found an average titre of mixed *L. acidophilus* agglutinins of 1–211 in 30 caries-free subjects but only 1–21 in 31 with caries. Attempts to use autogenous vaccines in children resulted in abscesses ascribed to the rough colony type acidophilus.

Macphee (1933) reported that with sera of 14 subjects a precipitin reaction was obtained with antigen prepared from carious dentin but little or no reaction with normal dentin.

Harrison (1939) and Williams and Harrison (1942) have prepared rabbit sera with immune factors to various strains of oral lactobacilli as tools for classification of the organisms and not as a means of prevention of development of these bacteria in rabbit mouths.

Canby and Bernier (1942) vaccinated 20 caries-susceptible men with vaccines prepared from strains of *L. acidophilus* isolated from carious dentin. The number of intracutaneous doses varied from 9 to 49 over periods of about 2 months. The average count of lactobacilli in the saliva was reduced in 19 subjects and increased in one. The agglutinin titre of serum was increased in 10, unchanged in 3 and undetermined in 8. (One subject was given 2 series of injections with no change in titre in the first.) There was no report on caries activity by direct observation.

Dietz, Williams and Lawton (1943) in a study of agglutinins for lactobacilli in 15 caries-susceptible and 15 caries-insusceptible men concluded "the highest titres are accompanied by a negative salivary lactobacillus count, irrespective of the caries experience. The low agglutinin titres in the two groups were not consistently accompanied by high salivary counts."

Williams (1944a, b) reported as stated in a condensed abstract: "Twenty-three volunteers were inoculated subcutaneously four times at weekly intervals with equal mixtures of two widely cross agglutinating organisms with both live and heat-killed vaccines. On the basis of this study it was concluded that salivary counts for lactobacilli are not appreciably affected by the increased blood agglutinin titres following subcutaneous injection of oral lactobacillus vaccines."

Discussion

It may be noted that the studies of specific immunity to caries-producing bacteria have been undertaken with two objectives. One of these has been to show that, because immune reactions are observed in caries-free individuals, *L. acidophilus* is the specific organism of dental caries. The other objective sought is immunization against dental caries on the basis that *L. acidophilus* is the cause of dental caries.

Proof of the existence of systemic immunity to *L. acidophilus* would be only indirect evidence that that organism is causal. While evidence exists that other bacteria may be associated with the carious process, studies of their immunity reactions are indicated before conclusions as to specificity of lactobacilli can be drawn. Even if

it were proved that *L. acidophilus* is the specific agent in caries, the evidence indicates that immunization against caries by vaccines would be attended with little or no success.

FERMENTABLE CARBOHYDRATE AND DENTAL CARIES

Kirk (1914) set forth the theory that a carbohydrate in the saliva sustained caries-producing bacteria and that the variation in the amount of this carbohydrate accounted for immunity to caries and fluctuations in intensity of the process. He also suggested that the dentinal fibrillae supplied carbohydrate to the organisms deep in the dentin, on the basis of the idea that cells seek a food supply rather than move from it as would be the case if living on carbohydrate drawn from the exterior of the tooth.

Bunting and Rickert (1914) could find no free reducing sugar in saliva but estimated the combined carbohydrate as averaging between 40 and 60 mg./100 cc.

Updegraff and Lewis (1924) found a "slight reduction" in saliva of 3 of 15 men and none in salivas of 7 women. The saliva was collected 1 to 1½ hours after a light breakfast and activated by chewing paraffin. There were in all 68 salivas tested. In the case of 3 normal individuals administration of 100 to 300 gm. of glucose produced a decided hyperglycemia but no increase in glucose in the saliva. Updegraff (1926) reviewed the literature of sugar in saliva and concluded it occurred rarely, even in the saliva of diabetics.

Morris and Way (1924) reported, from the study of the salivas of cases, including diabetics, in whom blood glucose ranged to as high as 5 times normal: "No matter how high the blood sugar concentration in the cases observed, the amount of reducing substance present in saliva was never more than a trace, nor was that trace more than was present in the saliva of subjects having normal blood sugar."

Van Huysen and Diefenbach (1944) found a mean of 7 mg. of reducing substance per 100 cc. of saliva in 191 samples from 8 individuals.

Walkhoff (1919) summarized extensive studies of the titratable acidity developed from various breads, flours, sugar and starch. In 72 hours, 0.5 gm. of bread incubated with 3 cc. of unsterilized saliva yielded acidity equivalent to from 1.2 to 6.8 cc. 0.1 N alkali; flours, 2.2 to 12.2 cc.; sugar, 0.7 to 0.9 cc.; wheat starch, 0.4 cc.; bran, 5.0 cc. Titration values at 3 and 24 hours were lower but with no essential change in the order of acid production.

Walkhoff (1917, 1919) exposed sound, whole incisors and cuspids from adults to bread and saliva mixtures. The teeth were coated with wax except for small areas. The exposure time was 6 months, with frequent changes of the fermenting mixture. He found cervical caries appeared first, initiated in the root, and enamel caries later. He said the cavities could not be distinguished from natural caries by macro- or microscopic means but he did not describe the course of enamel penetration. He could not find a difference in caries formation in the use of white or dark breads except in the pigmentation of the cavities.

Rypins (1922b) reported the incidence of carious mouths in 1068 preschool children of Kansas City, Missouri, in relation to the use (a) of detergent diets and (b) of sugar. His conclusions were mainly drawn from children 3 to 6 years of age. "Analysis of this period gives some support to the two theories. It presents a decreased incidence of carious mouths among the children on diets that fit the description of Wallace for 'physiological' oral hygiene (21.3 per cent less than on unhygienic diets). There is also a decreased incidence among the children using a minimal amount of sweets (21.0 per cent less than among those using excess of sweets).

"However, the validity of the findings is somewhat decreased by the presence of considerable caries among the children on the advocated diets; 38.2 per cent on 'physio-

logical' oral-hygiene diets showed caries; 32.4 per cent of those on a minimum of sweets; and 35.7 per cent of those on these diets in combination. Application of the theories did not completely protect against the disease." Rypins added in his conclusions, "The every-day findings of wholesale decay of teeth in a people living on our artificial dietaries, or who lavishly indulge in candies, jams and sugars, should not alone, or without carefully controlled comparison series, persuade us prematurely to conclusions on this important question."

Howe (1924a) stated on the basis of work with monkeys: "Since the idea that caries is due to local carbohydrate fermentation is so firmly fixed in the minds of many, we have again tried to produce caries by feeding sugar and white flour in such manner that it would adhere to the teeth for long periods. In addition, a candy made by boiling cane sugar and water was fed. In one case caries was found. In two animals that died after eight and a half months, the teeth were found to be covered with adhesive carbohydrate, so that they were cleaned with difficulty, but no caries was found." Howe (1924b) said of other tests with monkeys: "An excessive amount of sugar was fed to six animals. One of these developed caries is his superior left permanent molar, and two cavities in his lower molars. The other five showed nothing. So far as could be seen, it was only when sugar was fed in such a way as to affect the appetite and to produce a systemic derangement that any dental effect could be found."

Mecredy (1924) examined 4674 school children of New Zealand for dental caries. He listed 303 in a group of schools as being more than 2 miles removed from stores dispensing candy and sweets. They had an average of 5.2 carious teeth each and there were 9 perfect sets of teeth. In the schools near stores 701 children had an average of 6.8 carious teeth each and there were 6 with perfect teeth.

Wilkins (1927) was inclined to believe that greater frequency of eating and higher sugar consumption accounted for the higher rate of caries in New Zealand children than those of Birmingham, England. (See section on Fluorine and Dental Caries for data.) He said, "The greater prosperity, further, leads to New Zealand children having more pocket-money to spend on sweets and confectionery."

Schoenthal and Brodsky (1933) compared the daily expenditure of money for candy by 319 children with caries development over an average period of 13 months and found no relationship "to the number of cavities present at the beginning of the observation" and no influence on the development of new cavities.

Miller (1934) reported rampant decalcification of teeth by two individuals who habitually consumed hard candies or medicinal lozenges by sucking. Miller and Neuwirth (1935) reported six such cases including the two described by Miller (1934). Rosebury (1935) has remarked that such cases "are clearly to be regarded as exceptional, rather than representative of a general phenomenon."

Koehne, Bunting and Morrell (1934) observed 22 hospitalized girls over a period of 2½ years for various aspects of the relation of diet to caries. They found increase of caries activity, described mainly by increase in *L. acidophilus* counts in saliva but supplemented by clinical examination, as related only to increase in added sugar. The sugar was added in the form of candy to an "adequate diet" in amount equivalent to about 22 per cent of the energy value of the diet. Fudge, chocolate, caramels, soft sugar wafers and hard candies were used. "Three children failed to illustrate a relationship between the use of nutritionally adequate diets high in added sugar and the progress of caries activity." On the other hand, with 11 per cent of the caloric intake as added sugar with an adequate diet a low count of lactobacilli was found. But they were "unwilling to say that the use of nutritionally adequate diets in which less than 11 per cent of the calories are derived from added

sugar will *always* protect persons against caries. Five of a total of fourteen children who were fed such diets failed to show a decrease in caries activity." They also believed "that *when persons are susceptible to caries* the regular use of diets rich in artificially sweetened food will exaggerate the tendency in the majority of cases."

Koehne and Bunting (1934b) reported continued observations on 169 boys and girls of an orphanage, presumably at Maumee, Ohio. The low rate of caries and low activity of caries was ascribed to a low intake of artificially sweetened food and to the great regularity of meals and uniformity of the food. No correlation with dental caries was found between height, weight, sex, carbon dioxide-carrying power of saliva, intake of calcium, phosphorus or vitamin D and the order in which fibrous food was eaten. About half the caloric intake was from starchy foods.

Jay, Hadley and Bunting (1936) gave candy to a group of orphanage children (Maumee, Ohio) for comparison of caries activity with a control group. "The candy, which consisted of chocolate creams, fudge and hard candies, was supplied in wholesale lots and was dispensed by the matrons twice a day." Records of consumption were kept by the children. It was found that candy given by this system was transferred to the control group and in the final consideration of the data the controls were ignored. The caries activity of the 51 children who received candy for 5 months was compared with that recorded for the preceding 12 months. They found that 19 subjects with no clinical signs of caries developed active caries during the 5 months on candy. Two others not observed in the foreperiod showed active caries and two had active caries throughout. There were 25 children who had no caries activity either with or without candy. There were three children who had active caries in the foreperiod but developed no new lesions with the candy feeding. *L. acidophilus* counts were generally high during the candy feeding period, either with or without active caries being seen, and the counts dropped on cessation of the feeding of candy. In the control group of 128 children, who received some but much less candy, "there were increased acidophilus counts in only 26 per cent as compared to the 80 per cent in the candy group."

Landsman (1934) found extensive caries in children of St. Thomas, Virgin Islands, and ascribed the condition to the "presence and wholesale consumption of cane-sugar,* which the natives seem to be chewing, gnawing and sucking all the time."

Steggerda and Hill (1936) reported the incidence of caries of deciduous teeth in 1082 Navajos, 707 Mayas and 970 Dutch children (Michigan) ranging from 2 to 14 years. Just over half the Indians had caries; 90 per cent of the Dutch children were affected. The average numbers of teeth affected were: Dutch, 4.8; Navajo, 2.8; Maya, 2.3. Data on permanent teeth were given for 1358 Dutch, 944 Mayas, 1861 Navajos and 599 Jamaica Negroes and mulattoes. The percentage with caries was highest for the Jamaica and the Dutch groups, ranging up to 96.7 per cent affected and with about 25 per cent of the teeth affected. The two Indian groups were comparable with about 60 per cent with caries and 9 per cent of teeth affected. They stated that the diet of the Mayas was high in carbohydrate and that the decay occurred in the anterior teeth but that the Navajos were on a high protein diet and decay was largely confined to pits and fissures. They said, "When a disclosing solution is applied to the teeth of Navajos, plaque formation is always found." "In the Jamaica group, where sugars are extensively used and the chewing of sugar-cane is a common habit . . . there is very much caries of the anteriors."

Oranje, Noriskin and Osborn (1935) investigated the caries status of Bantu work-

*Dr. Landsman has stated to the reviewer that this should read "sugar cane."

ers. They found a higher incidence of caries in subjects who had worked longest in mines or towns, that is, who had been longest in contact with European type dietary. They concluded, "A notable increase in caries is associated with the consumption of sugar." They also concluded there was less caries associated with the consumption of sour milk and more caries in those groups using machine-ground mealie meal compared with whole mealies. The groups studied varied in number from 29 to 435 and percentage with caries ranged from 27 to 55.

Osborn, Noriskin and Staz (1937b) immersed undecayed teeth "in media obtained by chewing or rinsing the mouth with: a) normal saline, b) crude sugar-cane juice, c) a 12 per cent solution of refined white sugar, d) a cooked 15 per cent whole cereal . . . , e) a cooked 15 per cent 'porridge' made from best white flour or mealie." Incubation at 37°C was for 2 to 8 weeks. More teeth were decalcified by refined sugar than by sugar cane juice and it was indicated that whole wheat meal was less effective than white flour, but the data do not show significant difference by the chi square test. The cooked cereals were more active decalcifying agents than refined sugar. The pH attained from the fermenting mixtures was the same regardless of the constituents. The authors postulated a "protective agent" in natural foods that is removed by refining, but gave no indication of its nature.

Osborn, Noriskin and Staz (1937b) found that $CaHPO_4$ had little effect in retarding decalcification of teeth by fermenting sucrose or flour mixtures, but that calcium lactate plus sodium glycerophosphate or calcium glycerophosphate were inhibitive. These reagents were in 1 per cent concentration or more.

Belding and Belding (1938) compared acid formation on various media, notably those with a cereal base, by salivas of 20 caries-free men and of 20 with rampant caries. Practically all salivas reduced the pH of these media to 4.5 in a few hours. On the wheat medium the salivas of caries subjects produced a pH 4.5 earlier than those of the caries-free men. As sucrose-containing media were fermented more slowly than those with cereal base, Belding and Belding considered cereal carbohydrate as contributing more than sucrose in causing caries; streptococci were generally present and "in susceptible cases they were somewhat more prominent."

Read and Knowles (1938) selected 12 caries-free children and 12 with rampant caries after a study of caries in 2,894 children between the ages of 6 and 13 years. The selected children ranged in age from 3 to 14 years. It was found on inquiry as to past and present dietary history that the caries-free group had had good diet with respect to fat and protein but this aspect of the diet of the caries group was generally deficient. Carbohydrate in the latter group was listed frequently as excessive and sweets as "abundant daily." The caries-free groups had sweets "occasionally."

Jay (1940) cited two cases in which salivary *L. acidophilus* counts were reduced by restriction of carbohydrates. One of these needed only restriction of sugar; the second required a reduction of starch also to effect reduction of counts. No statement was made on the condition of the teeth.

Waugh and Waugh (1940) observed caries in 24 Eskimos before and after 18 to 31 feedings variously of preserved figs, candy bars, lollypops, sugar solutions and sugar cubes. All showed *L. acidophilus* after 5½ or 6 weeks. Of 22 reported at the end, 18 had increased caries. On the other hand, raisins, dried figs, maple syrup, honey and dates given in 13 to 56 feedings as "natural" sugars showed no change in caries status in 15 of 20 reported from an original group of 22, and one of the five showing increased caries admitted eating candy. The *L. acidophilus* counts in this group were scattered.

Miller, Muntz and Bradel (1940) found

that plaque material in aqueous suspension produces lactic acid from sucrose and maltose as readily as from glucose, and, to a lesser but still very definite extent, from lactose and starch substrates. The demonstration of acid formation from *starch* by plaques from 11 individuals, 4 being caries-free, is of particular interest in that the plaque as collected probably had a minimum content of saliva and, hence, of ptyalin. This point was not discussd by the authors.

Fancher and Fosdick (1941a) found the lactic acid content of the material from carious lesions was "increased from 50 to 200 per cent within 10 minutes after the ingestion of the sugar."

Fosdick, Campaigne and Fancher (1941) using a glass electrode, determined the pH of the saliva and 43 carious areas in 14 subjects with caries, before and after chewing candy. The pH of the saliva *increased* 10 minutes after ingestion of the sugar and returned to the initial value in 20 minutes. The pH of carious areas decreased from just under 6.0 initially to 5.0 in 20 minutes and in 30 minutes was about 5.6. (These values were plotted.) The pH of saliva from an "immune" subject showed the same trend as that from caries subjects; there was no change of significance in the pH of material from areas on the teeth of the caries-free subject. Samples of carious material were obtained from 59 controls and 105 experimental subjects 10 minutes after ingestion of candy. The lactic acid content was found to be about 0.2 per cent of the carious material of the controls and 0.3 per cent after ingestion of candy. Pyruvic acid was present in amount about equal to lactic acid. The authors postulated the formation of decalcifying acids from free sugar and stated "the ingestion of starches should not have any effect on susceptibility to caries in many cases."

Collins, Jensen and Becks (1942) classified 366 students into three equal groups, (a) caries-free (b) clinically caries-free but roentgenograms showing 4 to 28 caries areas and (c) 5 or more open caries areas. By interview they ascertained the food habits of each individual and classified each as to adequacy of protein, calcium, phosphorus, carotene, thiamine, ascorbic acid and vitamin D on the basis of the 1941 "yardstick" of the Food and Nutrition Board. They found no correlation with caries status. Analysis of the food records on the basis of the probable refined sugar content indicated daily ingestion from all sources of 10.4, 11.6 and 17.9 teaspoonfuls of sugar for the respective groups. There was a definite group association of caries with sugar intake but wide individual variation.

Boyd (1942) wrote: "A few isolated observations have been made to test specifically the effect of large amounts of sugar on the susceptibility of the teeth to decay. Children who have remained in hospital wards for periods of several months have been given diets which have included as much as eight ounces of sugar daily, together with other foods designed to provide an optimal intake of protein, minerals, vitamins and energy equivalent. Throughout the period of observation, no extension of caries or development of new areas of decay has been detectable, even though sucrose and dextrose have supplied almost half the energy content of the diet."

Fosdick (1942) attempted to "control" caries in 50 very susceptible subjects. "The degree of susceptibility was determined both by chemical analysis and by clinical observation. All patients had a full-mouth X-ray examination followed by a complete dental examination and each cavity and filling was charted. All cavities were filled, so that the mouth was free from caries. Each patient was then placed on the following oral hygiene regime: No attempt was made to alter or regulate the normal diet, which in some cases was high in free sugar. Immediately after the ingestion of food, each patient was required to rinse his mouth well with an antiseptic, chew a stick of antiseptic paraffin and brush his teeth with a

good brush and dentifrice. The procedure was so rigorous that only twenty of the patients continued the experiment for as long as six months. At intervals during the test, the susceptibility to caries was determined by means of the chemical test. At the conclusion of the test, another X-ray and oral examination was made." In every case susceptibility as indicated by solution of powdered enamel by glucose-saliva was diminished (though no consideration was given to the fact that cavities had been filled). Caries developed in seven cases, 4 with 1 cavity, 2 with 2 cavities and 1 with 7. In spite of this indifferent success, Fosdick recommended elimination of free sugar from the diet and immediate cleaning of the mouth after ingestion of food as a means of prevention of caries.

Whyte (1943b) observed the dental conditions and *L. acidophilus* counts of fifty boys under close institutional observation during 62-day periods of feeding and withholding of "macaroon bars" and "fudge tablets". These candies had 85.0 per cent and 93 per cent carbohydrates, respectively, with starch (43.0 and 52.2 per cent) sucrose (33.6 and 23.6 per cent) and glucose (8.4 and 17.2 per cent) being the actual constituents. He found the average caries figure increased by the carbohydrates feeding and asserted that this bore a relationship to *initiation* of caries. His counts of aciduric organisms were generally far lower than those of the Michigan group and in accord with King and Croll (1939). Whyte found subjects showing increased caries both with and without candy feeding, though without *L. acidophilus* positive at any time. He concluded there was no relationship between *L. acidophilus* and caries activity, arrest of caries or increased sugar of the diet.

Staz (1944b) reported: "In a group of Indian children the incidence of caries is 88.5 per cent. The highest incidence of caries is found in those children who are living on a relatively soft diet. Children who are frequent sweet-eaters are more susceptible to caries than those who only eat sweets occasionally or infrequently. Some of the children examined chew betel nut and eat other nuts. The habit of chewing betel nut seems to have no inhibitory effect on the incidence of caries, although most of the betel nut chewers are also heavy eaters of sweets."

Becks, Jensen and Millarr (1944b) observed the response of the *L. acidophilus* index and subsequent caries experience in 1250 individuals with rampant caries and in 265 completely free of caries, when refined carbohydrates were largely excluded from the diet. Observations were all for one year or more. They found a reduction in the index in 81.7 per cent of the rampant caries cases when the refined carbohydrate was replaced by meat, eggs, vegetables, milk and milk products. The clinical aspect was that of extensive arrest of caries. They remarked: "The reduction in intake of refined carbohydrates may have good clinical results, but it does not explain the cause of the disease.

"For instance, occasional observations were made to the effect that some individuals consumed large amounts of sugars without developing decay and others with a low sugar consumption developed rampant dental caries. This suggests that in addition to excessive refined carbohydrate ingestion, other factors have a bearing on this disease."

Volker and Pinkerton (1945) found the reducing substances of the salivas of 30 subjects averaged 20 mg. per 200 ml. When glucose was fed in the form of chewing gum, hard candy and "chewy" candy to 12 subjects, the rate of disappearance, as measured by return to normal of the reducing substance of the saliva, was variable but most rapid after gum chewing and least rapid after the "chewy" candy. "The maximum concentration of salivary sugar is reached within 5 minutes and the return to normal is usually complete after 30 minutes. Concentrations as great as 4,000 mg. per cent have been recorded for certain types of candy."

Volker and Pinkerton (1946) found comparable rates of fermentation of glucose and sucrose by saliva. The rate of fermentation of the sugars was greater than that of starch and of the latter greater than "certain of the dextrins."

Volker and Pinkerton (1947a) determined the level of reducing sugar in the saliva of 12 subjects at 0, 5, 15 and 30 minutes after the ingestion of various confections and foods. They found a maximum of 1.9 per cent reducing sugar 5 minutes after a "chewy candy" was consumed. Normal levels were found at 30 minutes.

Volker and Pinkerton (1947b), using 18 to 23 subjects, found reducing sugar of the saliva at a maximum of 3.3 per cent during solution of a 500 mg. wafer of glucose, compared with 1.4 per cent after glucose as a soft mass, 0.8 per cent after a solution and 0.7 per cent after chewing gum. Earliest return to normal was after use of the soft glucose mass.

Volker and Pinkerton (1947c) incubated pooled saliva of caries-susceptible subjects with varying amounts of glucose, sucrose, starch, fructose and dextrin. The rates of acid production from these carbohydrates were about the same except for delayed action on dextrin. No acid was produced from xylose and arabinose. There was no difference in rate of formation of acid from honey, raw cane juice, maple sugar or farina. Little acid was formed by the supernatant liquid of centrifuged saliva from glucose or sucrose, but the sediment rapidly formed acid.

Haggard and Greenberg (1949) found that after the ingestion of "a mixed meal, fresh orange juice, grapefruit juice, ice cream, crackers, chewing gum, caramel candy, and sweetened bottled soft drinks" the reducible sugar of the saliva rose to 550 –800 mg. per 100 ml. of saliva in 5 minutes. The concentration fell to normal more rapidly after the use of sweetened drinks than of solid materials.

Kitchin and Permar (1948) reported the progress of 422 of 559 individuals found to be caries active, that is with oral lactobacilli counts of 10,000 or higher, on diets with drastic carbohydrate restriction. Of the caries-active, 216 completed the low carbohydrate regimen, with 138 resuming moderate consumption of a variety of fruits and vegetables and the equivalent of one teaspoonful of sugar. Of the latter group 95 retained a low count. Fewer had gone on to increased use of higher sugar diets but with evidence of continued low lactobacilli counts.

Pearlman, Sprague and Best (1948, 1949) followed the change in lactobacillus counts in 25 mental patients undergoing therapeutic insulin coma. For control of coma these subjects received 3 to 6.5 pounds of sugar, mainly sucrose, per week. In 6 individuals with initially less than 10,000 lactobacilli per milliter of saliva, the counts remained low. There was an increase to a maximum in 5 weeks and then a decrease in 19 subjects, though many were not observed for the full 8 weeks recorded. The percentage whose counts showed an increase was not statistically significant. Net increase in DMF surfaces in the low count group was 10.3 mean for 3 persons and in the high count group 11.25 for 8 persons.

Schour and Massler (1947) reported the DMF rates of 3,905 subjects ranging in age from 6 to 60 years from four Italian cities. They found "the prevalence of caries is approximately two to seven times lower than that observed in the United States." They indicated their belief that the reduced rate was associated with a lower per capita consumption of sugar, the 1930–34 rate being 18 pounds compared with 103 pounds in the United States.

Bransby and Knowles (1949) reported examinations of the teeth of children of Guernsey and Jersey Islands in 1945 after the German occupation and again in 1947 after a partial return to pre-war dietaries. In the intervening 2 years caries rates increased in both islands. "A considerable

increase in the consumption of sugar, jam and confectionery", as well as increments in the protective foods, was noted.

Toverud (1949a, b) reported the changes in caries rates among 8000–9000 school children and 600–700 2½- to 7-year-old children of Norway in relation to dietary changes necessitated by war. There was "noted a reduction of 35 to 60 per cent in DMF teeth and of 60 to 80 per cent in DMF surfaces in Norwegian children 2½ to 14 years". In the younger group there was an increase in caries frequency in 1946–47 with return to pre-war dietary. "The most striking difference in food consumption and food habits as a whole in age groups up to 14 to 15 years during the war compared with that before the war is as follows: omission of fine and white flour; high reduction in sugar and sweets; increased consumption of fish and salted herring; increased consumption of potatoes and vegetables; reduced intake of fruits; reduced intake of meat; reduced between-meal eating" (Toverud 1949b). Toverud (1949a) after reviewing the effects of rationing of sugar wrote, "*It is safe to state that the most pronounced change in food and habit of eating during the war compared with the prewar period is to be found in the group of refined carbohydrates.* The same is true when we compare the postwar with the war period. The effect of this change on the teeth seems a double one, influencing the resistance as well as the factors acting directly."

Discussion

It is difficult to draw conclusions from the papers which give facts concerning the relation of carbohydrates to dental caries. Tests of caries activity have been substituted for direct observation of caries, and addition of sugar to a diet in any quantity involves reduction of some other constituent.

On the whole it is probable that the evidence sustains that very old belief that sugar has a direct relationship to the extent of caries. However, the relationship is probably one of acceleration of the carious process of sustaining the activities of acidogenic organisms. There is no evidence that sugar is involved in the *initiation* of caries. This interpretation is in accord with the observations on rat caries, namely, that fermentable sugars will not initiate caries in rats but will accelerate development of cavities initiated by coarse cereals.

The only satisfactory explanation of the formation of cavities in teeth is by acid solution of the inorganic components of enamel and dentin, the acid being formed by bacteria from carbohydrates, and proteolysis of the organic constituents. However, as Miller first showed, (and it has been confirmed by others) acid is formed in greater amount by oral bacteria acting on bread than on glucose or sucrose. Thus it would seem that cooked starch should be included as a carbohydrate ultimately fermentable in the mouth and conducive to the promotion of dental caries.

MISCELLANEOUS

Effect of Various Chemicals

Fosdick, Fancher and Calandra (1942) found in *in vitro* experiments that 1 mg. of synthetic vitamin K (2-methyl-1,4-naphthoquinone) per 100 cc. of saliva containing 10 per cent glucose, prevented formation of lactic acid. They found that it acted by preventing formation of phosphoglyceric acid from hexose phosphates. They suggested incorporation of synthetic vitamin K in sugar, candy and gum to inhibit dental caries and noted that "vitamin K is probably one of the substances removed from the sugar cane juice during the purification of sugar." Calandra and Fosdick (1943) stated that "Vitamin K inhibits the formation of acids from carbohydrates in the mouth."

Armstrong and Knutson (1943a, b) in a study of relative efficiency of 13 quinones in preventing acid formation in saliva-glucose mixtures, found 7 of them effective

and 1,4-naphthoquinone more active than the 2-methyl derivative used by Fosdick, Fancher and Calandra (1942). They found the quinones were bactericidal and this finding was further confirmed by Armstrong, Spink and Kahnke (1943). Armstrong and Knutson (1943a) pointed out that the bacteria-inhibiting property of the quinones was independent of vitamin K activity.

Hodge (1944) found that synthetic vitamain K fed to rats in dosages up to those with toxic effects had no effect in retarding caries induced by coarse cereal particles and promoted by sucrose.

Calandra, Fancher and Fosdick (1944) tested a series of quinones and compounds that "may affect the oxidation reduction systems involved in the formation of acids by fermentation". The quinones in general reduced solution of powdered human enamel suspended in the incubated saliva. Urea peroxide and sodium carbonate peroxide were effective.

Burrill, Calandra, Tilden and Fosdick (1945) reported an average of 0.42 new cavities in a year in 45 dental students who chewed gum containing 0.75 mg. of 2-methyl-1,4-naphthoquinone-sodium bisulphite compound, compared with 0.80 new cavities in 55 subjects who chewed a control gum. The incidences of new cavities in these subjects in 18 months were 0.64 and 1.10. "The use of the vitamin K gum produced, in most cases, a rapid drop in the *Lactobacillus acidophilus* count, and a concurrent though less pronounced drop in the enamel-dissolving power of the saliva. This response occurred, usually, within a week after the gum was first used. After the initial drop there was a gradual and inconstant rise in both bacterial count and the dissolving action. As time went on, the figures for the tests approached but seldom either reached or exceeded the values in the pre-gum tests."

Fosdick and Calandra (1947a, b) examined 31 compounds, including peroxides, quinones, guanidines and aldehydes, for inhibition of enzyme action as represented by lowered solution of calcium from powdered enamel suspended in saliva. They found the aldehydes, and particularly *dl*-glyceric aldehyde, showed "promise of being useful in the control of dental caries, as they are relatively non-toxic, are not objectionable to taste, and could be readily incorporated into sugar and sugar preparations."

Dreizen, Mann, Spies, Carson and Cline (1947) demonstrated that 7.5 mg. of sodium bisulfite in 10 ml. of saliva, from 11 subjects whose saliva showed fermentation related to caries activity, would inhibit acid formation. Similar action on saliva was obtained by chewing paraffin containing 7.5 mg. of the bisulfite.

Dreizen, Greene and Spies (1949a) tested the *in vivo* effects of the addition of 5-nitro-2-furaldehyde-2-hydroxyethyl semicarbazone and the analogous methyl derivative on acid production in the salivas of caries-active subjects. Saliva was collected after a control period of chewing paraffin and an experimental period in which 0.75 or 1.0 mg. of the compounds were incorporated in paraffin. Acid production was completely prevented in 38 of 42 tests and reduced in the other four.

Dreizen, Greene and Spies (1949b) tested 10 nitrofuran derivatives for inhibition of acid formation in saliva. "These compounds, when added separately in 500-microgram quantities", to 10 cc. of saliva "partially or completely prevented acid production, *in vitro*, in 174 of 176 caries-active aliquots tested."

Penicillin

Zander and Bibby (1947a, b) showed that a solution of 1000 units of penicillin in 20 ml. of water, when used as a mouth rinse, resulted for at least 2 hours in salivas which showed no drop in pH on incubation with glucose.

Hill and Kniesner (1947) determined the

effect on counts of *L. acidophilus* of the use twice daily of a dentifrice containing 500 units of penicillin per gram. In 5 months 21 per cent of 154 boys showed decrease to negative counts compared to 9 per cent of 86 in a control group. There was an increase in susceptibility in 20 per cent of the controls and in 4 per cent of the penicillin group.

Hill and Kniesner (1948) at the end of one year found 2.96 new carious surfaces in 108 boys who had used a tooth powder containing 1000 units of penicillin per gram; in a comparably aged group of 68 the increase was 2.90 new carious surfaces. Of those with high *L. acidophilus* counts, 42 per cent of the experimental and 28 per cent of the control group showed reduction of counts to 100 to 500 lactobacilli per ml.

White, Kniesner and Hill (1949) using the same group of 240 boys as Hill and Kniesner (1949) and a group of dental students, followed changes of oral flora with the use of a dental powder containing 1000 units of penicillin per gram. The number of lactobacilli was decreased and there was some sporadic increase in gram-negative bacilli of the Aerobacter and Escherichia types. "While the occurrence of gram-negative organisms was associated only with low or negative *L. acidophilus* counts, it appears indicative merely of a change in the balance of the normal flora."

Hill and Kniesner (1949) provided 240 institutionalized boys aged 8 to 15 with a control tooth powder and the same with 1000 units of penicillin per gram. They found, after one year, a shift of lactobacillus counts in the saliva to the lower classifications in both the control and experimental groups. Average increases in carious surfaces were 2.81 in the control and 2.97 in the experimental groups, respectively. They judged from the amount of tooth powder used "that most of these boys did not follow the instructions given them relative to brushing their teeth twice a day." In 13 dental students, selected for high caries susceptibility, only one carious lesion developed in 9 months with use of the penicillin tooth powder.

Zander (1950) tested the effect of a penicillin-containing dentifrice in reducing the incidence of new carious lesions in 6- to 14-year-old children. The penicillin group of 235 children were supplied with a tooth powder containing 500 units of potassium penicillin per gram, and 174 children in the control group, an identical powder without penicillin. Tooth-brushing was supervised once daily during the school term and the children were instructed to brush their teeth every morning and evening at home. At the end of a year significant reduction in decay was produced by the penicillin in both primary and permanent teeth measured in terms of (1) teeth (2) surfaces on previously undecayed teeth (3) surfaces on previously decayed teeth and (4) all surfaces. In the latter class the numbers were: 290 on penicillin, 486 in the control group in all teeth; 228 and 405 for permanent teeth only. At the end of a second year of study the values were 315 and 507 for all teeth including primary and permanent.

Removal of Mouth Organisms

Feirer and Leonard (1927) conducted experiments in the reduction of counts of oral flora that "indicated strongly that the various mechanical factors ordinarily involved in brushing the teeth, namely, the rinsing with water, the friction of the toothbrush bristles, the scouring action of the abrasive and the cleansing action of soap, even when combined, are entirely incapable of satisfactorily reducing the massive infection continuously present about the teeth and gums." Bactericidal agents in tooth pastes, however, reduced counts in mouth washings by about 70 per cent. In 129 counts of bacteria in 25 cc. of water used to rinse the mouths of 11 individuals, they found averages ranging from 434,000 to

43,600,000 organisms per cc., with an average for all of 14,000,000.

Feirer and Leonard (1931) found staphylococci to be the predominating microorganisms in cultures from the mouth. In 40 successive rinses of the mouth of one subject with 25 cc. portions of water they found initially 2,788,000 organisms, 1,255,000 in the twentieth and 1,040,000 in the fortieth. In individuals there was an increase of 300 per cent in the counts during a 7-hour fast. They designated this a "diurnal tide."

Crowley and Rickert (1935) broke up clumps of bacteria in mouth washings by a 30-minute spraying operation to effect a direct counting method for oral organisms reproducible within 13 per cent. They found that "after a meal the number of bacteria removed from the mouth decreased as much as 78 per cent." "Counts taken at different times of the day and on different days varied greatly in the same individual" and "No correlation was noted between counts made by the method described and the 'diurnal tide' of Feirer and Leonard" (1931).

Fosdick (1950) determined the number of tooth surfaces that became carious during a 2-year period among 523 subjects who brushed their teeth "within 10 minutes after each ingestion of food or sweets" and 423 control subjects who followed no regular pattern of tooth brushing. Significantly fewer surfaces became carious in the 523 experimental subjects.

Bacteria under Fillings

Besic (1943) by sealing cavities in teeth *in situ* with traces of carious dentin showed that *L. acidophilus* died out in 2 to 10 months, staphylococci remained viable in one case for 1 year and streptococci, the most prevalent form in deep carious dentin, could be cultured in ⅓ of the cases after 1 year. There was no evidence that caries had progressed in the sealed cavities.

Nutritional Requirements of Oral Bacteria

Hill and Kniesner (1941b) estimated the pantothenic acid content of 15 samples of saliva to range from 0.008 to 0.17 micrograms per cc. of saliva. They also showed that "pantothenic acid is a nutritional requirement for all common types of oral lactobacilli."

Hill and Kniesner (1942) determined the glucose and pantothenic acid requirements of Types I, II and III strains of oral lactobacilli. They found that 0.15 per cent glucose was adequate and a concentration of about 3 micrograms per 100 cc. of pantothenic acid was optimum.

Fancher, Calandra and Fosdick (1944) investigated the effects of the addition of vitamins to saliva on acid formation. The production of acid was measured by the increase in calcium content of the saliva while in contact with powdered human enamel. They concluded that cholesterol, a mixture of vitamins known as "Cerophyl", and possibly thiamine, stimulated acid formation. "Synthetic vitamin K (2-methyl-1,4-naphthoquinone) and natural vitamin K, (2-methyl-3-phythl-1,4-naphthoquinone) inhibited acid formation. There was no indication that this action has any relationship to its vitamin K activity."

Fosdick and Rapp (1944b) tested the effects of the addition of proteolytic enzymes to saliva on the solution of human dental enamel during fermentation. They had anticipated an inhibition of action on the enamel because of destruction of carbohydrate-degrading enzymes. On the contrary they found stimulation by trypsin and papain but no effect by pancreatin.

Kniesner, Mann and Spies (1942) examined human subjects with evidence of pellagra, beriberi and riboflavin deficiency. "The salivary pantothenic acid values of 29 patients with lactobacillus counts below 13,000 per cc. averaged 0.0880 micrograms per cc. of saliva, while 22 patients with counts above 30,000 averaged 0.0885 micrograms per cc. of saliva." These subjects had a low incidence of dental caries.

Dreizen, Mann, Cline and Spies (1946) examined the salivas of 16 nutritionally

deficient subjects relatively free of caries, 20 well nourished subjects with moderate caries activity and 14 well nourished individuals with rampant caries. They determined buffering value by titration to pH 4.0 with lactic acid. "Malnourished patients, relatively free from dental caries, had the highest buffer capacity in the saliva, the lowest number of *Lactobacilli acidophilus*, and the least active Fosdick determinations. In contrast, the well nourished patients with either moderate or rampant caries showed a progressive decrease in the buffer capacity of the saliva and corresponding progressive increases in the lactobacillus counts and the Fosdick determinations."

Mann, Dreizen, Spies and Hunt (1947) in a study of malnourished persons found an incidence of only 30.5 per cent of the amount of dental caries found in a control, well nourished group. They found in pellagra, anemia, initial nervous syndrome, and deficiencies of riboflavin, thiamine, and vitamins A and C, no cases with more than 2 plus reaction in the Fosdick, Hansen and Epple (1937) classification of caries activity. The maximum count of salivary lactobacilli was not over 20,000, with the majority less than 6,000.

Weisberger and Johnson (1946) found that "a medium consisting only of glucose, tryptophane, sodium acetate, potassium phosphates, thiamin hydrochloride, calcium pantothenate, nicotinic acid and vitamin-free casein hydrolysate is capable of providing for maximum acid production" with an oral strain of lactobacillus isolated from the mouths of 23 clinic patients.

Weisberger (1946a) substituted whole saliva and whole saliva concentrated to ¼ volume for the substances found by Weisberger and Johnson (1946) for acid formation by oral lactobacilli. The saliva was found to provide adequately for potassium phosphates but was incapable of supplying the amino acids represented by hydrolyzed casein and tryptophane even at 7-fold concentration. There was some replacement for nicotinic acid, pantothenic acid and thiamine hydrochloride.

Weisberger (1946b) showed that acid-hydrolyzed saliva could supply amino acids except tryptophane; alkali-hydrolyzed saliva supplied amino acids including tryptophane for acid production by oral lactobacilli. These findings indicate that the proteins of saliva are unavailable to lactobacilli.

Weisberger (1946c, 1947) found that by incubation of saliva with glucose sufficient hydrolysis of saliva protein occurred to provide tryptophane for acid production by lactobacilli.

Stephan (1949) found: "Manganese stimulated or was essential for the growth of 28 of 30 strains of lactobacilli, but was inhibitory to staphylococci and streptococci. Iron and zinc showed both stimulatory and inhibitory effects, whereas cobalt, copper, nickel, molybdenum, and fluorine showed only inhibitory effects for all organisms tested."

GENERAL CONCLUSIONS

1. There are several distinct species of microorganisms frequently found in human mouths and in carious teeth that *in vitro* can form acids from glucose, fructose, sucrose, lactose and maltose in sufficient concentration to decalcify dental enamel. Cooked starch probably is as available for fermentation as the sugars, but not as quickly converted to acid and especially so in individuals with low amylase activity.

2. Powdered enamel and enamel from which the outer surface has been removed are subject to solution by acids formed by bacteria, but intact enamel surfaces *in vitro* exhibit widely variable resistance to attack by acids produced by acidogenic bacteria.

3. There is no evidence that acidogenic bacteria and carbohydrates can initiate dental caries.

4. The progress of dental caries, once initiated, is effected by bacteria acting during the acidogenic phase on a carbohy-

drate substrate. Destruction of the organic matter of teeth is probably effected by proteolytic action by bacteria.

5. There is probably no specific microorganism associated with dental caries, but rather demineralization and proteolysis of tooth substance are effected in proportion to the total acidogenic and proteolytic powers of the organisms currently in equilibrium with oral environment.

6. The *aciduric* characteristic of a microorganism is secondary in importance to the *acidogenic rate* in the carious process.

7. The effective flora of dental caries may vary with the state of cavitation, such as being characterized by *L. acidophilus* in shallow cavities and by streptococci in the deep layers of carious dentin.

8. Lactic acid has been identified in carious lesions and phosphoglyceric, pyruvic and malic acid may be present.

9. The count of *L. acidophilus* in saliva by precise cultural methods, or by direct counting in plaques, may serve as a crude index of caries activity. Estimation of potential acid formation by the whole flora of saliva may also give some indication of caries activity. It may be that increased counts of *L. acidophilus* indicate places of lodgment of food rather than activity of caries.

10. Acid formation in plaques sufficient to decalcify enamel may develop within a period of several minutes after fermentable carbohydrates are taken into the mouth.

11. The caries-promoting potential of a plaque is determined by its flora.

12. There is no relation between the pH of total saliva and dental caries.

13. There is probably an inverse relationship between rate of cavitation and volume of secretion of saliva.

14. Saliva contains mild antibacterial agents that may affect rate of caries development.

15. The blood and saliva of various individuals differ in their immune reactions to *L. acidophilus*. There is insufficient evidence to justify the use of vaccine to control dental caries.

FLUORINE AND DENTAL CARIES

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CONTENTS

CONTENTS (*continued*)

FLUORINE AND DENTAL CARIES

The facts regarding fluorine in its relation to dental caries are so outstanding that a re-examination of all theory pertaining to the etiology of caries is desirable. Generally, attempts have been made to fit the facts to theories that have not been satisfactory even before the realization of the influence of fluorine on dental caries.

It is in this field in particular that discrete data are needed, not only as to which teeth decay but also as to what parts of each tooth become carious. These data are available in only a fragmentary way and consequently what may be entirely erroneous practices are being developed. For example, it is clearly indicated that the upper anterior teeth are very little affected by caries in fluoride districts, but no information is available on the extent of caries in the lingual, buccal and proximal surfaces of the molars.

Similarly, data on rate of decay in teeth in relation to fluorine in the enamel, dentin, saliva, water and foods are almost wholly lacking. It is indicated that pit and fissure caries is common in regions with high fluoride waters but that the cavities are small. Whether caries of this type is altogether identical with that in teeth in low fluoride districts as to flora or as to cavity pattern has had no illumination.

In this review, discussion of the methods used for the determination of fluorine has generally been omitted. The rapid advances in the knowledge of fluorine and living processes in the last 10 years have been made possible in no inconsiderable degree by the improvements in chemical analysis. Further development is urgently needed, as the methods still remain too detailed and too individualistic.

The most important contribution to the analysis for fluorine was the Willard and Winter (1933) method of isolation of fluorine by distillation as hydrofluosilicic acid. Little reliance can be placed on any quantitative analyses for fluorine reported prior to this date. Armstrong (1936) has further improved the distillation technic by removing the chloride ion and reducing the amount of perchloric acid that distills. McClure (1939a) has also provided a method of preventing distillation of chloride by means of silver sulfate. Churchill, Bridges and Rowley (1937) have distilled from sulfuric acid and then from perchloric acid to prevent interference from phosphates. Armstrong (1933a) improved the technic of titration of fluorine, and others have contributed to this aspect of the analysis until amounts of fluorine of the order of 5 micrograms can now be determined.

Thompson and Taylor (1933) devised a direct colorimetric method for fluorine in sea water and Sanchis (1934) adapted it successfully to the estimation of fluorine in most fresh waters.

Throughout this review constant attention has been given to the toxicology of fluorine. In lieu of a review of the extensive literature, citation is made below to reviews on the subject.

In the following list of review articles, the author, date, nature of the review and the number of references cited, are given as a rough estimate of the coverage of the literature. If titles are given in the bibliographies this is indicated by the letter "t." In reviews covering subjects not pertaining to fluorine the number of references to fluorine is given and this restriction indicated by an asterisk (*).

Adolph (1915), analytical methods, 89; Wofford (1923), mottled enamel, 14t; McKay (1926), mottled enamel, McKay (1930), mottled enamel, 47t; Velu (1932), mottled enamel; original, 50t; DeEds (1933), general, 128; McClure (1933), general, 127; Bazille (1936), toxicology; analytical methods; Hart and Elvehjem (1936), mineral metabolism, 19*; Dean (1936), mottled enamel, 42t; Roholm (1937a), toxicology, 118; Bouley (1937), toxicology, 115t;

Roholm (1937b), general; original, 893t; Dean (1938a), mottled enamel and caries, 13; Dean (1938b), mottled enamel, 80t; Dean (1938c), mottled enamel, 83t; Shortt *et al.* (1937), addendum of 63 references to Roholm (1937b); Schulz (1938), toxicology; original, 97t; Peirce (1939), fluorine and domestic animals, 122; Cox (1939), dental caries, 12; Dean (1939), mottled enamel, 12t; McClure (1939c), fluorides in food and water; original, 89t; Gettler and Ellerbrook (1939), toxicology, 77; Roholm (1939), toxicology, about 500t; Shohl (1939), mineral metabolism, 24t*; Dean (1940), mottled enamel and caries, 39; Cox (1940), dental caries, 30t; Hodge (1940), dental caries, 36; Greenwood (1940), toxicology, 380; Hawkins and Gordon (1940), mottled enamel, 65; Bibby (1941), dental caries; bacteria, 18*; Irving (1941), dental caries, 28; Volker and Bibby (1941), dental caries, 64; de Senarclens (1941), toxicology, about 150; Armstrong (1942), biochemistry of teeth, 28t*; Shils and McCollum (1942), trace elements, 43t*; Calvery (1942), trace elements, 10t*; Hodge (1942), dental caries, 10t; Mitchell (1942), fluorine in animal feeds, 29t; Knutson and Armstrong (1943a), dental caries, 40t; Ast (1943a), general and dental caries, 91t; Irving (1943), dental caries, 27.

"Fluorine and Dental Health," Publication No. 19 of the American Association for the Advancement of Science, edited by F. R. Moulton, presents a symposium on fluorine in relation to both mottled enamel and dental caries. Some original data are given.

In the review "Experimental Dental Caries in Rats" the following conclusions are stated:

"Fluorine, present in the diet in submottling levels, during the formation of the crowns, reduces susceptibility to decay.

"Fluorides, present in the caries-initiating ration in levels that interfere with bacterial action, retard the development of caries.

"Fluorine enters enamel posteruptively and enamel, so fluorosed, has increased resistance to caries."

The terms "fluorine," "fluoride," "fluoride ion" and "F" have been used to indicate the element "fluorine," but in no case is elementary, or free, fluorine meant or implied. This is an agreement with usage for other elements. For example, when calcium and phosphorus are discussed in their biological relations, the elementary substances are never implied. In most cases the terms, especially "fluorine" and "fluoride," have been used interchangeably and synonymously, but with preference for "fluorine."

THE DISCOVERY OF FLUORINE IN VARIOUS BIOLOGICAL TISSUES AND FLUIDS

Teeth and Bones

Morozzo (1802) described a fossilized elephant discovered near Rome. He later gave more details (1803) and submitted a tooth to the chemist Domenico Morichini. The latter found that both the enamel and the dentin of the tooth evolved "fluoric" acid on treatment with concentrated sulfuric acid. Identification was by etching glass and by precipitation of calcium fluoride. Morichini (1805) found fluorine in human enamel in a continuation of his study of the fossilized elephant tooth. He estimated phosphoric and "fluoric" acid to be 22 per cent of human enamel but could not estimate the relative proportions.

Klaproth (1804a, b) obtained samples of the fossil elephant molar described by Morozzo and analyzed by Morichini and confirmed the latter's finding of fluorine. He considered the fluorine to have arisen from phosphoric acid, as on current knowledge it did not exist in fresh bones.

Chenevix (1805) noted Klaproth's confirmation of Morichini's work but could not accept his idea that because "fluoric" acid did not exist in fresh bones it was a modification of phosphoric acid.

Gay-Lussac (1805), in a letter to Berthollet, told of the work of Morichini. Gay-Lussac himself found fluorine in ivory and in the tusk of a wild boar. He related how he had, with Morichini, confirmed the presence of "fluoric" acid in fossil ivory. Morichini had separated human enamel and found that it contained fluorine but ascribed the lessened etching power to interference by organic material. Gay-Lussac made a single experiment with the bones of a fish but withheld judgment as to the fluorine content.

Gay-Lussac was interested in the origin of fluorine in the bones. He was not inclined to believe that the "fluoric" acid was a form of phosphoric acid. (No reference was made to Klaproth.) A free translation of most of the above communication of Gay-Lussac appeared "as a supplement" to Klaproth's paper in Neues Allgemeines Journal der Chemie (Anonymous, 1805).

Fourcroy and Vauquelin (1806) failed to find fluorine in enamel, in recent ivory and in some fossil ivories. In two samples of fossil ivory they found fluorine but said that as calcium fluoride it could not exceed 3 or 4 per cent, in contrast to 22 per cent claimed. Several other chemists of Paris, they stated, had had results similar to theirs. (Their failure to find fluorine is probably because of the following faulty technic: They treated their samples in glass vessels, led the gases through glass tubes into milk of lime. Probably little or no hydrofluoric acid escaped conversion to fluosilicic acid (H_2SiF_6) and to soluble calcium fluosilicate. The true nature of hydrofluoric acid was not known at this time, and Fourcroy and Vauquelin discussed the possibility of phosphoric acid being changed to an acid with the properties of "fluoric" acid.)

Chevreuil (1806) detected fluorine in the bones and teeth of fossils. His procedure resembled that of Fourcroy and Vauquelin except that his delivery tube entered water instead of milk of lime. He observed decomposition of fluosilicic acid to give silicic acid.

Brande (1806) separated the enamel of human teeth, heated it to red heat, pulverized it and treated it with sulfuric acid. The whole was contained in a platinum crucible with a piece of glass rod "placed horizontally in such manner as to be about an inch and a half above the enamel." A glass plate covered the whole. Distillation was continued for a half hour during which time "white suffocating fumes were extricated." He found no etching of the glass. The evidence of leakage of fumes and the large area of glass exposed to the fluorine of 100 grains of enamel probably account for Brande's failure to find fluorine in human enamel.

Proust (1806) reported finding fluorine in the bones of an elephant found in excavations for a bridge over the Manzanares in Spain, in a molar tooth from Peru and in bone fragments and teeth of a horse and of an ass from Terruel in Aragon. He speculated on the association of fluorine and phosphorus in minerals.

Delamétherie (1806), in an editorial note on the report of Proust (above), pointed out that Morichini's discovery of fluorine in fossil bones had been confirmed by Fourcroy and Vauquelin, Klaproth and Chevreuil, and that Klaproth was concerned with the origin of fluorine in fossils, as it was not present in large amount in the bones of living animals.

Berzelius (1806a) confirmed Morichini by finding "fluorspar acid" in teeth, though at the highest not more than "0.03." He also found it in fresh bones, though in very small amount.

Berzelius (1807a) gave the details of his analyses of bones and teeth. His procedure for fluorine was to distill with water and sulfuric acid from a glass flask, filter off silicic acid and precipitate and weigh

calcium fluoride. He found in per cent as calcium fluoride the following:

Human bone ash	3.00
Fresh human bone	2.00
Human "bone substance of tooth," ash	3.00
Fresh human "bone substance of tooth"	2.10
Human enamel	3.20
Ox bone ash	4.25
Fresh ox bone	2.90
"Bone substance of tooth," ox	5.69
Ox enamel	4.00

Berzelius (1807b) wrote to Vauquelin that he had noticed the work of Morichini and that he had found fluorine in bones and enamel of man and ox. His estimates were as calcium fluoride: dry human bones, 2 per cent; human enamel, 3.2 per cent; ox bones, 9.3 per cent; ox enamel, 4 per cent.

In "Scientific News" (Anonymous, 1806) Nicholson's Journal discussed briefly Morichini's work as reported by Gay-Lussac but favored the findings of Fourcroy and Vauquelin that "fluoric" acid did not exist in teeth and bones. Nicholson's Journal later (Anonymous, 1807) discussed a letter of Gehlen concerning Berzelius' work on fluorine in bones and teeth. There is no mention here of the failure of Fourcroy and Vauquelin to find fluorine in such material, but Brande's negative findings are given by reference.

Thus by 1807 it was well established that fluorine existed in fossil bones and teeth and there was good evidence that it could be found in fresh bones and teeth.

John (1812), interested in Klaproth's suggestion of transformation of phosphoric acid to fluoric acid, sought more evidence of the existence of fluorine in fossils. He found fluorine in the enamel of a fossil elephant tooth from Siberia but none in other parts of the tooth. He found magnesium present and believed that he was the first to find it in teeth.

An abstract of a paper by Rees (1839a) reported that he "had endeavoured to obtain proofs of the existence of fluoride of calcium while analyzing human bone; but in no instance could he detect the action of fluoric acid upon glass. Having failed to detect it in bone, he next applied himself to ascertain if fluoric acid existed in the enamel of teeth, in recent ivory and in the precipitate obtained from urine by means of lime water, but in neither case did he succeed in detecting traces of fluoric acid. The author, however, states that he had detected it in fossil teeth." (The preceding is the entire abstract.)

Rees (1839b) could not find any trace of fluorine in recent human bones or enamel, or in recent ivory. However, he readily detected it in fossil ivory by the procedures that failed on fresh bones and teeth. He believed that previous experimenters had observed the etching of glass by phosphoric acid. He concluded that fluorine of fossil bones had been introduced in the process of mineralization and "that fluoride of calcium should be expunged from the list of the constituents of animal substances."

Erdmann (1840), stimulated by Rees, examined the ash of fresh human long bones and of human teeth. He pointed out that the hydrofluoric acid is given off at once on treatment of bone ash with sulfuric acid and, if a cover glass is not promptly clapped on, the etching reaction will not be obtained. He found fluorine in both bones and teeth and said the reaction from teeth was not stronger than that from bones.

Marchand (1842) found human bones to contain fluorine equivalent to 1 per cent as calcium fluoride. In fossil bones of a bear and of a deer he found about 2 per cent calcium fluoride.

Von Bibra (1844) reviewed the work of Rees. Von Bibra found fluorine in all bones examined, the higher content being in fossil bones. He identified fluorine, not only by etching of flasks and glass plates, but also by the odor of fluosilicic acid. He excluded etching by sulfuric acid and phosphoric acid by comparison of the type

and location of markings and concluded that without doubt fluorine was present in bones. He tested the bones of man, herbivora, frog, fresh and seawater fish and the shell of a turtle.

Daubeny (1844) found fluorine in the ash of various fossil bones, in the vertebra of a recently killed ox, in the tibia from "a human subject from an anatomical cabinet," in teeth of an ox and in human teeth of recent date. The etching of glass was varied, being deepest with the fossil bones. Since he found carbonates and gelatin interfered with the etch test, he decided that these accounted for the failure of others to detect fluorine in recent bones. He believed such traces as were present were lost in the ashing process.

Middleton (1844) found fluorine in human bones from the dissecting room. Included were the occiput, vertebrae, humerus, femur, teeth and the "femur of a foetus of 6½ months." But he could find none in bone from a fetus 3½ months old.

Smith (1845) said he found fluorine present in recent bones, with decided evidence in some cases and in others no trace. But he attributed failure to find fluorine "more to the minuteness of the quantity than to the total absence of it."

Wilson (1846a, b) reviewed the technics that had been applied to the detection of fluorine in bones and, in view of the simplicity of the etching tests, was inclined to believe that variability of fluorine content accounted for the failure of some to find it in recent bones. Wilson found fluorine in "recent human bones, male, female and foetal, which were obtained, without special selection, from the dissecting room."

Wilson pointed out that ground waters could remove fluorine from bones as well as add it to them in the process of fossilization, as indicated by his findings on the solubility of calcium fluoride. But, "I am inclined to think that there is a double phosphate of lime and fluoride of calcium, much less soluble than the latter salt is; and that production of this compound fixes the fluoride and prevents its abstraction by water." He could find nothing to support the recently revived idea of transmutation of phosphate to fluoride.

Heintz (1849) found 3.88 per cent calcium fluoride in the ash of ox bones; 4.05 per cent in the ash of sheep bones; and 3.5 per cent and 3.24 per cent in human bone ash. His values are by difference, though he established fluorine as qualitatively present.

Fremy (1855) considered that he had settled the question of the presence of fluorine in fresh bones when he obtained SiF_4 from all bone powders and from ash obtained by incineration or by extraction with dilute alkali. His quantitative data included calcium fluoride with siliceous and other constituents. Since 1855 no effective dissent has been voiced against the occurrence of fluorine in bones, though as late as in 1943 Linsman and McMurray used a method for fluorine that failed to disclose its presence in normal human bones and teeth.

Summary. The investigators in the first half of the 19th century (a) established the following facts of modern importance or (b) recognized difficulties that have largely been resolved only in the past decade.

1. Fluorine occurs in teeth and bone.
2. The fluorine content of teeth and bone is variable.
3. The fluorine content of fossil teeth and bone is higher than that of fresh tissues.
4. The association of fluorine with phosphorus was noted.
5. The increased fluorine of fossils was suggested to be caused by abstraction of fluoride from ground waters by the calcium phosphate of the hard tissues.
6. The difficulties of analysis of fluorine were exemplified and the interference by organic matter with analytical technics was suggested.
7. A far higher content of fluorine of bones and teeth was estimated than that which has been found with modern methods.

8. Variability of fluorine content with age of living bone was detected.

Water

It was very early considered that water was the source of the fluorine that appeared in the bones and teeth. Berzelius (1822) first reported fluorine in water, finding 3.31 p.p.m. as calcium fluoride in the Sprudsl Spring at Carlsbad. He commented on the volcanic nature of the terrain. He found no fluorine in two mineral samples from Auvergne in France. Later Berzelius (1823) reported more details of finding fluorine in the waters of springs at Carlsbad and in the mineral deposits at the mouth of the springs. He estimated the waters contained 3.2 p.p.m. calcium fluoride. He found no trace of fluorine in the waters of Teplitz, Königswart or Auvergne.

Hünefeld (1828), working with the advice of Berzelius, reported a trace of calcium fluoride in the residue of 1900 gm. of water from the Gastein Spring. He assumed the presence of "fluoric" acid because of etching of his glass vessels.

Daubney (1844) speculated on the enrichment of fossils by ground waters but found it difficult to account for such accumulations in view of the "rarity of the mineral itself in the waters of springs."

Middleton (1845), on finding differences of fluorine in fossil and modern bones, was "led to suspect that water might be the agent producing this apparent change." He noted the association of the fluorides and phosphates of calcium and found them together in water-deposited minerals except in "a pure but incompact stalactite of carbonate of lime." An editorial footnote said that Middleton later had "ascertained the presence of fluoride of calcium in the deposit obtained by boiling the ordinary pipe-water supplied to the houses in London." (Murray and Wilson 1942.)

Silliman (1846) wrote: "I have never failed to obtain evidence of the presence of fluorine in any coral . . ." Contrary to his expectations, "the fluorine is present in much larger proportion than phosphoric acid." He stated: "It need hardly be said that the existence in sea water of all the matters noted in these analyses is a just inference."

Wilson (1846a, b) showed that calcium fluoride was appreciably soluble in distilled water and thus cleared one difficulty in the explanation of the origin of fluorine in fossils. He found fluorine present in wells of Edinburgh and in sea water from the Firth of Forth.

Wilson (1849) reported to the British Association for the Advancement of Science on the finding of fluorine in various ocean waters. He noted that Middleton (1845) had concluded from analysis of marine shells that fluorine must be present in sea water, and Silliman (1846) independently reached the same conclusion from analysis of corals. Wilson found fluorine in walrus tusks and in kelp and offered these findings as further evidence that fluorine must be recognized as a constituent of sea water.

Nickles (1857) said the fluorine of blood, urine and bones was derived from water and plant foods. In the matter of the fluorine content of water he found minimum quantities in the Seine, the Rhine and the Somme Rivers. He found large and variable amounts of fluorine in mineral waters. He said the water of the Atlantic contained very little fluorine, estimated at one three-hundredth that of the concentration of some of the mineral waters.

Mène (1860), by use of the residue from evaporation of 50 liters of water, found fluorine in the waters of the Rhone, Saone and the Loire Rivers. He treated this water residue in glass with concentrated sulfuric acid and conducted the gases into water, where he observed decomposition to silicic acid. The alkaline filtrate from the latter was evaporated and fluorine verified by the usual etching procedure. This method ensured finding fluorine in the presence of the silica originally present in the water.

After about 1850 there were numerous reports of the presence of fluorine in water, mainly by French investigators. In the United States there seems to have been only the report of Hillebrand (1893) who found 5.2 p.p.m. fluorine (10.7 as CaF_2) in a thermal spring near Taos, New Mexico. This lack of data in the American literature possibly hindered the long search by McKay for the cause of mottled enamel.

It is of interest to note that fluorine was found in London in water in 1845. This fact must be considered in evaluating all studies involving conditions of the teeth of London subjects.

Plants

Will appended a footnote (Will and Fresenius, 1844) to a paper on the inorganic constituents of plants which said, in part: "The existence of fluorine in some vegetable ashes, although extremely probable, has not been proved until recently. Careful experiments, conducted under my own superintendence, by Messrs. James, Müller and Blake severally, have shown that the ashes of French barley, grown in Switzerland, contain very distinct traces of it; both straw and grain were employed. The presence of silica in considerable quantity renders the detection of fluorine very difficult." Also: "Fluorine occurs in the teeth and bones of animals, having been derived by them from vegetable food."

Daubeny (1844) could not assign an origin of bone fluoride in plants, as he found no trace of fluorine in the ash of 12 pounds of barley. He believed such traces as were present were lost in the ashing process.

Wilson (1846a, b) credited Will with being the first to find fluorine in plants. Wilson obtained from one pound of plant potash sufficient fluorine to etch glass.

Wilson (1852a) was able to detect fluorine in the presence of silica by (a) precipitating K_2SiF_6 or (b) precipitating silica with ammonia. He preferred the latter. With this method he was able to find fluorine in various siliceous minerals and in coal and plant ashes. He said: "The presence of fluorine in animals may now be fully accounted for; it not only enters their bodies in the water they drink, but it is contained in the vegetable food. . . ." Wilson (1852b) found fluorine in the ashes of a wide variety of plants but chiefly in the grasses.

Blood, Milk, and Miscellaneous Fluids and Tissues

Gay-Lussac (1805) had indicated a belief that fluorine must be present in blood to account for its occurrence in bones and teeth. Middleton (1845) reasoned similarly.

Wilson (1846a, b) stated that he had etched glass with the fluoride of the ash from 128 ounces of ox blood and, after five unsuccessful trials, had found fluorine in cheese. He believed this to be the first demonstration of the presence of fluorine "in the two great formative liquids of the animal body, blood and milk." He said: "Whether this fluorine be supposed simply to travel through the organism, dissolved in the circulating fluid as fluoride of calcium, or as some other salt, and to quit the body as it entered it, without serving any purpose therein; or be imagined to fulfill some important end in relation to the functions of life, it must be expected to shew itself as a very frequent, if not constant, ingredient in the bones."

Wilson (1850) ashed 26 imperial pints of freshly-drawn ox blood, the process requiring a month. Sodium chloride was extracted with water and the residue treated with sulfuric acid in a lead vessel over a period of 10 days, with the whole of the sample being used. The glass plate, used to cover the dish, bore these words which had been scratched through the wax coating: "Blood, 5th July, 1850." The words were deeply etched.

But from 20 pints of cow's milk from a town dairy, milk which "left a suspiciously

small residue of solid matter," Wilson failed to obtain fluorine. He believed the main cause of failure was his neglect to remove the sodium chloride. But from 9 pints of "rich milk from a country farm" he obtained ashes which distinctly etched glass. Ashes from 12 pounds of new skim-milk cheese produced deep etching. Wilson believed the fluoride of calcium separated with the casein.

Nickles (1856) verified the presence of fluorine in bones and also found notable quantities in blood of man, mammals and birds. Wilson (1857), who questioned Nickles' priority in the matter of discovery of fluorine in blood, translated Nickles brief paper in its entirety. So, apparently, in 1857 it was recognized that there was fluorine in the blood and "there is fluorine in the bile, in the albumen of eggs, in the saliva, in the urine, in the hair; in the hairs of animals (ox, cow, calf); in a word, the organism is penetrated by fluorine, and we may expect to find some in all the liquids with which it is impregnated." There was no statement made by Nickles as to details of discovery of fluorine in bile, albumen, saliva or hair or whether these conclusions were merely deductions from its demonstrated presence in other tissues.

Horsford (1869) ashed human brain and showed qualitatively that fluorine was present.

Tammann (1888) found little fluorine in egg shells, definite amounts in egg white and about 10 p.p.m. in egg yolk. He calculated this was more than double the amount necessary for the chick bones, using Zaleski's (1866) values for bone fluoride. He found about 8 p.p.m. fluorine in calf brain, 0.4 p.p.m. in cow's milk, and definite traces in cow's blood.

Urine

Gay-Lussac (1805) considered the enamel to be built by nutritive means which supplied the fluorine and, since other bone constituents were present in urine, he expected fluorine would also occur there. He said, "The discovery is extremely important and one from which we rightfully can expect great consequences." (Translated)

Berzelius (1806, and 1807a, b) also reasoned that the bones were active in metabolism and hence he sought fluorine in urine. He succeeded in isolating calcium fluoride by precipitating it from urine with lime water.

Rees (1839b), who was unable to find fluorine in bones, also failed to find it in urine, but Wilson (1846a, b) confirmed Berzelius' results.

Summary

1. Fluorine was first reported present in animal tissues in 1803, being found in the enamel and dentin of a molar of a fossil elephant.

2. The variability of fluorine in bones and teeth was early noted and also the comparatively higher content of fluorine in fossil remains.

3. Fluorine was reported to be present in urine in 1806; in water, 1822; in plants, 1844; in blood, 1846; in milk, 1846; in brain, 1869.

4. Though no details were given, it was stated in 1857 that fluorine occurs in saliva, hair, bile and eggs.

5. The variability of the fluorine content of water was known in 1823.

REACTION OF FLUORIDE WITH BONES AND TEETH

Fluorine and Fossils

Girardin and Preisser (1842) considered the alterations which take place in bones buried in different soils or in caverns. They believed that, with proper precaution, the fluorine content would indicate whether or not a bone was old or of recent origin, since fluorine was invariably found in fossil bones but was absent or very low in fresh bones. Girardin and Preisser (1843) gave the data of chemical composition of various fossils to sustain the above arguments.

They found calcium fluoride around 1 to 2 per cent in true fossils and as high as 9.12 per cent in the case of a tertiary period manatee.

Daubeny (1844) speculated on the enrichment of fossils by ground waters but found it difficult to account for such accumulations in view of the "rarity of the mineral itself in the waters of springs."

Middleton (1844), on finding more fluorine in fossils than in recent bones, remarked: "It occurred to me that ordinary water might be the vehicle, and if so, the presence of fluorine in recent bones would not only be accounted for, but also its accumulation in fossil bones, being filtered from the moisture circulating in the earth's crust." He found fluorine in certain stalactites and in encrustations after the evaporation of water. Middleton (1845) found 11 per cent calcium fluoride in bones from India, 5 per cent in bones of an ancient Greek, but only 2 per cent in the bones of an Egyptian mummy. He calculated the age of fossil bones on the basis of a ratio of fluorine to phosphorus and assigned an age of 2000 years to the Greek bones. This procedure gave an age of 7700 years to the bones from India and 24,200 years to an anoplotherium bone described by Lassaigne as containing 15 per cent calcium fluoride.

Wilson (1846b) said: "Water, as my own experiments prove, may carry away fluoride of calcium from osseous remains, as well as transport it to them. We require to account for its detention in bones, as well as for its conveyance to them. From an experiment made in the laboratory, as well as from their association in nature, I am inclined to think that there is a double phosphate of lime and fluoride of calcium, much less soluble than the latter salt is, and that the production of this compound fixes the fluoride, and prevents its abstraction by water. Further researches will decide this point."

Hoppe (1862) recorded that he could not find fluorine in the undeveloped enamel of swine but found it present in the mature enamel of man, swine and a fossil rhinoceros. He estimated the amount present as less than 2 per cent stated as calcium fluoride. He was led by the constant presence of carbon dioxide and chlorine in enamel, as well as fluorine, and by the insolubility of the compound that held these ions, to consider them to exist in enamel as an apatite.

Aeby (1873) considered that in fossil bones the ratio of calcium to phosphorus remained constant and that the carbonate declined inversely as the fluorine increased. He therefore concluded that in the metamorphosis of bones fluorine replaced carbonate, rather than that an addition of calcium fluoride took place.

Carnot (1892a) further developed the idea that the fluorine content of bones is a rough measure of their antiquity. He found for modern bones 0.20 to 0.39 per cent calcium fluoride; in fossil bones, 0.88 to 6.21 per cent, proportional to the estimated geologic age. Carnot (1892b) showed that the ratio of fluorine to phosphorus in very old fossils was approximately that of fluorapatite but that it decreased through the geologic series to about one-fifteenth that value in modern bones. Carnot (1892c) applied the above method to animal and human bones found in the same deposit. The animal bones had 1.43 and 1.84 per cent fluorine; the human bone, 0.17 per cent. The ratio equivalents of fluorine to phosphorus were 0.469, 0.578 and 0.066. He concluded the human bones were modern.

Zaborowski (1893) submitted ancient human bones to Carnot for clarification of their relative ages. The analyses for fluorine and the ratio to phosphorus were in agreement with Zaborowski's opinion derived from other considerations. Gautier (1893) observed 1.714 per cent calcium fluoride in fossil bones found in a cavern in the Department of Herault in southern France. He remarked on the replacement of carbonate by fluorine. He was interested in the origin of phosphorites.

Wilson (1895) gave an extensive review of the use of the fluorine content of fossil bones for the determination of age, particularly the work of Carnot (1893), which he translated. He applied Carnot's method to a fragment of a human pelvis and a bone of a mylodon found together in Mississippi. The fluorine contents were 0.38 and 0.28 per cent respectively and, in consideration of ratios with phosphorus, Wilson concluded the age was intermediate between the Quaternary Period and the Modern.

Gautier and Clausmann (1913b) found about 5 times as much fluorine in the diaphyses of long bones as in the epiphyses and more in adult than in young animals and man. Their values ranged from 37 p.p.m. fluorine in the fresh epiphyses of a human infant to 800 p.p.m. in the fresh diaphysis of an ox. The skeleton of a shad, fresh, contained 497 p.p.m. and its scales 488. They emphasized the latter similar values. Fresh cartilage from the head of the femur of a 65-year-old man was found to have 14 p.p.m. fluorine. The tendons of a 2- to 3-months-old calf had 3.5 p.p.m.

Bones in Water Treatment

On evidence of Carnot (1893) of removal of fluorine from water by bone, H. V. Smith (1935) made a single trial in which "67.5 mg. of bone was used per liter of water, the original fluorine concentration of 2.5 p.p.m. was reduced to 1.7 p.p.m." Sanchis, in discussing this paper, suggested "the introduction of substances containing fluoride antagonistic ions in the diet as a logical solution of the problem when a better source of supply cannot be obtained," but he did not name any such ions. Bone might be effective.

Smith and Smith (1937) found that crushed bones, which had been boiled with water to remove fat and protein and then further treated by boiling with 2 N sodium hydroxide, would reduce the fluorine content of natural waters to 0.1 or 0. p.p.m. The degree of fineness of the bone, ratio of fluorine to bone and degree of contact were factors. "For example, standing for 96 hours in contact with one gram of prepared bone, reduced the concentration of fluorine from 2 p.p.m. to zero as compared with a concentration of 0.5 p.p.m. remaining in the sample of water shaken for a two-hour period." The effluent from 5 pounds of such bone produced a water with 0.2 p.p.m. fluorine when natural water, with 3.5 p.p.m. fluorine, was passed through it at the rate of 30 gallons per hour to a total of 140 gallons. When 70 gallons had passed the fluorine level was 0.1 p.p.m.

MacIntire and Hammond (1938) have shown that true tricalcium phosphate can reduce the fluorine content of natural waters from 6 p.p.m. to concentrations of the order of 0.1 p.p.m. Adler, Klein and Lindsay (1938) found fluorine was removed from natural waters by a precipitated dried tricalcium phosphate to the extent of 3.7 gm. of fluorine per kg. of the phosphate. The effluents, at the rate of 4 to 8 gallons per square foot of filter bed, had less than 1 p.p.m. fluorine. The pH of the effluent was 7.0 irrespective of the pH of the influent. They tentatively considered the reaction as one of adsorption.

Bone Fluorine Derived from Food, with Special Reference to Dietary Bone Meal

Bethke, Kick, Edgington and Wilder (1929) fed supplements of limestone with and without (a) sodium fluoride, (b) rock phosphate and (c) steamed bone meal to groups of 8 swine. They found an increase in the fluorine content of the bones in amounts roughly proportional to the fluorine content of the ration, the maximum being 11,000 p.p.m. from feed containing 100 gm. of sodium fluoride per 100 pounds of feed. The breaking strength of the femurs was very much reduced, being 393 pounds on the above dosage of sodium fluoride and 841 pounds on bone meal. "The pigs fed either 2 parts rock phosphate or 60 or 100 grams sodium fluoride showed signs of stiffness."

Ellis and Maynard (1936) tested the comparative absorption of fluorine from sodium fluoride and from bone meal. They used small groups of rats that had been raised from weaning to 53 days of age on the Sherman diet of two-thirds wheat, one-third whole milk and 1.3 per cent of salt. This ration also served as the basal ration of the experiment and contained approximately 3 p.p.m. of fluorine.

When fluorine was incorporated in the ration at a level of 14 p.p.m. of added fluorine for 62 days, the fluorine content of leg bones was more than trebled as compared with the controls. The incisor pigment showed lightening. There was no difference in the effects of sodium fluoride and bone meal.

In further experimentation, 8 and 12 p.p.m. were added as sodium fluoride and bone meal for experimental periods of 56 and 168 days. Lightening of the incisors was detectable at the lower level. The bone fluoride of the rats was increased by both forms of added fluoride without appreciable difference. In the older rats the level of bone fluoride was higher than that of the younger for both experimental and control rats. Analyses of the teeth, presumably the incisors, of the rats killed after 56 days, showed fluorine changes parallel to those found in the bones. Fluorine as p.p.m. in the teeth was:

Controls	81
Sodium fluoride, 8 p.p.m.	228
Bone meal, 8 p.p.m.	220
Sodium fluoride, 12 p.p.m.	246
Bone meal, 12 p.p.m.	276

Evans and Phillips (1938) tested the skeletal storage of fluorine from 4 samples of bone meal containing different levels of fluorine. For each test they used 8 rats 30 to 50 gm. in weight. Their basal ration was:

Yellow corn	76
Crude casein	15
Yeast	3
Cod liver oil	1
Iodized salt	1

The ration itself contained 4.8 p.p.m. of fluorine, determined by Armstrong's method (1936). From calf bone (which showed by analysis 20 p.p.m.) 36 to 54 p.p.m. of fluorine was found in the femurs of the rats after 8 weeks. The fluorine level in the ration was 6 p.p.m. in this test. No results were reported on the basal ration with an equivalent of bone salts.

With bone meals that raised the fluorine content of the ration to 29.9, 26.0, 29.0 and 16.9 p.p.m., respectively, 288, 288, 236 and 293 p.p.m. of fluorine in the femurs were found.

The authors did not find any effects on the incisors of the rats.

Lee and Nilson (1939) compared the storage by young rats of fluorine from canned salmon and mackerel with that from inorganic fluorides in synthetic diets. The salmon as fed, without drying, contained 5.77 p.p.m. of fluorine and the mackerel 26.89 p.p.m., the fluorine being mainly in the bones. The mean fluorine (p.p.m.) content of the diets and the rat carcasses after 12 weeks were as follows:

	In diet	*In rat carcass*
Fresh salmon	5.77	18.47
Dried salmon	19.34	18.24
Fresh mackerel	26.89	71.74
Dried mackerel	84.47	75.82
Basal synthetic diet	1.28	6.94
Basal + NaF	2.74	8.91
Basal + CaF_2	2.63	8.60

Typical striations of the lower incisors were observed in rats fed the canned mackerel but none in those on the salmon diets. The authors concluded: "Only 21 per cent of the fluorine from canned salmon or mackerel is stored, indicating that the naturally occurring fluorine from these fish is stored at about one-third of the rate of added inorganic fluorides." No determination of fecal fluorides was made.

Tisdall, Drake and Brown (1930), in compounding a cereal mixture for infant feeding, included 2 per cent bone meal in

preference to a pure calcium phosphate for traces of other minerals. The resulting mixture contained 0.78 per cent calcium and 0.62 per cent phosphorus. They reported satisfactory use of the cereal mixture over a period of 3 months in a children's hospital of 300 beds. Brown and Tisdall (1933) reported extensive use of this cereal mixture for over 2 years. In a test with 24 children, better growth was obtained in comparison with "ordinary cereals." No direct study of utilization of the bone meal was made.

Marcovitch, Shuey and Stanley (1937) reported that a prepared baby food, "found to contain 2 per cent bone meal, or 12 p.p.m. of fluorine," when fed alone to rats produced visible striations in one of them but none when a mixture of 2 parts baby food and 1 part milk powder was fed.

The same authors reported the following for the p.p.m. fluorine content of various bone-containing foods:

Sardines	7.30
Fish (no bones)	0.00
Mackerel (with bones)	3.90
Salmon (canned)	4.50
Prepared baby food containing 2 per cent bone meal	12.00
Bone meal	450.00

Stearns and Jeans (1934) found tricalcium phosphate, either as the salt or purified bone meal, a satisfactory source of calcium and phosphorus for children.

Boissevain and Drea (1933) found recent human bones from Colorado containing 6000 to 8000 p.p.m. by spectroscopic analysis and 1200 to 3500 p.p.m. by chemical assay. Bones from eastern United States had 500 to 3000 p.p.m. and 100 to 1600 p.p.m. respectively. Whale bone contained 1200 and 4800 p.p.m. by the two methods; it was pointed out that sea water contains 1.2 p.p.m.

Glock, Lowater and Murray (1941) found by spectrographic and chemical analysis of human ribs that the fluorine content rises with increase in age. They found values ranging from 200 p.p.m., in fat-free bone in a stillborn infant, to 3100 p.p.m. in the case of an adult, age 37. Rats, on a ration with 500 p.p.m. added sodium fluoride, showed 190 p.p.m. in fat-free bone for a control at 6 weeks, and 7050 p.p.m. after 84 weeks on the ration. The fluorine content of rat bones declined on withdrawal of NaF. Rats 46 weeks of age with 6000 p.p.m. fluorine in bones showed 2870 p.p.m. after 14 weeks with no added fluorine. A second series, age 38 weeks, showed a decline from 9430 p.p.m. to 3020 p.p.m. in 12 weeks.

Sharpless and McCollum (1933) observed that rats on a low fluorine diet for 3 to 4 months had 6 to 27 p.p.m. fluorine in dry bone. Marcovitch, Shuey and Stanley (1937) found 36 to 65 p.p.m. fluorine in the bones of rats on a rice and milk ration for 35 days. Evans and Phillips (1939) found skeletal fluorine to average 13 p.p.m. with a range from 1 to 31 p.p.m. in adult rats raised on a mineralized milk diet averaging 1.6 p.p.m. of fluorine on a dry basis. McClendon and Foster (1941) found 40 to 100 p.p.m. fluorine in the femurs of 5 rats that had received a ration containing 0.3 p.p.m. of fluorine from weaning to an unstated age. Enamel fluorine ranged from 40 to 100 p.p.m. and dentin fluorine from 70 to 100 p.p.m.

McClure (1944b) has reported height, weight and bone-fracture experience in relation to fluorine ingestion of 1,458 high school boys and 2,529 men taking physical examinations for induction into the armed forces of the United States. The subjects interviewed were from the fluoride areas of Illinois, Oklahoma and Texas; subjects from Indiana, New Hampshire and Washington, D. C., represented exposure to non-fluoride waters. Approximately 20 per cent of the subjects had had one or more bone fractures, but no relation to fluoride exposure was revealed. Likewise, there was no relation of height and weight to fluoride exposure, though the men from Texas and

Oklahoma averaged more than two inches taller than men from New Hampshire.

Fluorine and Teeth

Gabriel (1893) could account for only 98.85 per cent of bone ash by chemical analysis for common constituents. By the etch test for fluorine he concluded that the deficit was not due entirely to that element, since he could not find more than 0.05 per cent in the ash of teeth or in separated enamel and dentin.

Bertz (1898) separated enamel and dentin of human teeth and analyzed for fluorine. He found 0.471 per cent fluorine in dentin, on dry basis, and 1.089 per cent in enamel. In embryonic calf enamel he found 0.771 per cent fluorine. Though he was aware of the antifermentative and bactericidal theory of fluorine in teeth as protecting from caries he does not reveal whether the teeth he analyzed were sound or carious.

Harms (1899) analyzed the bones and teeth of various animals. He found for the ash of teeth of a calf 50 p.p.m.; man, 60 p.p.m.; pig, 180 p.p.m.; dog, 90 p.p.m. He pointed out that others had found higher values which he considered in error. He regarded fluorine as only an accessory constituent of the teeth.

Jodlbauer (1902–1903) found more fluorine in the crowns of teeth than in the roots. He separated enamel from dentin and found enamel richer in fluorine, containing 3700 p.p.m.

Gautier and Clausmann (1913a) reported the fluorine of the enamel of a 1-year-old dog as 1660 p.p.m., a 7- to 8-year-old dog as 1160 p.p.m. and of a hippopotamus as 1777 p.p.m. They (1913b) found the dentin of a 6- to 7-year-old dog to have 560 p.p.m. fluorine.

Trebitsch (1927), in introductory remarks, said fluorine in the teeth has especial significance in view of the antiseptic properties of its soluble salts. He found 2900 to 5900 p.p.m. fluorine in human teeth.

Boissevain and Drea (1933) analyzed human teeth for fluorine by a spectroscopic method and a modification of Willard and Winter's chemical method (1933). They found no fluorine in enamel of teeth formed in New York City but 680 p.p.m. in the dentin; Colorado Springs enamel showed 650 p.p.m. and the dentin, 1120 p.p.m. fluorine. Chemical analysis of the whole teeth from New York gave 630 p.p.m.; Colorado Springs whole teeth contained 940 p.p.m.

Klement (1933) found, with the Armstrong (1933b) modification of the Willard and Winter (1933) method, 300 p.p.m. fluorine in human teeth and 490 p.p.m. in ox enamel. In dolphin and shark teeth the fluorine content was very much higher, being 6900 to 7400 p.p.m. The tooth of a fossil shark had 28,400 p.p.m. He considered that these values represented variable replacement of hydroxyl by fluorine in hydroxyapatite.

Chang, Phillips, Hart and Bohstedt (1934) added fluorine as rock phosphate to the ration of cows at 220, 440 and 880 p.p.m. levels. After about 4½ years the animals were sacrificed and various tissues analyzed for fluorine. They found in the controls 267 p.p.m. fluorine in enamel, 623 p.p.m. in dentin and 584 p.p.m. in bones. The fluorine content of these tissues in the experimental cows was increased 16 to 25 times over the controls by the fluorine feeding, but the relative order of fluorine content was not changed.

Bowes and Murray (1935b) obtained human enamel by filing off the outer layers of teeth and drilling out the dentin so that a shell of the tooth remained. Complete chemical analysis of enamel and dentin was made. The results for fluorine were derived from Bowes and Murray (1935a) though they stated that more recently fluorine was distilled as H_2SiF_6 before estimation by the zirconium-alizarin method. They gave a value 250 p.p.m. for fluorine but expressed

the opinion: "F is probably not present in all teeth." Chlorine was present, 3000 p.p.m., and was in a form not extractable by hot water from powdered enamel. As apatites these amount to 0.663 and 4.397 per cent fluorapatite and chloroapatite, respectively. The authors calculated 12.06 per cent carbonatoapatite and 75.04 per cent hydroxyapatite as present in the same enamel sample. For dentin they found the same amount of fluorine but no chlorine. The teeth used by Bowes and Murray were sound permanent premolars removed from 14-year-old children for orthodontic reasons. The teeth were of the class that show the mildest form of Mellanby's hypoplasia.

Bowes and Murray (1935a), by means of a modification of the zirconium-alizarin method in which interfering ions were removed by precipitation, estimated the fluorine content of human enamel of the London area to be 200 p.p.m. Also, they found rats' teeth to contain 200 to 300 p.p.m. fluorine.

Muñoz (1936) found 340, 430 and 444 p.p.m. fluorine in normal human enamel and 340, 390, 650 and 730 p.p.m. in mottled enamel. In normal dentin he found 330 and 430 p.p.m.; in dentin from a mottled tooth, 390 p.p.m.

Lowater and Murray (1937) examined teeth spectrographically with especial interest in fluorine. Human enamel and dentin of sound teeth from London were listed as showing a possible trace of fluorine. Mottled enamel and dentin from Malden, Essex, showed positive evidence of fluorine. None was found in dog dentin or in the incisor enamel of control rats. The enamel of fluoride-fed rats showed a positive test. It is of interest to note that potassium was not found in normal teeth but was present in enamel and dentin of mottled teeth of man and rat.

Von Fellenberg (1937) found 49 p.p.m. fluorine in each of two whole caries-free deciduous incisors from two children of the same family. In one tooth, said to be a six-year molar, caries-free, he found 79 p.p.m. in the dentin, 64 p.p.m. in the enamel and 106 p.p.m. in the root.

Armstrong and Brekhus (1938a) reported the mean fluorine content of the enamel of sound permanent teeth as 111 p.p.m., and of dentin as 169 p.p.m. They observed 250 and 360 p.p.m. in mottled enamel. Armstrong (1937), Armstrong and Brekhus (1937) and Armstrong and Brekhus (1938b) found the mean fluorine content of sound enamel from carious teeth to be 69 p.p.m. In three subjects, the enamel from sound teeth was found invariably to have a higher content of fluorine than that of carious teeth from the same mouths.

McClure (1948) analyzed the enamel and dentin of 262 sound and 248 carious teeth from 91 human subjects, 44 of them being from Northern Illinois, with a variable exposure to 1.5 to 2.5 p.p.m. fluorine in the drinking water. None of the teeth had mottled enamel. He found the fluorine content of the enamel to be of the order of 100 p.p.m., and of dentin, 250 p.p.m. There was no significant difference between the fluorine content of carious and non-carious enamel or dentin or teeth from the same 19 individuals nor was such difference indicated by the general findings. "Therefore, it does not appear that the caries history of one tooth as compared with another tooth in the same dentition was related to its fluorine content." Variation was found for the different teeth of the human dentition but there were no indications of significance.

Danckwortt (1941) found the fluorine of normal whole teeth of cattle to average 443 p.p.m.; the whole third molar of a man, 106 p.p.m.; the tooth of a deer, 170 p.p.m. The content of normal cattle bones was from 130 to 910 p.p.m.; of fossil cattle bone, 6200 p.p.m. Cattle poisoned by fluorine showed 5 to 10 times as much fluorine in teeth and bones as in the normal animal. A whole

human tooth, presumably mottled, had 1100 p.p.m.

Volker, Hodge, Wilson, and Van Voorhis (1940) demonstrated, by means of radioactive fluorine, *adsorption* of this element on enamel, dentin and bone powdered to pass a 60-mesh screen, and on hydroxyapatite. Solutions of fluoride varying in concentration by powers of 10 from 1:1,000 to 1:1,000,000 were used. The adsorption was in accordance with the Freundlich adsorption isotherm. "A few experiments were done in which the crowns of teeth were dipped for 30 minutes at 40° into solutions of sodium fluoride containing radioactive fluorine. The enamel was partially dissolved off by dipping the tooth into a solution of hydrochloric acid. Small but detectable amounts of fluorine were adsorbed by these intact enamel surfaces; the maximum amount of fluorine adsorbed on a single tooth was of the order of 0.02 mg." They suggested: "The local application of solutions of fluorides to teeth *in situ* might add sufficient fluorine to the enamel surface to decrease its susceptibility to dental caries." Hodge (1940) further discussed the "Freundlich adsorption isotherm" as a means of distinction between chemical reaction and surface reaction. The brief half-life of radioactive fluorine necessitates short time experiments. Volker *et al.* accordingly said: "In fact, the evidence of a surface reaction may have been an artifact contributed by the relatively short exposure of the calcium phosphates to the fluoride solutions." (See also Volker (1939).

Volker, Sognnaes and Bibby (1941) found that radioactive fluorine injected in rats was deposited in the bones and in the molar crowns. They failed to find it in the incisal third of the incisors. Fluoride injected into four cats was found deposited in the bones, more in those sacrificed after 2 hours than in two killed after 30 minutes. "The failure to find significant amounts of the labeled fluoride in the dental tissues well removed from the tooth pulp does not support the possibility that fluorine may be deposited via the circulation in the dental enamel . . . However, the possibility still exists that the ingested fluoride is first stored in the bones and may later be released for circulation where it could conceivably be deposited in the more inert portion of the teeth. Unfortunately, the short half-life of the radioactive isotope will not permit studies to test that possibility."

Perry and Armstrong (1941) showed by chemical analysis that a posteruptive increase in the fluorine content of rat molar enamel can be effected by 20 p.p.m. of fluorine in the drinking water. Arnold and McClure (1941a, b) have confirmed this finding with injected fluoride and with 10 p.p.m. fluorine in the drinking water of rats.

McClure (1942) gave fluoride in food and water to a mature dog over a period of about a year and a half. Teeth were extracted at intervals and analyzed for fluorine. The successive values for dentin were 180, 220, 390, 590, and 720 p.p.m. and for enamel 60, 70, 90, 70 and 110 p.p.m.

Short (1944) found a significant delay of eruption of permanent human teeth in Colorado Springs with 2.6 p.p.m. fluorine in the community water supply, as compared with Illinois cities with 0.0 to 0.5 p.p.m. and Pueblo, Colorado, with 0.6 p.p.m. No significant differences were found for cities with up to 1.8 p.p.m. fluorine. The criterion was eruption of the whole dentition rather than of specific teeth.

Plater (1945) examined 49 children of Union Grove, and 320 of Madison, Wisconsin, for malocclusion. The percentage distribution of malocclusion for Union Grove (1.0 p.p.M.F) and Madison (0.05 p.p.M.F) respectively was: normal, 40 and 12; Group 1, 30 and 50; Group 2, 30 and 36; Group 3, 0 and 2. Loss of permanent teeth was found to occur at the age of 7 in Madison; the earliest loss in Union Grove was at 15.

Summary

1. Fluorine will deposit slowly as fluorapatite throughout whole bones exposed to water (such as fossils), proportionally to the length of time of exposure and probably in proportion to the fluoride content of the water.

2. Bone powder will rapidly adsorb fluorine from water, probably as a strictly surface reaction, until the fluorine content of the water is reduced to about 0.1 p.p.m.

3. The fluorine of bones in the diet is available as shown by deposition in the bones of experimental animals and it will cause toxic effects as shown by striations produced on rat teeth.

4. Fluorine is deposited in animal bones in proportion to duration of exposure and the amount of fluoride ingested.

5. No ration for experimental animals has yet been devised with such a low fluorine content that no fluorine is deposited in the bones.

6. The fluorine content of human enamel varies widely and is probably related to the fluorine of food and water during the time of formation.

7. The fluorine content of deciduous enamel is probably lower than that of permanent tooth enamel in the same districts.

8. Mottled enamel has a higher content of fluorine than "normal" enamel.

9. The fluorine content of teeth that decay is probably lower than that of sound teeth.

10. Fluoride adsorbs on enamel powder.

11. Fluoride fed posteruptively to rats and to dogs will increase the fluorine content of the enamel.

METABOLISM OF FLUORINE WITH SPECIAL REFERENCE TO ITS QUANTITATIVE PRESENCE IN SOFT TISSUES AND FLUIDS

Animal Tissues

Gautier and Clausmann (1913a) found the p.p.m. fluorine content of the following fresh and dry tissues from various animals as shown below:

Skin	23-year-old man	19	45
	54-year-old woman	16	40
	New-born girl	6.66	12.76
	Calf, 2 months old	3.20	8.40
	Calf, new-born	5	19.60
	Pig, 1 year old	4.80	10.70
	Human epidermis, 70-year-old man	146	164
Scales	Pongolin manis	175	199
	Armadillo	205	228
	Fish	486	599
Hair	Black, man, 16 years	150	172
	Blonde, man, 22 years	113	130
	Gray, man, 74 years	53.20	61
	Black, dog, adult	165	197
	Gray, dog, very old	75	89
Horn	Ram, 4 years old	19	24
Nails	Mixed human	80	94
Feathers	Quill, hen, 2–3 years	54	72
	Tufts, same hen	107	118
	Quill, ostrich, age unknown	44	50
	Tufts, same ostrich	68	79
	Down, duck	89	98

Gautier and Clausmann concluded that fluorine varied from tissue to tissue, that the content decreased with age and that it was proportional to the phosphate content of the tissue.

Gautier and Clausmann (1913c) gave an extensive tabulation of their results of analysis for fluorine of soft tissues, organs, blood, secretions and excretions of man and animals. Their data are generally very high in comparison with modern results for comparable tissues, but they have value in their relations. They summarized their ideas on fluorine, such as: a fairly constant ratio of phosphorus to fluorine, the higher content of fluorine in older bone, the high fluorine of bone and enamel, the low fluorine of muscle, milk and urine but the medium values for blood, brain and some glands.

Gautier (1914a, b) discussed his finding of fluorine in animals under three classifications. In the active tissues and fluids (muscles, glands, nerve tissue, blood, milk) the ratio of phosphorus to fluorine is high, from 350 to 750. In tissues of slower metabolism (bones, cartilage, tendons) the ratio is intermediate, from 130 to 180. In the protective tissues of little or no metabolism (hair, fur, feathers, nails, epidermis) the ratio approaches that of apatite, 4.89, being of the order of 3.5 to 7.5. He suggested these latter may be a path of excretion of fluorine and drew the analogy to copper and arsenic.

Chang, Phillips, Hart and Bohstedt (1934) said, "It was impossible for us to demonstrate large amounts of fluorine in washed hair" of cows fed for 4½ years with a ration containing fluorine as high as 880 p.p.m. The hair of the controls had about 6 p.p.m. and of the fluorine-fed animals about twice that amount. A similar increase occurred in hoofs.

Gautier and Clausmann (1916) reported the analysis of a large number of plants, with particular emphasis on those used as food by man. They sought the distribution of fluorine in the parts of the plants and the ratio of phosphorus to fluorine. They could find no general law for plants on the occurrence of fluorine corresponding to that which they had found for animal tissues but concluded that in general fluorine and phosphorus increased and decreased somewhat together.

It is interesting to note that they found less fluorine in wheat bran than in wheat flour, which is the opposite of the observation of Wilson (1852b) upon which were based the speculations of Crichton-Browne (1892).

McClure (1939c) has summarized the reports on the fluorine contents of food since 1933. The fluorine values in p.p.m. with sources of data for "some of the more common foods, especially those consumed by infants and children" are shown below:

Milk	0.07–0.22	Phillips, Hart, Bohstedt (1934a)
Egg white	.2–.3	Phillips, Halpin, Hart (1935)
Egg yolk	.8–1.2	Phillips, Halpin, Hart (1935)
Potatoes	.2	Churchill, Bridges, Rowley (1937)
Wheat	1.0	Churchill, Bridges, Rowley (1937)
Wheat	.3–1.0	Hart, Phillips, Bohstedt (1934)
Crushed oats	.2	Churchill, Bridges, Rowley (1937)
Oats	.7–1.7	Hart, Phillips, Bohstedt (1934)
Corn	.2	Churchill, Bridges, Rowley (1937)
Rice	1.0	Churchill, Bridges, Rowley (1937)
Tomatoes (dry substance)	.6–.9	Dahle (1936)
Lettuce (dry substance)	.6–.8	Dahle (1936)
Carrots (dry substance)	.2	Churchill, Bridges, Rowley (1937)
Turnips (dry substance)	.2	Churchill, Bridges, Rowley (1937)
Apples (whole)	.8	Marcovitch, Shuey, Stanley (1937)
Spinach (dry substance)	6.3–8.6	Winter, Butler (1933)
Beef	.2	Churchill, Bridges, Rowley (1937)
Mutton	.2	Churchill, Bridges, Rowley (1937)
Pork	.2	Churchill, Bridges, Rowley (1937)
Canned corn	.2	Churchill, Bridges, Rowley (1937)
Dried beans	.2	Churchill, Bridges, Rowley (1937)
Fish foods	1.5–12.5	Tolman (1937)
Fish foods	3.9–7.3	Churchill, Bridges, Rowley (1937)
Teas	13.1–178.8	Reid (1936)
Teas	41–67	Churchill, Bridges, Rowley (1937)

Machle, Scott and Treon (1939) analyzed a wide variety of foods purchased in Cincinnati and of plant foods grown in fluoride areas of Arizona. In general the values of foods from Arizona were lower than those purchased in Cincinnati. Most values were less than 1 p.p.m., the means being 0.452 p.p.m. for Arizona and 0.730 p.p.m. for Cincinnati. No valid conclusions could be drawn, as different methods of analysis were used for the foods from the two sources. The authors wrote: "Our failure to find abnormal amounts of fluorine in foodstuffs from Arizona indicates that there is little or no liklihood of the increment of fluorine from food playing an important role in the production of mottled enamel in the areas under study."

Armstrong and Knowlton (1942) found 0.27 to 0.32 mg. of fluorine in the daily food of the house staff of the Minnesota General Hospitals and considered that to be the average fluorine intake from sources other than the water.

Fluoride of Blood and Blood Clotting

Arthus and Pages (1890) found the clotting of blood was prevented by 0.15 per cent of sodium fluoride as compared with 0.10 per cent of oxalic acid and explained the action as due to precipitation of calcium.

Arthus (1891) found that 0.4 per cent of sodium fluoride inhibited glycolysis in blood.

Stuber and Lang (1928) proposed a theory of blood clotting based on the parallel activities of glycolysis and coagulation. They considered clotting as a result of lactic acid formation. In the blood of hemophiliacs they found fluorine high, and it was also high in the blood of geese which show normally a delayed coagulation. Their values for p.p.m. fluorine and clotting time from various subjects were:

Normal man	0	
Dog	0	5 min. 10 sec.
Cat	0	4 min. 45 sec.
Rabbit	0.75	15 min. 30 sec.
Hen	1.20	4 min. 40 sec.
Duck	0.51	21 min.
Goose I	1.50	252 min.
Goose II	1.03	92 min.
Hemophiliac I	2.925	180 min.
Hemophiliac II	3.965	150 min.

Taege (1929) reported analyses of the blood of 10 geese and found the fluorine content to range from 4 to 14 mg. per 100 cc., i.e., 40 to 140 p.p.m.

Hoff and May (1929–1930) found only traces of fluorine in normal human blood. Three samples of blood from a hemophilia patient, under a course of treatment which reduced clotting time, showed no difference from normal blood in respect to fluorine content.

Stuber and Lang (1929) found that healthy residents of Kiel had a blood coagulation time of 10 to 17 minutes, compared with 6 to 9 minutes in Freiburg. One subject, presumably Lang, had shown coagulation in 6 minutes 25 seconds in Freiburg, 12 minutes 45 seconds after 3 months residence in Kiel, and 16 minutes 40 seconds after 6 months in Kiel. They found fluorine in the blood of 18 out of 20 patients in Kiel but found it in the blood of none, except hemophiliacs, in Freiburg. Analysis of Kiel water showed 0.165 p.p.m. fluorine and of milk, 1.1, 1.6, and 2 p.p.m. Analyses of Freiburg water showed 0.028 p.p.m. and of milk, no fluorine. They ascribed the increased coagulation time in Kiel to the blood fluoride derived from water and milk. They considered that fluorine is possibly present in hemophiliac blood in an unionized form.

Feissly, Fried and Oehrli (1931) found that 100 mg. of ammonium fluoride per 100 cc. of blood delayed blood clotting to about 50 minutes, whereas Stuber and Lang found a delay of 6 to 12 hours in clotting of

blood of hemophiliacs with 2 to 4 mg. of fluorine per 100 cc. of blood. Feissly *et al.* found generally less than 1 mg. per 100 cc. of blood in 5 hemophiliacs and concluded that fluorine is not a factor in this condition in either delay of glycolysis or blood coagulation.

Foit (1931), in a study of the mechanism of blood clotting, found 20 to 30 p.p.m. of fluoride ion tended to oppose the action of antithrombin. Coagulation was prevented by 800 p.p.m. fluorine and was checked significantly at 450 p.p.m. Foit found the blood fluoride of two hemophiliacs to be in the normal range. Blood calcium in rabbits was diminished by the injection of fluoride solution. No coagulation was produced by adding lactic acid to fluoride plasma. Addition of tissue extracts to recalcified plasma caused coagulation but only indefinite effects on glycolysis. Symptoms of acute toxicity of injected sodium fluoride in rabbits included hemorrhage in the thymus.

Gautier and Clausmann (1913c) found 4.6 p.p.m. fluorine in human blood.

Purjesz, Berkessy, Gönczi and Kovacs-Oskolás (1934) could find no fluorine in the blood of normal man.

Goldemberg and Schraiber (1935a) found the fluorine of whole human blood and of plasma to be of the order of about 0.5 to 0.8 p.p.m. There was less or no fluorine in the erythrocytes. In one or two samples of cerebrospinal, ascitic, and pleural fluids, plasma levels of fluorine were found. Bazille (1935) found more fluorine in plasma than in erythrocytes.

Hartmann, Chytrek and Ammon (1940) found the blood fluoride content of 10 normal human subjects to vary from 27 to 74 micrograms per 100 cc. or 0.27 to 0.74 p.p.m. They separated the fluorine of blood to alcohol soluble and insoluble fractions, but found such variation they could draw no conclusions.

Fluorine in Saliva

Boissevain and Drea (1933) found no fluorine in human saliva by spectrographic analysis.

Goldemberg and Schraiber (1935b) said that saliva is the secretion with the lowest fluorine content. They gave no figures. Cox (1940) wrote: "Cox and Matuschak have analyzed a single sample of stimulated saliva pooled from three children each from Galesburg and Quincy, finding 0.095 and 0.12 parts per million of fluorine, respectively."

McClure (1941a) analyzed pooled stimulated salivas from groups of 40 to 200 children and from 100 cc. samples from individuals. For 11 individual children of Amarillo, Texas, where the community water supply had 3.8 to 4.0 p.p.m. fluorine, the fluoride of saliva ranged from 0.13 to 0.24 p.p.m. Ten samples from adults in a community with a fluoride-free water supply ranged from 0.04 to 0.14 p.p.m. fluorine. The pooled salivas ranged from 0.06 to 0.16 p.p.m. fluorine, with samples from Galesburg showing 0.16 and those from Quincy 0.14 p.p.m. Because the sensitivity of the analytical method did "not permit any significance to be attached to differences of 0.05 parts per million or less," McClure did not conclude there was any relation between the level of fluorine in saliva and the level of drinking water between 0.0 and 1.8 p.p.m. Also, in considering the caries rates of Elmhurst, Joliet, Maywood, Aurora, Evanston and Oak Park, McClure said: "According to the results for the fluorine content of the saliva of subjects representing these respective cities, an increased amount of fluorine in the saliva is not responsible for the observed differences in the caries rates of these cities."

Wills (1940) injected intravenously 10 mg. of sodium fluoride, containing radioactive fluorine for tracing purposes, into cats and then stimulated salivary secretion.

He found radioactive fluorine in the saliva within one minute.

Volker, Sognnaes and Bibby (1941) injected intravenously into four cats 10 mg. of fluorine as sodium fluoride containing radioactive fluorine (half-life 112 minutes). The excretion in the urine ranged from 10.4 to 22.7 per cent of the total dose; that in the saliva, 0.0540 to 0.1102 per cent. "Urinary and salivary secretion of the isotope occurred in appreciable amounts only when blood concentration was elevated."

Placental and Mammary Transmission of Fluorine

Gautier and Clausmann (1913c) reported the mean fluorine content of four samples of human milk to be 0.48 p.p.m.; in cow's milk they found 1.8 p.p.m. Stuber and Lang (1929) could find no fluorine in cow's milk. Boissevain and Drea (1933) failed to find fluorine in milk.

Phillips, Hart and Bohstedt (1934a) fed rock phosphate, yielding up to 880 p.p.m. of fluorine, in the ration of cows. Milk from these cows was fed to rats for a period of 140 days. The analyses of the milk and of the rat carcasses show little or no transfer of fluorine. The authors concluded that the fluorine content of normal cow's milk may vary from 0.05 to 0.25 p.p.m., and that fluorine in concentrations over 0.1 p.p.m. "has no essential function in the metabolism of the rat." Their only test of function was growth of 36 rats divided into 12 lots.

Constantini (1934) gave 1.5 gm. of NaF daily to a 54-kg. lactating goat. This dosage, about 30 mg. per kg., caused milk production to drop in 7 days from 600 cc. to 15 cc. per day. Fluorine was found in traces in the milk, and Constantini expressed the opinion that its concentration was of the order of 10 p.p.m. He ascribed no importance to such transmission of fluorine through milk as a cause of toxicity.

M. Ç. Smith and Smith (1935b) stated that they have observed mottled enamel of the deciduous teeth of breast-fed children. They gave no details other than that the mothers were drinking water with an extremely high fluorine content.

Smith (1935) said: "Our experiments with rats have shown that even though we feed a high concentration of fluorine (0.05 per cent NaF in the diet) to pregnant females, their offspring have normal teeth at the time of weaning, which later become mottled when the young rats have access to the food of the mother animals."

Reid and Cheng (1937) fed from 1.8 to 9.0 mg. of fluorine per 100 gm. of food to rats during pregnancy and lactation. The offspring at birth had, as a mean of 20 rats, 0.024 mg. of fluorine per animal and were given no added fluorine. At 2 to 3 weeks of age the fluorine content ranged from 0.300 mg. per 100 gm. for control rats to 2.340 mg. for offspring of mothers receiving the highest dosage. A linear relationship was found for intermediate amounts of fluorine fed.

Von Fellenberg (1937) found 0.14 p.p.m. fluorine in milk. McClure's (1941a) analyses showed 0.16, 0.13, and 0.14 p.p.m. fluorine in cow's milk. McClendon, Foster and Supplee (1942) found the fluorine of milk solids to vary from 0.4 to 4.4 p.p.m., with a mean of 1.2 p.p.m. for states north of 40° latitude and 1.7 p.p.m. for those south. (The difference of the extreme values, 4.0 p.p.m., is equivalent to 0.5 p.p.m. in liquid milk; between the means, 0.5 p.p.m. on the solid basis is equivalent to 0.06 p.p.m. for the liquid basis.)

Murray (1936) found the mean fluorine content of the wet weight of 5 litters of newborn rats to be 1.1 p.p.m.; in 5 litters from mothers receiving a ration with 500 p.p.m. NaF (about 225 p.p.m. F) 5.1 p.p.m. was found. By transferring newborn rats from fluorine-fed mothers to controls and vice versa, she also showed, by analysis of the bones of the suckling rats (killed at 21 days), that fluorine was transmitted in rat milk. The incisors of young rats from mothers fed with 500 p.p.m. NaF during

both pregnancy and lactation were "slightly opaque and whiter" than those of control rats. Her data indicated that approximately the same amount of fluorine was acquired by placental and by mammary transmission.

Evans, Phillips and Hart (1938) analyzed the bones of embryo (2 to 10 weeks), fetal (4 to 10 months) and term veal calves for fluorine. On the basis of bone the fluorine content was comparable and of the order of about 35 p.p.m. On the basis of ash, however, it was highest for the embryo stage (92, 64 and 55 p.p.m., respectively). Fetal cartilage contained 10 p.p.m. F on dry weight basis. The residue from mature cow bone extracted with HCl to 0.4 per cent ash contained 4 p.p.m., but 1000 p.p.m. were found in the ash of the residue. Mature cow bone showed 190 p.p.m. F in fresh bone; in samples of bone meal, 460 p.p.m. Evans *et al.* concluded that small amounts of fluorine are transferred through the placenta under normal conditions and also that small amounts pass into milk.

Knouff, Edwards, Preston and Kitchin (1936) could not detect any fluorine in the whole carcass of a term fetus from a bitch fed 5 p.p.m. fluorine during the last 8 weeks of pregnancy. When 25 p.p.m. was fed, the fetus contained 730 p.p.m. In a fetus aborted because of increasing doses of sodium fluoride, 3250 p.p.m. was found.

Evans and Phillips (1939) found an average of 10 micrograms of fluorine in newborn rats from mothers on a low fluorine diet. This had increased to 22.5 micrograms at weaning, in 21 days. Addition of fluorine to the basal ration increased placental transfer but, up to 20 p.p.m., had no effect on mammary transmission of fluorine. They calculated 33 per cent of milk fluorine appeared in the newborn rats, although only 5.1 per cent of the total solids of milk ingested by the mother had so appeared. This suggested a preferential storage. The fluorine of rats that had produced litters was lower than that of lot mates with no litters.

Halpin and Lamb (1932) fed to growing chicks a ration containing rock phosphate as 1, 2 and 3 per cent. The rock phosphate contained 3.52 per cent fluorine and hence the rations 352, 704 and 1056 p.p.m. respectively. "The data from three growth experiments show quite definitely that there was no harmful effect at the 1 per cent level with the basal ration used. The 2 per cent level showed some depression of growth, and the 3 per cent level was seriously harmful." The two lower levels had no effect on 5-months egg production, but the rock phosphate "apparently decreased egg production." There was no "measurable effect on the ash content of tibiae of the birds at 17 to 20 weeks of age. There was, however, a considerable incidence of crooked breastbone in one experiment at the 2 per cent and 3 per cent levels."

Phillips, Halpin and Hart (1935) found a maximum of 3.3 p.p.m. of fluorine in egg yolks of hens on rations containing up to 1050 p.p.m. of fluorine as rock phosphate. Their results indicated that "3 to 5 dozen eggs from hens fed 3 per cent of rock phosphate would furnish about 1 mg. of fluorine." The fluorine was concentrated in the yolk, the whites containing 0.1 to 0.5 p.p.m.

Purjesz, Berkessy, Gönczi, and Kovács-Oskolás (1934) could find no fluorine in normal hen eggs. Following injection of 30 mg. of NaF daily per hen, they found a maximum of 0.463 p.p.m.

Fluorine in Urine

Gautier and Clausmann (1913c) reported human urine to contain 0.18 p.p.m. and the urine of a cow and of a calf to have 0.13 and 0.11 p.p.m., respectively. Goldemberg and Schraiber (1935b) estimated that residents of Buenos Aires eliminate 0.9 to 1.1 mg. of fluorine per 24 hours, 80 per cent being in the urine and 20 per cent in the feces.

Machle (1936) determined the fluorine concentration in the urines of 101 adult subjects from widely scattered low-fluoride

areas of the United States and Canada. Similarly, analyses were made of the urines of 19 women and 19 children, hospitalized for various reasons. There was no difference in the distributions of the results. The means were 1.07, 1.11, and 1.08 p.p.m. of fluorine, respectively, with four subjects exceeding 2 p.p.m. Since these subjects, especially the women and children, were using waters with fluorine content of the order of 0.1 p.p.m., Machle indicated that the fluorine in these cases must be derived mainly from the food. No difference in metabolism of fluorine by children and adults was suggested.

Machle, Scott and Treon (1939) found a mean fluorine content of 17 urine samples from Arizona as 3.51 p.p.m. They found a positive correlation of the fluorine content of the drinking water and the concentration of fluorine in the urine.

Machle, Scott and Largent (1942) determined the fluorine balance of a normal adult subject over a period of 20 weeks. The mean daily intake of fluorine from foods was 0.155 mg., from drink 0.299, or a total of 0.457 mg. The output of fluorine per day was 0.059 mg. in the feces and 0.415 mg. in the urine. They concluded there was no measurable storage of fluorine.

Machle, Scott and Largent estimated the fluorine concentration of sweat, obtained under forced conditions, as one-sixth that of urine (0.514 p.p.m. compared with 3.256 p.p.m.). "Under conditions of excessive sweating, therefore, the possibility of definite loss of fluoride must be considered." They found the fluoride content of inguinal and axillary sweat the highest. In 0.35 gm. of finger nail clippings they found 7 micrograms of fluorine, or 20 p.p.m.

Machle and Largent (1943) gave a single subject 2 mg. of fluorine 3 times daily as: sodium fluoride (14 weeks); calcium fluoride, in solution (4 weeks); calcium fluoride, solid (3 weeks); bone meal (5 weeks); and cryolite (3 weeks). A control period of 20 weeks preceded the sodium fluoride period and intermediate control periods of from 4 to 12 weeks were used between experimental periods.

It was found that absorption of added soluble fluorides was practically complete, as indicated by only a fourfold increase in fecal fluoride, which was only 0.04 to 0.06 mg. per day during the control periods. Absorption of fluorine from solid cryolite was 77 per cent, from solid CaF_2, 62 per cent, and from bone meal, 37 per cent. Of the absorbed fluoride, 52 per cent was retained, presumably in the bones, irrespective of the source of the fluorine. There was a lag of a few days in the excretion of fluorine at the beginning of each experimental period and a similar lag in decrease to normal at the end. Machle and Largent explained this as mainly saturation and desaturation of the soft tissues. They derived linear relations between absorption, storage and urinary excretion and concluded, "Within limits, urinary fluoride excretion may, therefore, be used as a measure of fluoride storage."

Largent and Moses (1943) observed an excretion of fluorine in the urine of a single subject averaging 0.666, 0.554 and 0.562 mg. per day for 7, 5 and 23 periods, respectively. The average daily fluorine of the feces was 0.055 mg.

McClure and Kinser (1944) compared the concentrations of fluoride in the pooled urines and in the drinking waters of over 1900 young men. They found that when the fluoride content of the water was above 0.5 p.p.m. the concentration of fluoride in the urine was approximately that of the water. Attainment of this equilibrium was achieved immediately, in the experience of McClure, as he moved from one area to another. In areas where the fluoride content of the water was below 0.5 p.p.m., the fluoride concentration in the urine was 0.3 to 0.5 p.p.m. McClure and Kinser ascribed this excretion to fluorine from foods and regarded it as independent of the locality.

Summary

1. Skin, hair, nails, scales, horn, hoofs and feathers may have a fluorine content somewhat higher than that of the soft tissues but lower than that of bone, dentin and enamel.

2. Modern values of the fluorine contents of plant foods indicate that most of them are less than 1 p.p.m. and that the fluorine content of an average mixed diet from plant sources would be under 0.5 p.p.m.

3. The fluorine content of normal human blood is of the order of 0.5 p.p.m. and is mainly in the plasma.

4. It is doubtful that the clotting time of blood is related to its fluorine content.

5. The fluorine content of human saliva is of the order of 0.1 p.p.m. and is uninfluenced by the fluorine content of the drinking water up to 1.8 p.p.m.

6. There is a slight but definite transfer of fluorine by the placenta, and it is somewhat proportional to dietary fluorine.

7. There is a very slight transfer of fluorine to eggs, even with a high fluorine content of the diet of the hen.

8. The fluorine content of cow's milk is of the order of 0.15 p.p.m. The mammary transfer of fluorine may be increased by increasing the fluorine content of the ration.

9. Urinary fluorine bears a constant relation to fluorine absorption.

10. The fluorine of feces is relatively constant and of the order of 0.05 mg. per day on a normal soluble fluorine intake.

MOTTLED ENAMEL WITH SPECIAL REFERENCE TO DENTAL CARIES

Mottled Enamel Observations Prior to About 1915

Magitot (1878) considered erosion, now designated "hypoplasia," as conducive to caries. It is possible that some of the teeth with yellowish and brownish color described by Magitot were affected by mottled enamel.

Kühns (1888) reported the observation of black spots in the teeth of a family from Durango, Mexico. He associated the condition with water from an iron-bearing hot spring and with a nearby iron mountain, the Cerro del Mercado. The spots could not be removed by bleaching. He considered the color to be manganese salts converted to manganese dioxide under the influence of iron and light. The effect of light was introduced because the anterior teeth were the ones affected. He said the occurrence of these black teeth in Durango was known throughout Mexico. He stressed the fact that the spots were in and not on the teeth.

Vainicher (1891) recorded on page 55 of his book on caries, and showed in a plate facing page 56, a form of erosion. Vainicher being a Neapolitan, it is possible that he observed mottled enamel, since the condition was later found to be common in the vicinity of Naples.

Spokes (1897) discussed hypoplasia in 2000 children of London. The incidence was 7.0 and 7.5 per cent for 841 boys and 622 girls, respectively, from the City, Southwark and St. Savious's Parish, and 16.5 per cent and 13.7 per cent for 103 boys and 80 girls from Mile End, Poplar and Bethnal Green. The first group of districts are in central London and the latter group in East End. Hypoplasia was seen in deciduous teeth. Grevers, of Amsterdam, in the discussion of Spokes' paper, said: "I may say the children in England are better off than they are in Holland, for in an orphan asylum which I have visited there, I have found the large majority of children affected with hypoplastic teeth," and, "In the higher classes of society in Amsterdam, I found the very large majority having hypoplastic teeth, and I was surprised to hear Mr. Spokes say that in England it was only 4 per cent. I am sure that is not the condition in Holland."

Eager (1901) reported on the occurrence of black teeth (denti neri or denti di Chiaie) among the natives of Pozzuoli, 5 miles from Naples. "This defect was first de-

scribed by Prof. Stefano Chiaie, a celebrated Neapolitan, and bears his name." Another form was "denti scritti" or writing on the teeth found in the teeth of children who summered in Pozzuoli. The defect was popularly ascribed to the use of waters "charged under pressure with volcanic fumes" or to the fumes themselves. Eager said: "Strong well-formed teeth not particularly prone to decay appear to be the rule among young Italians when they have not been subjected to the influence during infancy of the causes of Chiaie's disease."

The above brief report by Eager was abstracted in Dental Cosmos (44: 300–301, 1902) and thus came to the attention of McGhee (1912), who referred to the abstract in a routine statement on pigmented teeth. The brief mention of "brown or black discoloration of the enamel" was seen by McKay with Black (1916c) and thus the discovery of Eager's contribution was consummated, though McKay cited only the abstract, quoting it in full.

Fynn (1910) published a paper which had been read before the Colorado State Dental Association on July 13, 1909, on a defect in the teeth of 87.5 per cent of the children born and raised in Colorado Springs. He said: "Dr. Black has shown that in those cases which we are considering it is the cementing substance that is defective . . ." He considered three items as local: water, milk, and vegetables. He eliminated water because "analysis shows it to be exceptionally pure," and ascribed the cause to lack of calcium in local soils and consequent deficiencies in milk and food. There was no demonstration of such calcium deficiencies by analysis.

Noyes (1912) wrote: "The most recent work of Dr. Black shows the brown and mottled enamel of certain localities to be found associated with greatly freckled skin. Enamel, therefore, must be considered as epithelial in origin and ultimately from the epiblast, while all other calcified tissues are connective tissue and ultimately of mesoblastic origin."[1]

Bampton (1914) described cases of brown coloration and some roughness and pitting of enamel in two families (English). The condition was traced through five generations of one family and three generations in the other. It was said that the deciduous incisors of the first generation female of the first family were present at birth and were brown. No other deciduous teeth of this family were definitely brown. However, in the other family first and third generation deciduous teeth, as well as the permanent teeth, were brown.

Of the first family it was said: "The drinking water was originally of a 'hard' nature, but for the last few years has been 'soft'." Little decay was noted, but in two instances artificial dentures had been obtained for esthetic reasons.

Nothing was said of continuity of residence of either family but the statement on the water supply suggests that all members with brown teeth had used the same water. Bampton called the condition hereditary; if it were indeed mottled enamel it would be another instance of confusing a long continued dietary characteristic with hereditary influences.

Gasparrini and Piergili (1916) in a paper dated December 1915 described "denti scritti," a condition found at Capua and Naples and near Rome. They were interested in bleaching stained teeth and this particular condition responded well to their treatment. They were led to inquire into its origin and concluded it was due to the use of water with a low content of calcium. The condition did not exist in the deciduous teeth; they reasoned that this was because of the use of milk at this period. But the action of low calcium water on the permanent teeth during the act of drinking water was advanced as the cause. The analogy of building stones, which are readily susceptible

[1] See McKay, with Black (1916b), and McKay (1917).

to dissolution by water when under ground but harden on exposure to air, was offered to explain resistance of teeth of people not native to the endemic areas. They asserted that the condition never appeared in those who used spring water but was common among those using cistern water.

Gasparrini and Piergili undoubtedly were dealing with mottled enamel, but their observations on association with water were not accurate. McKay (1918) vigorously attacked their idea that "the superior incisors—the teeth most in contact with the water in drinking" are the most affected for that reason. He said: "Surely a water which was powerful enough to produce such a change by mere contact with, or laving of, the teeth would hardly have its action limited to or exhausted by so small an area of the denture, and surely so powerful a water would exert some similar influence upon the rest of the teeth."

Dick (1916) reported observations on the teeth in rickets in 1000 school children of the East End of London. The children were mostly Jewish and Dick pointed out that the dietary was especially rich in fats. He stated that "80 per cent showed distinct evidence of rickets" and also that "over 80 per cent of the children were breast-fed for from twelve to eighteen months." Dick observed that: "In the typical form of hypoplasia commonly met with, the teeth affected are the central and lateral incisors, the tips of the canines, and the crown of the first molars." He said: "In judging of the presence of hypoplasia, it was found practically impossible to make accurate observations on the temporary teeth of infants of school age. Caries was so universal and extensive that it completely masked the hypoplasia. It was evident that a hypoplastic condition of the teeth was common in the temporary set, and was, in all probability, the chief factor in bringing about premature decay. For the purposes of statistics only the records of the permanent dentition have been taken, and in marking a case as one of hypoplasia, only the severer forms are admitted, and cases of slight pitting, chalky-looking patches in the enamel, and so on have been excluded, although microscopically such teeth would be found markedly defective and liable to disintegration. Of the 586 rickety cases in which a record of the permanent teeth could be taken, 42 per cent had normal teeth and 58 per cent had defective teeth; 20 per cent of these showed hypoplasia frequently combined with decay, and 38 per cent had decayed teeth. This is not equivalent to saying that 42 per cent of school children have normal teeth. As already pointed out, all infants with a record only of temporary teeth have been excluded, because these teeth were so universally decayed as to make accurate observations on the structure impossible. Again, most of the children with records of the permanent teeth were about the ages of 12 or 13 years, when all the permanent teeth have been erupted except the third molars, and caries has least time to make its appearance. It is a somewhat quaint commentary on the general state of the teeth of the community to have to explain why only 58 per cent of the individuals are given as having defective teeth.

"Of the cases with carious teeth, the lower first molar was decayed in 80 per cent, the upper first molar was decayed in 30 per cent, one or more lower premolars in 30 per cent, and one or more upper premolars in 12.5 per cent. The incisors, canines, and second molars were seldom decayed."

Dick also stated: "Careful search for a hypoplastic condition of the milk incisors during a long period both in school children and in the babies who have attended the Hackney Mothers' Centre has failed to detect a case."

Sim Wallace, in the discussion of Dick's report, pointed out that, although rickets was very common, only 7 per cent of the children showed hypoplasia (corrected by Dick as 10 per cent). He also pointed out

that Dick had not compared the caries rate between the children with and without rickets. Also, "caries frequently does not affect hypoplastic teeth or teeth due to rickets—if that is a cause."

Dick (1918) studied "403 children of 11 years of age taken from the ordinary London County Council schools, but of these, 281 were children in schools where the nutrition was distinctly below the average." These latter were from the East End of London; the 122 from good-class schools were from North London. The distribution and classes of hypoplasia were: In 281 poor-class children, opaque chalky enamel 28.5 per cent, brown line of Retzius 13.0 per cent, hypoplasia 8.5 per cent, honeycombed teeth 2.5 per cent; in 122 good-class children the corresponding values were 9 per cent, 1 case, 5 per cent, 0.

The percentages of opaque chalky enamel affected teeth were: Central incisors 78 per cent, lateral incisors 55 per cent, canines 30 per cent; premolars 20 per cent, first molars 10 per cent, second molars 5 per cent.

"The first molars were frequently badly decayed or the tooth had been extracted." No further statement was made on caries. In the 38 cases with brown lines of Retzius, 36 had the central incisors affected.

Since Murray and Wilson (1942) found in the examination of 1400 London school children that "including all grades of fluorosis but excluding the questionable, 28 per cent of 589 London children between the ages of 10 and 15 had some mottled teeth," it would seem that most of the hypoplasias noted by Spokes (1897) and by Dick (1916, 1918) were mottled enamel and were not due primarily to rickets. It is of historical interest that Middleton reported fluorides in deposits from London water in 1845.

Mottled Enamel, 1915–1930

McKay, in collaboration with Black (1915), described "mottled enamel," a term introduced by Black as "suggested by the appearance of the teeth." Points emphasized were: (a) sharp restriction to certain geographical areas; (b) absence from deciduous dentition; (c) graduation of severity from paper-white teeth and white flecks to black stained and heavily pitted teeth; (d) the fundamental lesion as an absence of interprismatic substance; and (e) absence of the lesion from teeth formed outside the endemic regions. Though the studies made by McKay and Black were based on endemic areas in the Rocky Mountains, among other affected regions recognized was a "small district near Naples, Italy. . . . The etiology of this condition has not yet been determined, but the investigation has been prosecuted upon the assumption that water is the causative factor."

In the discussion, J. R. McQueen told of about 150 cases he had seen in Benton, California, where the water was supplied from hot springs. McQueen said, "I find these teeth are soft, ordinarily, but they withstand decay very well."

Rodriguez (1915) described "mottled enamel," or "brown stain," among the Pima Indians of Arizona. The subjects were also examined by McKay. Rodriguez said that "the stain is not causative of disintegration or related in any way to other dental affections." He pointed out that samples of the teeth were hard to obtain and high prices had been offered for extracted teeth. He threatened court action if anyone obtained Indian teeth by "any clandestine methods."

In 1906 dentists of the Rocky Mountain region told G. V. Black of a localized condition which they called Colorado brown stain. Black, in collaboration with McKay (1916), described the condition. He reported the gradation of color from paper white to black. Nasmyth's membrane was found to be normal. Only the outer third of the enamel seemed to be affected. "It was particularly notable that the lines and depths of the abnormal condition had no reference whatever to the lines of accretion

or growth in the formation of the enamel, thus showing a remarkable difference from the contemporaneous accretional deformaties of the enamel, in which the lines of accretion in the growth of the tooth are very closely followed." He found the cementing substance was lacking, the inter-rod space being either empty or filled with "brownin."

Black dissolved "brownin" from sections of mottled enamel with a sequence of absolute alcohol and gasoline and found the paper-white condition. This enamel would then be stained by a procedure that did not affect normal enamel.

Black said: "As to caries, the teeth of these children compare favorably with those of other communities where endemic mottled enamel is unknown. They have a mild climate and almost continuous sunshine during the day. The children are out practically every day the year round, and this in itself certainly has its effects in limiting the amount of dental caries. But when the teeth do decay, the frail condition of the enamel makes it extremely difficult to make good and effective fillings. For this reason many individuals will lose their teeth because of caries, though the number of carious cavities is fewer than elsewhere."

McKay, in collaboration with Black, (1916a) stressed the endemic nature of mottled enamel. He stated that he had never observed mottled enamel in deciduous teeth, and described the contrast of the temporary molars adjacent to newly erupted first permanent molars with mottled enamel. He said: "This mottled condition, in itself, does not seem to increase the susceptibility of the teeth to decay, which is perhaps contrary to what might be expected, because the enamel surface is much more corrugated and rougher than normal enamel. It is recognized, however, by dental practitioners dealing with this sort of enamel, that, caries having occurred, it is difficult at times to find enamel sufficiently dense in which to lay cavity margins."

An examination of 2945 children in relation to their history of residence established the fact of the connection of nativity with endemic mottled enamel.

McKay, in collaboration with Black (1916b), stated that "the clinical experience during this investigation does not confirm or support" the association of freckles and mottled enamel (Noyes, 1912). Data were given of the association of mottled enamel with residence of 3239 children. It was shown that, in children who entered endemic regions at various ages, there was a pattern of mottled enamel conforming to formation of enamel during residence in the area.

McKay said: "Even from the beginning of the notice taken of this lesion and before any definite steps were taken to study it, the sentiment of both the profession and the laity in the areas of susceptibility was that the water was in some way responsible." Analyses of the water of a city deriving its supply from melting snows high in the mountains indicated a low calcium content, but many other localities with high incidence of mottled enamel had high calcium water.

McKay, in collaboration with Black (1916c), continued the investigation of mottled enamel with especial reference to the water supply. He reported two cities "in one of the southern states along the Atlantic seaboard" as having mottled enamel, popularly believed caused by water from artesian wells drilled about 20 to 25 years earlier. To a question of whether there was connection between dental caries and mottled enamel, the answer given from one was "No" and from the other, "Teeth seem to be average in quality."

McKay cited the abstract of Eager's observations (1902) and revealed correspondence with Vincenzo Guerini of Naples, who wrote, "I can assure you, before all, that the name 'Chiaie' is not at all the name of an author, but that of a quarter of Naples. . . ." Guerini had observed mottled enamel, and mentioned it in a pamphlet *Odontologia Forense*. McKay reported extension of endemic areas to Texas. Photographs and a

description of mottled enamel sent to Carl Röse of Germany brought the reply: "The cause of the teeth becoming black . . . is not difficult of solution. I may tell you at once in advance that you have probably a very soft drinking water, poor in lime." McKay recorded his disappointment in Röse's reply, as he could find no correlation of calcium content of water with the dystrophy.

McKay, in collaboration with Black (1916d) reported other endemic areas of mottled enamel, particularly inhabited by Indians. He also considered "a possible relation which might exist between this enamel lesion and some corresponding lesion in bony development" but concluded "that the existence of this lesion of the enamel does not in any way imply any similar bony lesion."

McKay presented a summary of water analyses which indicated nothing but contradictions. He wrote: "There are present, however, in waters certain other elements of rarer varieties that exist only in traces, the determination of which requires much more elaborate technique and spectroscopic and polariscopic tests, which are beyond the capacities of the ordinary chemical laboratories."

The foregoing articles of Black and of McKay were ably summed by F. C. Smith (1916). He noted the implied association, also made by Eager, of mottled enamel with hot springs and vulcanism and the suggestion of trace elements as being involved in the etiology.

After the death of Black, McKay continued the investigation of mottled enamel. In 1917 he reported on further cases and stated his continued conviction that analysis of the enamel and of the water used during the development of the enamel would give the clue to the solution of the problem. He was concerned with the composition and origin of "brownin" but dismissed a suggestion that it may be associated in some common cause with freckled skin, as two of three cases cited in this paper had no freckles. In 1918 he reported on the almost perfect association of mottled enamel in South Dakota with the use of water from artesian wells. He traced the origin of the water in the Dakota sandstone to the Rockies and linked this same formation with the Texas endemic areas.

McKay (1918) records a communication from Prof. John E. Grevers of Utrecht concerning the occurrence of mottled enamel in Holland and his studies on rats raised with various waters. He quotes from Grevers: "In comparing the teeth of these rats with those of wild rats, one is struck with the whiteness and lack of lustre."

McKay (1919) examined cases of mottled enamel in Virginia associated with both dug wells and artesian supplies. Also on Nantucket Island, Massachusetts, he saw the worst cases of mottled enamel in Portuguese immigrants from Cape Verde Islands. He established by correspondence that it was only on the Island of Brava that mottled enamel was found, and it was associated by some with water supply. Analyses of waters of endemic regions in the United States were continued with no clue to the causative factor. No mention was made of caries.

Wofford (1923) reviewed the knowledge of mottled enamel. He made no statement on dental caries.

Akeroyd (1923) found mottled enamel in all children born or raised in Columbus, New Mexico, and stressed the point that it appeared only on the second molars of children who entered Columbus at about nine years of age. He outlined, among other features: "Deciduous teeth are never stained. . . . Lack of dental caries very noticeable. However, when caries is present the frail chalk-like condition of enamel and the apparent relative hardness of the underlying dentine make it extremely difficult to insert good and effective fillings." He also said, using italics: "*Prevention is assured only by distillation of available water or importation of water from non-endemic*

district." He gave no data to support this statement, which is apparently only a hypothesis.

Williams (1923) studied sections of mottled enamel teeth obtained from McKay. He found silver nitrate penetrated the defective enamel comparatively rapidly. The stain at the zone where normal and mottled enamel met was found surrounding the rods. Williams saw under high magnification defective formation of the enamel rods. The granules and spherical bodies were imperfectly fused.

Velu (1923) reported an enamel condition of man and animals which affected the second dentition, later identified by Velu and Balozet (1931b) as "darmous." The condition was found to be regional. Velu dismissed congenital effects, water and pasturage as causal and entertained the idea that gaseous emanations from the soil were the causative factors.

Grabham (1923) associated the very low caries rate of the inhabitants of Porto Santo with the water supply. His entire paper on page 480 of the Report of the British Association for the Advancement of Science, is as follows:

"The object of this paper is to stimulate inquiry and to suggest that the mineral waters of the island possess some influence in resisting the development of caries. Porto Santo is a small island of the Madeira Archipelago, and its water-springs are highly mineralized with chlorides, carbonates and sulfates in contrast to the sweet waters of the principal island. The outstanding features of the people's diet are that the food is taken cold, no green vegetables or milk are included, and there is nothing to require grinding mastication. The people drink moderately. Consumption is frequently present, while there is no scurvy, no alimentary disorders, and no malignant disease as far as the author can ascertain. But the teeth of the district are characterized by a thin yellow line across the upper incisors, which in after-life spreads and stains the teeth generally. The stain is unknown in Madeira. The writer states with confidence that the yellow stain on the incisors is a sure indication of a sound set of teeth, and its regular occurrence is believed to furnish conclusive evidence of the permeation of the blood fluids in the interstices of the columnar enamel, and is certainly due to the same constituent in the local and highly mineralized water."

A statement in the British Medical Journal (Anonymous, 1923a) included the foregoing but added some further details. Grabham examined 600 of the 3000 inhabitants. Among them he found "28 cases of well established caries. All these people except seven had come from Madeira, and only two of the seven showed the sign which characterizes the Porto Santo dentition." It was noted that "Trachyte is the main feature of the Porto Santo volcanic ejecta, as compared with the basalts of Madeira."

Harris (1924) recorded mottled enamel in Massachusetts but in subjects of origin elsewhere. Ten were from the Azores Islands, six from Cape Verde Islands, and one from Nova Scotia. Those from the islands had been reared on water from lava formations. He said: "In all cases the permanent teeth were the only ones affected and the superior incisors were commonly darker in color than any other teeth affected. In general, dental caries is more prevalent than can be considered usual." It is interesting to note that, in a brief passing statement of the composition of average enamel, he listed "Calcium Phosphate and traces of Fluorid."

Fossum (1924) reasoned that enamel must be formed perfectly or not at all, and therefore mottled enamel could only be caused by some postformative agent. He decided that acid was the only possible explanation of the corrosion of enamel and ascribed its source and assigned its site of action as the gum line. According to Fossum, the erosion occurred as the teeth were in the process of eruption. The acid was said to be

formed during the childhood diseases, and measles, whooping cough, tonsilitis, chicken pox, typhoid fever and "flu" were mentioned. Though Fossum recognized that his observations were restricted to South Dakota, he offered no explanation as to why mottled enamel did not occur in the non-endemic regions. He dismissed water as having any relation to mottled enamel because for 10 years he had exposed a tooth to artesian water, with repeated changes, but no mottling resulted. This paper was reprinted in essentially the same form 4 years later (Fossum, 1928).

Schulz and Lamb (1925) found that the upper incisors of rats fed 0.05, 0.10 and 0.15 per cent of sodium fluoride in the ration "showed an excessive growth."

McCollum, Simmonds, Becker and Bunting (1925) "were led to consider whether perhaps a deficiency of fluorine in the food might lead to the formation of teeth which had poor structure, and consequently possess little power to resist the agencies which lead to decay." They fed 226 p.p.m. of fluorine as sodium fluoride to rats. This amount of fluorine was "comparable to what might be expected to occur in natural foods." Their assumption was presumably based on reports such as that of Gautier and Clausmann, who reported 5.9 to 1,380 p.p.m. of fluorine in 63 plant tissues, with a mean of 265 p.p.m.

McCollum, Simmonds, Becker and Bunting reported "marked overgrowth" of the maxillary incisors of fluoride-fed rats. They found the incisors to be "of a dull, opaque white color" and to "lack the natural polish of well formed tooth substance. In certain areas they are corrugated transversely, suggesting intermittent interferences with development." McCollum *et al.* suggested that the excessive length of the upper incisors resulted from lack of natural wear by contact with the lower incisors, which were friable and had been fractured. The molars were normal and showed "no evidences of caries." It is interesting to note that in reference to the faulty incisors they used the term "mottled areas."

McKay (1925) reported mottled enamel in Benton, California, with the history of use of water from a warm spring. At Oakley, Idaho, McKay participated in a mass meeting the day preceding an election involving a bond issue to change a water supply, of which he said: "I believe it to be the first instance in the annals of dentistry where a policy of municipal economics of such magnitude as is involved in the abandonment of a water system and requiring a vote of the people, has been determined solely by and upon a dental aspect." On the recommendation of McKay, a spring was then used that by biological test, namely, no mottled enamel in children who had used the water, promised to be non-injurious. (See p. 364 for sequela.)

McKay in this paper (1925) again discussed dental caries but now on the basis "that the incidence of caries in mottled enamel was no greater than in 'normal' enamel," though he also said: "It has been repeatedly noticed in the examinations of afflicted districts during the past ten years, not only by myself but by others, that there was a curious absence of decay." He asked: "Why is it, then, if the integrity of the enamel is held to be the determining factor in caries, that mottled enamel, which is so obviously defective, is able to escape carious attack with not more than average frequency of incidence?"

Beust (1925) presented histologic evidence that the mottled enamel originates from the outside of erupted teeth. He partially decalcified enamel experimentally "by immersion in chlorin, sodium and sulphur mixtures. . . . Such altered enamel manifests itself as does mottled enamel, the initial attack occurring on irregular spots or areas, gradually affecting larger surfaces as the exposure to the agencies is prolonged. . . . My conception is that mottled enamel is enamel not fully calcified that has been attacked during or shortly after the

eruption of the tooth." In conclusion, Beust stated he had produced artificial mottling with (a) sodium bisulphite, (b) sulfurous acid, (c) hydrogen sulfide, (d) hydrochloric acid, (e) acetic acid and (f) chromic acid; but in verbal discussion recorded at the end of the paper he said he "was in hopes some remarks would be made concerning the possibility of elements in the water that might produce such changes."

Beust (1926) advanced his theory of the chemicotraumatic origin of mottled enamel, contending that: "The agency causing the lesion enters the enamel from without centripetally, traverses these capillaries, etches their walls and apparently dilates them."

Pierle (1926), by questionnaire methods, mapped an area of mottled enamel of about 70,000 square miles in Texas, with a total population of about 450,000. He summarized many of the facts about mottled enamel, notably that (a) mottled enamel is very rarely seen on deciduous teeth, (b) teeth erupt with the paper-white aspect, (c) these teeth later become stained brown and (d) children who move into endemic areas are not affected in teeth of which the crowns are already formed. He believed the dystrophy was one of a calcium deficiency, and he produced mottled teeth in rats born and raised on a calcium-deficiency diet of meat, whole maize, wheat bran and butter fat, in unstated proportions, with green vegetables twice a week and water from wells which produced human mottled enamel. The "mottling took the form of white lines, in spots visible along the sides of the upper incisors." The upper incisors of one of the rats were "pulled out of alignment to such a degree that the animal ate with difficulty." The water contained 243 p.p.m. CaO. A second water with 14.6 p.p.m. produced mottling, but two other waters with 127.3 and 18.8 p.p.m. were without effect. Apparently only one female was used with each experiment, though several litters from each were studied.

The above papers of Beust and of Pierle were discussed together. It is of interest that Bunting, who had been associated with McCollum in producing overgrown incisors in rats with fluoride, participated in the discussion but only dealt with Beust's paper. Price, who had been associated with McKay in his early work through the Research Commission, said, in regard to water samples analyzed for McKay, "we found a wide variance in chemical content, and the probabilities were then, in the light of our knowledge, that it was the absence of something in the water."

McKay (1926), at the invitation of the editor of Water Works Engineering, reviewed the evidence that mottled enamel was caused by something related to the water supply and asked for more information from water engineers and for interpretation of water analyses. He pointed out that no relation had been established with calcium content nor any relation of iron content of water to staining.

The article above drew replies from 18 water works men (Anonymous, 1926b) and editorial comment (1926). Notable among the replies was that of Frank Hannan, chemist, Filtration Plant, Toronto, Ont. (p. 934, Anonymous, 1926). He said in part: "Since the enamel is essentially mineral in composition and the water definitely incriminated, the mineral content of the water seems the probable source of the trouble. Of the mineral elements at present known to be common to both water and enamel, the chief are calcium, phosphorus, and fluorine. For our intake of phosphorus, we are independent of the small proportion found in water; the same can be asserted of calcium with perhaps a shade less certainty, a dietary deficient in this element being not altogether unusual. But when we consider fluorine, all is at present shrouded in obscurity. The detection and estimation of small traces of fluorine are tedious and

troublesome and quite outside the province of the ordinary water works chemist who has to handle many thousands of samples in a year. The French chemist Gautier found fluorine practically universally present in water. That it cannot exist there in more than traces is fortunately secured to us by the low solubility product of calcium fluoride, fluorine in sizable doses being a rather powerful poison." He continued by suggesting that mottled enamel was caused by a deficiency of fluorine in the waters.

The others suggested, variously, acid, manganese, magnesium, radioactive substances, and trace elements in general. Some maintained it was caused by foods.

McKay (1926) replied to the suggestions and criticisms but not to the fluorine idea contributed by Hannan.

Bergara (1927a, b) fed three dosages, namely, 64, 285 and 625 mg. of sodium fluoride per kg., daily, to rats for four months. The outstanding new dental manifestation observed was the appearance of alternate white and chocolate-colored horizontal striations in the enamel of both upper and lower incisors. He noted that the upper incisors tended to become longer and the lowers, shorter.

McKay (1927) reported that R. S. Weston of Boston had analyzed brown stain from mottled incisors from Oakley, Idaho. McKay pointed out that specimens of mottled teeth were hard to obtain. These teeth were extracted because of an accident, and by chance McKay had a photograph of the subject before the teeth were lost. Weston reported that the stain contained manganese. McKay was not too convinced and indicated that if manganese were the stain or cause of the stain, it might have come from food or other source subsequent to preeruptive effects of another water constituent.

McKay (1928) reported that on a visit to Naples and Pozzuoli, Italy, he found evidence, which he could not positively confirm, that the water supplies of these cities had been changed since Eager's (1901) report. Mottled enamel was no longer seen in children. But at Resina, on the slopes of Vesuvius, mottled enamel was to be seen in the adults only. He established that water had been piped into Resina in recent years and saw the evidence that wells in the city had been abandoned. In the country immediately surrounding Resina the people drank water from wells dug into the lava flows that buried Herculaneum. Both children and adults exhibited severely mottled enamel. McKay made no comment on caries.

McKay (1928), in combating the idea that structure influences caries, said, "The astounding fact is that mottled enamel shows no greater susceptibility to the onset of caries than does enamel that may be considered to have been normally or perfectly calcified," and, "My testimony has been supplemented by that of others, who report that these mottled enamel cases, in the various districts, are singularly free from caries."

Bunting, Crowley, Hard and Keller (1928) reported the results of a survey of mottled enamel in 103 children of Minonk, Illinois. Frederick S. McKay, Frederick Noyes, E. H. Hatton, Philip Jay and Martha Jones collaborated. "The purpose of the survey was to determine the relationship of mottled tooth formations to the prevalence of dental caries and the presence of *B. acidophilus* in the mouth." They found that "the prevalence of dental caries among these children, the percentage of those affected, was about the same as would be found in any other community, but although caries occurred in the mouths of most children, its extent and activity were remarkably limited. The great majority of cavities consisted of small pit and fissure lesions in the molars and seldom did caries extend beyond that stage. In this respect the behavior of dental caries in the mouths of these children is distinctly different to that which usually occurs.

"The bacterial cultures taken from this

group did not show the same correlation with the activity of dental caries as is found in other communities. So far as may be seen from the tabulations, the deviations from that which usually occurs are not in any one particular direction."

McKay (1929) said of the Minonk, Illinois, survey: "So far as determining the prevalence of caries in mottled enamel as compared with normal enamel, the result of this examination was inconclusive, for the reason that we made no attempt to differentiate as to whether caries had occurred in the *mottled* enamel or in *normal* enamel."

McKay (1929) reported the data of mottled enamel and dental caries as found in children of Towner, Colorado, 55 persons examined; Bruneau, Idaho, 54 persons examined; and Pima Indian School of Sacaton, Arizona, 78 persons examined. He said: "My own conviction, prior to these examinations and based on the observations of the past several years, was that mottled enamel was not more liable to decay than was normal enamel, but to find it consistently less liable in these communities was a complete surprise."

It was noted that there was very little decay in the incisors. The indications of the above analysis are that such decay as did occur in the other teeth was more extensive than in those with mottled enamel, but definite conclusions cannot be drawn from the data presented, one reason being that there was so little decay in any of the teeth.

McKay said, using italics: "*It is not to be understood that in presenting these findings there is any intention to establish that mottled enamel should necessarily be any the less liable to decay.*

"The evidence presented is to show that mottled enamel, by reason of its defective structure, *is not thereby rendered more liable to decay than is normal enamel*, but that when it does decay the same surfaces are involved as in normal teeth, and for precisely the same reasons."

McKay pointed out the opportunities for the study of caries in such populations. It is to be noted that teeth designated as "normal" by McKay were really "not mottled."

Kempf and McKay (1930)[2] investigated the severe mottled enamel in Bauxite, Arkansas. The condition was directly related to wells drilled in 1909 and later. The records and rent rolls of the Aluminum Company of America provided an accurate means of determining the place and duration of residence of each family. Many specific variations of residence made it possible for Kempf and McKay to conclude that mottled enamel was present only in teeth that were formed during residence in Bauxite. Children who had been away from Bauxite for a period of years and then had returned showed normal enamel in the teeth formed in the interim. Children of Benton, Arkansas, about five miles away, had normal teeth unless they had been former residents of Bauxite. Third molars were mottled by residence after about 11 years of age. Kempf and McKay recorded that Bauxite changed its water supply to the same source as that of Benton.

Kempf and McKay appended a bibliography of known papers relating to mottled enamel.

McKay (1930) reviewed the known geographical distribution of mottled enamel and gave a bibliography of papers (47 entries) that was practically a duplicate of the one given by Kempf and McKay (1930).

Anderson and Stevenson (1930) reported that mottled enamel was a very common condition in North China. Their data suggested a decrease in incidence with ageing. They considered mottled enamel "part of

[2] In the introductory paragraph Kempf and McKay (1930) referred to "denti di Chiaie" as applying to Stefano Chiaie "who first described the condition" indicating that McKay had not accepted Guerini's explanation of the name.

a syndrome of constitutional defect, probably of early malnutritional origin, and the diminished occurrence of the condition among those of the more advanced age-groups merely reflects the selective discrimination of diseases in general against the portion of the population thus affected."

They recorded chalkiness and "brownin" stain separately, in a scale of 0, 1, 2, 3 and 4.

The paper of Kehr (1931) is of particular interest as having been published in February, just prior to the first announcements of fluorine as the causal agent of mottled enamel. Kehr concluded that mottled enamel of children of several Kansas communities was definitely associated with the water supply. Water analyses gave no clue as to cause. Manganese was absent in two analyses and present in two others, at less than 0.1 p.p.m. and 0.25 p.p.m. respectively. He could find no reason in health records to support Fossum's (1924) theory of childhood diseases as causal. Wolman, who discussed the paper, said: "No definite conclusions can be deduced at the present time other than that this enamel defect occurs in certain areas of the United States, and the causal factors seem to be definitely associated with the water supply of these areas."

Observations on Mottled Enamel after 1930

Theriault (1931), as secretary of the Water, Sewage and Sanitation Chemistry Section of the American Chemical Society, reported (April 10, 1931): "Particular interest was manifested in a discussion of the tooth defect known as mottling. The dental condition is characterized by the formation of a heavy brown stain on the teeth, which have the appearance of being corroded. The structure of the teeth indicates decalcification. This disfiguring defect has long been recognized by the dental profession and, on the basis of epidemiological evidence, has more recently been ascribed to some undetermined constituent or characteristic of the water supplies. On the basis of spectrographic and other evidence, H. V. Churchill, chief chemist of the Aluminum Company of America, reports the presence of fluorine in waters from the affected localities. This unusual constituent of water supplies appears to be present as calcium fluoride. The amounts present range from 2 to 17 parts per million in samples collected from water supplies in Arkansas, Colorado, North Dakota, and South Dakota, where 'mottling' is prevalent. If sulfuric acid is added to these waters, the water will etch glass because of the evolution of hydrofluoric acid. While no definite causal connection has been established between the presence of fluorine in the water and the dental defect, the severity of the attack appears to vary directly with the amount of fluorine found. Ordinary methods of water analysis would fail to reveal the presence of fluorine in the water. Analyses of water from 40 representative American cities show that fluorine is present in only 11 cases and then only in traces. Investigation by dental authorities is being undertaken to determine the possible influence of large amounts of fluorides on dental structures."

In a communication dated April 27, 1931, Smith, Lantz and Smith (1931a) stated: "By several lines of evidence in our laboratory the destructive action of the water upon the developing enamel of the teeth has been shown to be due to its content of fluorine. A condition resembling mottled enamel has been produced in the incisors of rats by the use of water obtained from St. David, Arizona, an endemic community. This water was reduced to one-eighth of its original volume by evaporation and given to rats to drink. Water residues were incorporated in the rations of other rats. In both cases a defect of the teeth was produced which was similar, if not identical, to that condition produced in litter mates by the addition of sodium fluoride to their ration. The teeth were chalky white, and in many cases decidedly pitted."

They identified the condition of the teeth with that observed by McCollum, Simmonds, Becker and Bunting (1925), produced by fluoride feeding.

Churchill (1931) gave the details of his studies and reported the fluoride data by spectrograph of the waters of Bauxite, Arkansas; Colorado Springs, Colorado; Kidder, South Dakota; Lidgewood, North Dakota; and Oakley, Idaho; all these were cities with mottled enamel. The fluorine content was estimated as ranging from 2 p.p.m. at Colorado Springs to 13.7 p.p.m. at Bauxite. Fluorine was reported as present in the water supply of 16 large cities and absent from that of 10. No fluoride was found east of the Appalachian Mountains. He said: "It is well to emphasize the fact that no precise correlation between the fluoride content of these waters and the mottled enamel has been established. All that is shown is the presence of a hitherto unsuspected common constituent of the waters from endemic areas. However, it is of interest to note that apparently the relative severity of the defect in these various areas seems to follow the fluoride concentration."

Churchill credits his associate, A. W. Petrey, of the research laboratories of the Aluminum Company of America, with the original observation of fluorine in the waters of mottled-enamel regions.

Smith, Lantz and Smith (1931b) gave the detailed evidence from 39 families of St. David, Arizona, representing about 250 individuals of the population, showing the almost perfect association of mottled enamel with formation of enamel in an endemic zone.

Analyses of waters for the usual constituents gave no clue to the cause of mottled enamel. The water produced no effect on rat teeth, but when it was concentrated to one-tenth volume and provided all the drinking water for rats, they showed lesions on the incisors within a week. "Production of mottled enamel on the teeth of the experimental animals was accomplished in February, 1931." The chalky white, corroded appearance was identified with the effects produced by fluorine by Schulz and Lamb (1925) and by McCollum, Simmonds, Becker and Bunting (1925). Analyses of St. David waters, associated with mottled enamel, showed in all cases fluorine; analyses from water not associated showed little or no fluorine. Waters from other parts of Arizona showed a similar association of fluorine and mottled enamel. The waters which caused mottled enamel contained 3.8 to 7.15 p.p.m. fluorine; those which were harmless were estimated at 0 to 0.3 p.p.m.

McKay (1932) recorded that Churchill (1931) had written to him in January 1931 telling of his spectroscopic detection of fluorine in the water supply of a community (Bauxite, Arkansas) with mottled enamel. Churchill also pointed out that "the only known deposit of cryolite in the United States" occurs "on Pikes Peak in Colorado," the source of water for Colorado Springs, where mottled enamel was first observed by McKay. Churchill also pointed out that "fluorides are very often found in the vicinity of volcanic activity, and in those localities where hot or warm springs are encountered." McKay further recorded correspondence from Churchill, under date of March 5, 1931, on the quantitative analyses of some water associated with mottled enamel.

The discovery of fluorine by Petrey in Churchill's laboratory was made only incidentally to an examination of the water for aluminum. (Furthermore, it may be pointed out here, the detection of fluorine was possible because the water contained sufficient calcium to yield the broad calcium fluoride absorption band.)

The work of Smith, Lantz and Smith (1931b) was critically reviewed by McKay, and he was apparently completely satisfied that the trace element he had so long

considered as the possible etiological agent of mottled enamel is fluorine.

On May 18, 1931 the Pittsburgh Section of the International Association for Dental Research (Van Kirk, 1932, and Friesell, 1932) met to hear the reports of Churchill (1932) and Smith, Lantz and Smith (1932). Churchill reported the presence of fluorine in waters of endemic regions as being 2 p.p.m. or higher, and of non-endemic, as below 1 p.p.m. Smith, Lantz and Smith reported that water of St. David, Arizona, where severe mottled enamel was endemic, was 4 to 7 p.p.m.; water from non-endemic areas in Arizona had 0 to 0.72 p.p.m. St. David water concentrated to one-tenth mottled rat incisors. "The cause of 'brown stain' associated with mottled enamel was not discovered. Fluoride did not cause the stain. Manganese, reputed to induce it, was absent from the drinking waters of St. David, where the stain is conspicuously present in the mottled enamel."

Consequent to this meeting a somewhat condensed version of the paper of Kempf and McKay (1930), the entire paper of Churchill (1931) and a modified version of the report of Smith, Lantz and Smith (1931b) were printed. (These respective authors, 1932.) The latter authors in "Summary and conclusions," not reprinted from their previous work, stated that St. David water had 3.8 to 7.15 p.p.m. and that non-endemic communities had 0 to 0.3 p.p.m.

Velu and Balozet (1931a) produced "darmous" as observed by Velu (1923) in the permanent teeth of 5 sheep by daily feeding of 7.5 gm. of natural phosphate. The incisors formed prior to the phosphate feeding were normal. The first of the middle teeth were yellow and more or less eroded; the second middle teeth were much more affected. The incisors were delayed in eruption, said by Velu and Balozet to be characteristic of darmous. Velu (1931a) following the dietary procedure of McCollum, Simmonds, Becker and Bunting (1925), produced in rats severe fluorine intoxication, fluoride cachexia, with 15 mg. of calcium fluoride per rat per day. Velu and Balozet (1931b) produced darmous in 6 sheep, identical in all respects with that seen naturally, by giving them water saturated with natural phosphate. These authors related the observations of Velu in finding darmous associated only with waters drawn from strata adjacent to phosphate beds. (A discussion by Marchoux pointed out that they had not excluded some possible infection.) Velu (1931b) produced in rats all symptoms of fluoride cachexia observed by American authors by feeding Moroccan and Algerian natural phosphates. Velu (1931c) gave rats waters saturated with natural phosphates of Morocco and of Algiers and found the characteristic lesions of fluorine poisoning. He concluded that darmous is a chronic fluorosis of animal and man derivable from fluoride-containing waters. Velu (1931d) observed darmous in sheep in regions in which water was drawn from aquifers near phosphate strata but not in areas where aquifers were separated from the phosphate beds by impermeable formations. He found the dental lesions in the owners of the flocks and in their children. He also described darmous in a horse. Velu (1931e) produced darmous in sheep by giving them water saturated with natural phosphate. He pointed out the losses caused in flocks by use of such waters, losses due to inanition and diminished fertility. Velu (1931f) found that when all drinking water had been saturated with natural phosphate female rats no longer cast litters after 5 months. The animals also failed to gain weight. He said the oestrus cycle was suppressed.

Velu (1932a) noted the seasonal nature of darmous in grazing animals, resulting from the fact that the animals found enough water in food and streams in winter but in summer were supplied with water from wells. However, in man he found no such seasonal variation, since well water was used all the year round. He asserted that if children

were removed from the influences of the toxic water during the period of formation of enamel their teeth would be normal. He pointed out that water supplies must now be analyzed routinely for fluorine. Velu and Zottner (1932) described lesions in the livers of sheep severely poisoned by the fluorine of natural phosphates. Velu (1932a) reviewed the literature of fluorosis and presented his investigations and those of his colleagues, with some additional details. Velu was apparently unaware of the work of Churchill and of Smith and Smith in identifying fluorine as the causal agent of mottled enamel. Fray (Velu 1932b) summarized Velu's paper (Velu 1932a).

Velu (1933a, b) demonstrated that fluorosis produced in sheep by calcium fluoride was partially alleviated by the addition of pure calcium phosphate to the diet. He concluded that mineral deficiencies contribute to the severity of fluorosis. Velu pointed out that a normal tooth frequently exists between two fluorosed teeth in natural darmous, because of seasonal variation in fluorine intake. Velu (1933c) observed in the horse that placental transfer of fluorine to the offspring occurred to such an extent as to mottle the middle teeth slightly and the canines severely. The central incisors were normal.

Masaki (1931) said of caries in mottled enamel areas of Japan: "It is also remarkable that the percentage of dental caries is comparatively small among those who suffer from this abnormality and the eruption of the permanent teeth, especially the first molar, is very late. The average age of eruption of the first molar is 8 years, while it erupts in average in 5 years in other districts in Japan."

Smith and Lantz (1932) found no effect on the calcium and phosphorus content of rat incisors from a 60-day or 120-day feeding of 0.05 per cent sodium fluoride.

M. C. Smith and H. V. Smith (1932) reported that they had analyzed by the Fairchild method approximately 110 public and 55 private water supplies in Arizona, finding mottled enamel associated with fluorine exceeding 1.5 p.p.m.

H. V. Smith and M. C. Smith (1932b) analyzed the waters of 110 public and 75 private water supplies for fluorine with the Fairchild method.[3] They found from 0 to 12.6 parts per million. They associated values above 2.7 p.p.m. with mottled enamel, although they noted white flecks in teeth at 1.8 p.p.m. They said: "The evidence indicates that waters having a fluoride concentration varying from 2.0 down to 0 p.p.m. do not interfere with normal development of the enamel to the extent of causing observable mottled enamel."[4]

Chaneles (1932) concurred in the finding of less caries in mottled enamel but also expressed the opinion that mottled teeth are not more susceptible to caries than normal teeth. He noted that the mottled teeth were relatively soft under dental instruments.

Anderson (1932a) reported mottled enamel in all of 54 persons examined in a village of 250 near Peiping, China. In his scale of mottling (Anderson and Stevenson, 1930) there was more chalkiness of enamel than "brownin-stained" teeth. The water of the village was obtained from shallow, open wells. Anderson stated that he intended to have the water analyzed for fluorine but had no analyses at the time of publication.

Anderson (1932b) recorded the incidence of dental caries in 1000 Chinese at Peiping Medical Union College. Anderson and Stevenson (1930) as a result of observations of over 5000 patients, had reported from the same dental clinic "the exceedingly common occurrence of mottled enamel among the Chinese, at least in Northern China. . . ." Certain descriptions of groups suggest that many of their subjects were identical in their separately reported studies of (a) caries and (b) mottled enamel. If this be

[3] See Smith (1935).

[4] Compare Smith, Smith, and Foster (1936).

true,[5] the data of Anderson on caries in the separate teeth in a mottled-enamel region are very valuable in some aspects of caries etiology. Anderson records that he examined each of the five surfaces of every tooth, but his reported data are for decay in whole teeth. The pattern of decay in the *permanent* teeth was suggestive of that observed by Bunting, Crowley, Hard and Keller (1928) at Minonk, Illinois, and also by later authors: that is, little or no decay in the upper incisors. The decay rate was also low in the molars and if, as Bunting, Crowley, Hard and Keller observed, it was mainly confined to pit and fissure caries, there would be very little interproximal decay. It is worthy of note that the *anterior upper deciduous* teeth had a relatively high rate of decay, in fact greater than that of the posterior teeth, but the pattern of decay in the *lower deciduous anterior* teeth resembled that of the *lower permanent* dentition.

Agnew and Agnew (1943) wrote: "In West China and Eastern Tibet, we have found that the incidence of caries among groups who have mottled enamel, or who live in areas where this condition is endemic, was equal to the incidence among those not suffering from mottled enamel or living in areas where this condition is not endemic." They stated that the incidence of caries in West China was 30 to 37 per cent and in Eastern Tibet was 31 per cent.

Reed (1932) discussed the origin of the stain of mottled enamel as resulting from exposure to "actinic rays of sunlight," thus explaining absence of stain on lower incisors and teeth back of the cuspids.

Montelius, McIntosh and Ma (1933) analyzed mottled enamel for organic matter, calcium, phosphorus and iron. They found no variation from normal values as reported in the literature or from their own normals for iron and therefore could not account for the origin of the brown stain.

McKay (1933a, b) reported the reexamination of children in Oakley, Idaho, approximately 7½ years after the change of water supply had been made as described by McKay in 1925. In all, 94 children were examined, ranging *in age at time of water change* (July 1, 1925) from "born 1½ years after change" to 6½ years. General mottling began in the series in children 4½ years old at the time of change. Furthermore, a line of demarcation was found on teeth formed presumably at the time of water change, there being both mottling and normal enamel on the same tooth. McKay said: "It was not that the old water at Oakley failed to contribute some element necessary to the proper calcification of the enamel, but rather that it did contribute something that interfered with or prevented proper calcification." He also said: "As far as has been made apparent, mottling is the one outstanding lesion of the enamel that is traceable to a specific error in the diet, and, as has been shown, this has been produced by the presence of a particular substance, fluorine, in the water. It would be distinctly informative if any lesion of the enamel could be presented that could be traced definitely to the absence of any particular essential ingredient of the diet."

McKay (1933c) briefly reviewed evidence of fluorine in causation of mottled enamel. In comment on McKay's report, Churchill (1933) pointed out that in metallurgy, traces of metals profoundly influence various properties. By analogy he suggested of fluorine: "In some way or other it functions to disturb or influence the way in which the real structural material of the teeth is built up into teeth." He suggested statistical comparison of enamel structure to detect degrees of variation associated with the fluorine of the water.

Ainsworth (1928–29, 1933) found mottled enamel in children born and raised in Maldon, Essex, England where the water con-

[5] Dr. Anderson has stated in a private communication to the reviewer that the data of caries and of mottled enamel were from the same group of subjects.

tained 4.5 to 5.5 p.p.m. of fluorine. In Witham, a few miles distant, where no mottled enamel was found, the fluorine of the water was 0.5 p.p.m. He said of the Maldon children: "The condition of their teeth generally was good, well above the average for council schools. There was relatively little caries: 7.9 per cent of the permanent teeth were carious, as compared with an average in all districts examined of 13.1 per cent; and 12.9 per cent of deciduous teeth were carious against 43.3 in all districts." He recorded that the teeth were hard to get for histological studies.

Ainsworth's histological studies led him to conclude that pitting of mottled enamel results from fracture of the enamel rods. Ainsworth's comments on fluorine were added in his 1933 paper. He did not relate fluorine *per se* to the reduced caries rate.

The findings of Ainsworth in Maldon, Essex, England cast some light on a summation (Anonymous, 1926) of some data of Ainsworth and Young (Committee for the Investigation of Dental Disease, 1925) which gave selected figures of highest and lowest caries rates in England for comparison with Birmingham: The respective percentages of carious permanent teeth and carious deciduous teeth (upper jaw) were: for Birmingham, 11.2 and 32.4; for London, 13.0 and 44.7; for Sheffield, 14.6 and 40.1; for country schools in Norfolk and Essex, 8.0 and 25.9; for country schools in Yorkshire and Cumberland, 16.1 and 55.1.

Boissevain (1933) analyzed the water supplies of 169 towns in Colorado. He found fluorine in 80 and more than 1 p.p.m. in 25 of them. Mottled enamel was associated with fluorine in nearly every case. He regarded the presence of 1 p.p.m. or more as causative. He said: "McKay (1929), after inspecting many thousands of mottled teeth, thinks that they are not more prone to decay than normal teeth. This is also Akeroyd's (1923) opinion, and my own impression. Once a mottled tooth starts to decay, however, it deteriorates rapidly, as they are difficult to repair because of the brittle enamel and hard dentin." (See page 354 for quotation from Akeroyd, 1923. Note that it points out *lack* of caries instead of *no increase*.)

Boissevain noted that fluorine could be removed from water by means of animal charcoal or bone meal but that it was "simpler and cheaper to buy distilled water from one of the artificial ice companies." Boissevain gave a table identifying the Colorado towns referred to by key letters in various papers by McKay.

Montelius (1933) found 3.2 per cent of Chinese from South China with mottled enamel compared with 16.6 per cent in North China. The percentages with chalky enamel were 2.4 and 13.0, respectively. He also gave detailed statistics on dental caries in the 4,474 individuals he examined but did not relate incidence to geographical areas. Only 43.5 per cent of the males were free of caries and 30.4 per cent of the females. In the upper central incisors, decay was found in 3 per cent of the teeth on the right side and 2.7 per cent of those on the left side; the respective incidences in the upper lateral incisors were 1.2 per cent and 1.5 per cent.

Hyatt (1933) has written: "When the term 'structure' is used in connection with inorganic matter, it refers to the hardness, compactness, looseness, density, softness, etc. Formation, on the other hand, refers to the shape or form. With this understanding of the meaning of these terms when applied to the teeth, we are enabled to distinguish what constitutes the difference between mottled teeth and normal teeth. The formation or shape of the tooth in both cases is the same. The structure is different. Even though the structure is different, this does not increase, nor is it related to, the liability to decay. When decay occurs in mottled teeth, it starts at identically the same point as it does in normal teeth, and these places have identi-

cally the same shape or form in both mottled and normal teeth. When the structure surrounding the point of liability is hard and dense, the beginning and the progress of decay are slower. When the structure surrounding the point of liability is soft and less dense, then the beginning and progress of decay are more rapid."

Koehne and Bunting (1934), in a general review on nutrition and dental conditions, made no mention of dental caries in the section on mottled enamel. Instead they noted that "Sebrell and colleagues (1933) and Sharpless and McCollum (1933) stated that small amounts of fluorine are not necessary to the formation of good teeth and that a low fluorine content of the bone is not disadvantageous."

Dean (1933) reported the results of a country-wide questionnaire with respect to mottled enamel in continental United States. To the 70 areas reported in the literature he added 73 more, 5 of which he confirmed by visit. General absence of the dystrophy from the more populous areas was indicated.

Dean (1934) outlined a system of classification of mottled enamel that has been widely used. The seven classes were: (1) normal, (2) questionable, (3) very mild, (4) mild, (5) moderate, (6) moderately severe and (7) severe. The conditions were illustrated. Brown stain began at the "moderate" stage. Dean remarked that determination of the "questionable" stage was particularly difficult and indicated that he used groups of about 25 children to establish this community index because, "In such areas, occasional minute flecks and small white spots on the enamel often show in an unusually high percentage of children." He said: "In areas of high endemicity, mottled enamel is occasionally observed on the deciduous molars, generally the second deciduous molars. In my experience, mottling of the enamel on deciduous teeth is almost invariably of the very mild type, even though the permanent teeth of the same individual may show a severe mottling."

Dean, Dixon and Cohen (1935) established a community mottled-enamel index based on the classification by Dean (1934). The community index was considered negative "when less than 10 per cent of the children show 'very mild' or more severe types of mottled enamel." Dean and Elvove (1935) classified Pueblo, Colorado, with a mean fluoride content of water of 0.57 p.p.m., as "negative" and Galesburg and Monmouth, Illinois, with 1.86 and 1.7 p.p.m., respectively, as "slight" in the community index scale. Dean and Elvove (1937) classified East Moline, Illinois, with 1.5 p.p.m. fluorine in the water supply, as "border line" and Junction City, Kansas (0.7 p.p.m.) as "negative." With data from 15 communities where the fluorine content of water ranged from 0.6 to 8.0 p.p.m., they showed the increasing severity of mottled enamel with increasing fluorine content of the water supply.

Coumoulos (1949) classified mottled enamel in deciduous teeth as:

"0 Non-mottled enamel.

"1 Any slight aberration from the normal translucency of the enamel ranging from flecks to small areas occupying up to one-quarter of the buccal surface.

"2 Opaque areas occupying more than one-quarter and up to one-half of the buccal surface.

"3 Opaque areas occupying more than one-half and up to three-quarters of the buccal surface.

"4 Opaque areas occupying more than three-quarters of the buccal surface including cases in which all the surface was relatively opaque."

The letters a, b, c and d were appended to the above numbers "to denote *density* of the opaque areas."

Lemmon (1934) expressed the opinion that babies born in the mottled enamel area of the Texas Panhandle were slow in

erupting the deciduous teeth. "These temporary teeth are scarcely ever mottled." He believed also that the toxic fluorides, by interfering with bone metabolism, accentuated rickets. No data were given.

A study of freedom from caries was initiated in 1931 in England (Anonymous, 1935a). The response from dental examiners was poor and, of 13 areas reported, only 6 were tabulated. The free-from-caries percentages were: Carmarthenshire, 0.2; Enfield, 1.2; Willesden, 1.4; Shropshire, 2.9; Great Yarmouth, 3.2; and West Morland, 10.3. No positive conclusions were drawn concerning the data, but Wilson (1941b) pointed out that Westmorland is a fluorspar area.

Erausquin (1935) noted an inverse relationship between mottled enamel and dental caries in 2,838 subjects of all ages in Argentina. A number of observers contributed the data, and the caries estimations appear variable in relation to mottling.

Brown (1935) remarked of the children of Kenhardt, South Africa, with mottled enamel: "The teeth, though discoloured and unsightly, are strong and resistant to caries."

Smith and Smith (1935a, b) reported severe mottled enamel in the deciduous teeth of 2 children aged 5 and 7. The water supply contained 12 p.p.m. fluorine. A 2½-year-old boy and his 4½-year-old sister, who had been artificially fed with a milk formula made up with water containing 13.5 p.p.m. fluorine, had mottled enamel. Children who consumed water with 12 to 18 p.p.m. fluorine only as it occurred in cooked foods developed mottled enamel in permanent teeth. Smith and Smith added that they had observed mottled enamel of dedicuous teeth in breast-fed children in areas where the fluorine of the water was very high. They suggested: "The rapidity of calcification of the temporary teeth as compared with that of the permanent set may explain why *only* the use of water containing extremely high concentrations of fluorine will produce mottling of the enamel of the deciduous teeth."

Neff (1935) found the pulp cavities of the teeth of *Gambusia affinis* (mosquito-fish) "became broader or wider in proportion to the length of the teeth, and the teeth took on an increasing 'roughened' appearance, the roughening being extreme in some cases" in passing from a region of normal enamel to one of severe mottled enamel in children. He suggested the use of fish "as a criterion of fluoride ion concentration."

M. C. Smith (1936) reported the study of mineral, vitamin and protein variation on mottled enamel in the incisors of an aggregate of 1500 rats and guinea pigs, the latter being used for vitamin C studies. Variations of calcium and phosphorus and vitamin D were without effect. The dental effects of deficiencies of vitamins A and C and of calcium and phosphorus were judged to be additive to those produced by fluorine. No effect on mottled enamel through variation of protein supply was noted.

R. R. Smith (1936) reported 24 cases of mottled enamel seen in private practice and asserted not to be associated with fluoride-containing waters. Smith provided no evidence of analysis of the water. He suggested the source of the fluorine was from insecticide sprayed on apples and indicated that "one large apple could contain approximately as much fluorine as three or four glasses of water containing the damaging concentration of one part per million of fluorine." He gave no data on the analysis of such apples.

Dean (1936) summarized the knowledge of mottled enamel. He made no mention of the relation of mottled enamel or of fluorine to dental caries. In fact, he suggested for prevention of mottled enamel: "It is possible in many cases to provide distilled or cistern water for drinking purposes during the susceptible period, the first eight years of life." The change in Dean's view toward

fluorine, mottled enamel and caries, as influenced by his subsequent extensive studies, is indicated later in this section.

Smith, Smith and Foster (1936) stated: "Mottled teeth are not only ugly to look at but are structurally weak. The enamel chips off and the teeth cannot be successfully filled when decayed. Mottled teeth of the most severe type usually break down and must of necessity be replaced by false teeth in early adult life."

In their summary they said: "The effects of drinking fluorine-containing water on enamel formation of children's teeth—i.e., production of mottled enamel—are described:

a. 0 to 0.8 p.p.m. of fluorine produces no noticeable effect.
b. 0.9 p.p.m. usually produces very mild mottled enamel.
c. 1.0 to 2.0 p.p.m. produces mild to moderate mottled enamel"

and statements are made of the lesions up to and over 6 p.p.m.

Mansbach (1937b) stated that an examination of 7000 Jewish children born in Palestine showed that about 40 per cent were free of caries, but their emigrant parents had a very high rate of caries. He ascribed the one-generation change of caries rate to sunlight and high vitamin diets and explained increased rates in some groups by higher sugar consumption and vitamin C deficiency. (Compare Clawson, Kalifah and Perks (1940).)

Scott, Kimberly, Van Horn, Ey and Waring (1937) reported between 1.0 and 2.0 p.p.m. fluorine in well waters in 29 Ohio communities, 2.0 to 3.0 p.p.m. in 17, and 3.0 to 4.0 p.p.m. in 3. The higher levels were in the northwestern part of the state near Toledo. They noted that, in water treatment, fluoride was reduced as a function of magnesium removal. It is apropos to note that Doremus (1890) proposed the use of sodium fluoride as a water softener, having found it specially "thorough and noteworthy" in the precipitation of magnesium.

Arnim, Aberle and Pitney (1937) examined 204 Indian children of New Mexico pueblo villages. The children were from 7 to 11 years old. Where the water contained 2.4 p.p.m. fluorine, 64 per cent of the central incisors had white spots; only 0.92 per cent were mottled with 1.1 and 1.2 p.p.m. fluorine. Of the whole group, 64.2 per cent had no caries of the permanent teeth, compared with 47 per cent observed by Eliot, Souther, Anderson and Arnim (1934) by comparable methods in New Haven, Connecticut, in children 5 to 12 years old. Only 12.7 per cent of the permanent molars were decayed, compared with 36 per cent observed by Eliot. "No carious lesions were observed in 1,605 permanent incisors." They remarked: "The observations recorded herein add another group of teeth, structurally defective, in which the incidence of caries is not increased."

Blue (1938) reported in general on the examination of about 400 school children in the Oklahoma Panhandle. In Guymon, with 1.45 p.p.m. of fluorine, 19 of 82 children had normal enamel, the others very mild to moderately severe. In Texhoma, with 1.25 p.p.m., 12 of 33 children had normal enamel, the others being similar to those of Guymon. For the entire group of 400 he found 9.9 per cent had had one or more bone fractures, 20.25 per cent had poor posture, 15.3 per cent had definite signs of rickets and 43 per cent were underweight. He said: "Although no definite tabulations were made on the percentage or incidence of dental caries. . . . I believe it can be truthfully stated that the incidence of caries is high especially in those cases which are beyond the questionable and very mild classifications of mottled enamel." He had no controls on any of his observations.

Armstrong and Brekhus (1938a) found a mean content of fluorine in 42 specimens of sound human enamel of 0.0111 per cent (111 p.p.m.) with a range from 0.0073 to

0.0167 per cent (73 to 167 p.p.m.). "Specimens of sound deciduous teeth have been difficult to obtain. The pooled enamel separated from the crowns of three such teeth whose roots had been physiologically resorbed was found to contain 0.0072 per cent fluorine."

Armstrong and Brekhus (1938a) found about 250 p.p.m. of fluorine in the enamel of the second bicuspids of a 16-year-old subject with most of the teeth slightly mottled. The dentin had from 371 to 425 p.p.m. fluorine. The enamel from a 41-year-old subject with severely mottled and pitted enamel had from 333 to 361 p.p.m. fluorine. It is of interest that this latter subject "had lived during the first 19 years of life at Durango, Mexico." Armstrong and Brekhus said: "The high fluorine content of the enamel and dentin of mottled teeth is direct evidence that fluorine is the etiological factor in the production of the condition."

Houser and Knox (1939) examined 27,379 school children of 323 Ohio communities for mottled enamel in relation to the fluorine content of the water. They classed as "Non Res" children from farm areas attending village schools and gave the opinion that the fluorine of their farm water supplies was generally lower than that of the town water. Houser and Knox advanced the theory that "possibly some other factor than fluorides may cause the 'very mild' mottling which cannot be differentiated from true mottling caused by fluorides." They stated that the Ohio State Department of Health had "decided that a fluoride content greater than 1.5 p.p.m. justifies the installation of treatment devices to reduce the fluoride content of the water." They stated also that some communities had adopted surface water supplies and abandoned ground waters because of the fluorine content.

Hurme (1949) investigated the frequency of occurrence of "white spots" in the enamel of teeth in New England, where the fluorine of water supplies does not exceed 0.25 p.p.m. The defects were described as "discrete opaque areas in the enamel which, unless pigmented, appear white or ashy gray, in definite contrast to the surrounding translucent tissue. They are usually small, with an irregular, rounded, cloudlike shape, and they seldom involve more than a fraction of the total area of a crown surface. The smaller opacities appear to be covered with the same dense, smooth-appearing material as the rest of the enamel, but the larger ones may lack the glazed surface in the central portion. In such cases it is relatively easy to press the point of a sharp instrument into the soft chalk-like tissue in the center of the opaque area. The small opacities, which are met with frequently, are seldom pigmented, whereas the large ones, which are much less common, are often discolored yellow, brown, or orange. The discoloration is present at the time the tooth erupts. It may be distributed evenly, but in the majority of cases it is most intense near the center of the defective area. If a tooth with an opaque 'spot' in the enamel is placed in an acid solution for a few hours, it will be observed that the imperfect tissue corrodes more rapidly than the sound tissue." He stated this type of white spot tended to disappear with age. He found them present in the dentition of 142 of the 170 subjects examined.

Wilson and De Eds (1939) fed rats rations with 0.0031, 0.0062, 0.0125, 0.025 and 0.05 per cent of cadmium and observed mottling of enamel, at all levels, apparently identical with that caused by fluorine. Wilson, De-Eds and Cox (1941) showed that mottling of rat incisors was produced also by a concentration of cadmium as low as 0.0016 per cent. Joint tests of cadmium with fluoride did not indicate that cadmium sensitized the animals to fluorine but rather suggested that cadmium *per se* caused the effect. The rapidity of bleaching of the teeth was greater by cadmium than by fluorine. Since cadmium inhibited bone

phosphatase activity in concentrations as low as 10^7 N, it was further indicated that the fluoride and cadmium activities were exerted in the same way.

Smith and Smith (1940) said of residents of St. David, Arizona, with mottled enamel: "That decay was widespread and repair was highly unsuccessful among the young adults is shown by an incidence of more than 50 per cent of false teeth in the age group 24 to 26 years. This high incidence of false teeth appeared in all subsequent age groups. Very rarely, adults were found whose teeth, though mottled, were free from caries. It was the exception rather than the rule to find dentitions from which there had been no extractions because of inability to repair carious teeth successfully." The numbers examined in each of their age groups were not given and there was no statement concerning extractions performed for esthetic reasons.

Wilson (1939) recorded observation of mottled enamel in various areas of England. She stated that in 9000 children of the Punjab in India she found evidence of mottled enamel in both deciduous and permanent teeth. She described the Punjab area, where mottled enamel was endemic, as an alluvium overlying a buried ridge. The latter was the source of the fluorine in the wells. One of the villages was Kasur (see next paragraph). Waters from its wells had from nil to 4 p.p.m. of fluorine.

Day (1940) found mottled enamel in all of 203 children examined in Kasur. The deciduous dentition was affected in all cases, the molars showing the greatest defects. The fluorine content of the well waters was variable, ranging from 0.7 to 6.4 p.p.m. The climate of the Punjab is very hot and dry, and Day estimated 8 to 10 large glasses of water of over 1 pound each would be consumed daily. As to caries, Day found no cavities in 1352 permanent incisors and restriction in general of decay to pits and fissures. There were 2.23 decayed teeth per child and only 8.67 per cent of all teeth were decayed. Of the 203 children, 42 per cent were caries-free. There was no evidence of delayed eruption or of abnormal degree of malocclusion. Day indicated a belief in the placental and mammary transmission of fluorine.

Taylor and Day (1940) said that dental fluorosis was very common in the plains regions of the Punjab regions of Northern India but that in the Kangra district of the Punjab "dental fluorosis is conspicuous by its absence." They therefore did not believe fluorine had anything to do with the low caries rate they found in children of the Kangra district. Their association, however, was with frank fluorosis, the possibility of a submottling level not being considered. Therefore, no conclusions either way can be rightfully drawn concerning fluorine and the caries rate in this case.

Day and Tandan (1940) examined caries incidence in 756 boys of Lahore in the Punjab district of India. Although 94 per cent were affected by caries, the pattern of decay was quite different from that of other areas, in Day's experience, with a similar high percentage. In Lahore there were 4.73 carious teeth per mouth, compared with 14.43 in a comparable age group in Rochester, N. Y. Only 4 fillings were found and "the number of extracted permanent teeth was negligible." Most of the decay was occlusal, there being only 7.88 per cent of mesial, distal and proximal surfaces affected, compared with 41.30 per cent in American school children. Although Day (1940) stated that he found no mottled enamel in Lahore in 1000 children, he also reported that the fluorine content of a well in Lahore had 0.6 p.p.m. fluorine. It is possible that the dental caries pattern and low incidence of caries in Lahore is associated with a protective dosage of fluorine derived through high consumption of water with a relatively low fluorine content. Lahore is 35 miles from Kasur. (See Wilson, 1939, and Day, 1940). Wilson (1941) said: "In the course of nutritional examinations in Punjabi vil-

lages situated on the margin of the buried ridge containing fluorine, I found that the children of agricultural families whose dietary included milk and milk products, although they showed no macroscopic mottling, usually had large and well-formed teeth and very little caries compared with children of the same social class living in villages distant from a source of fluorine."

Day (1944), in a study primarily concerned with caries incidence in relation to severe deficiencies of vitamins A, D and C and of calcium and animal proteins, observed mottled enamel and an average of only 0.62 cavities per child. The fluorine content of the waters averaged 1.62 p.p.m. "In the total of 8,312 permanent and deciduous teeth examined in 314 children, only 196 cavities were found despite the fact that a great number of deciduous teeth were retained long beyond their normal term." There were 117 cavities in 6,936 permanent teeth (1.7 per cent) and 79 in 1,376 deciduous teeth (5.7 per cent). There were only 24 "open" cavities in the permanent teeth (0.35 per cent). "Not one cavity was found in either the upper or the lower incisor teeth." As the average age of the group was 11.87 years and there were only 1,376 deciduous teeth among 314 children (4.4 teeth each, on the average) it is unlikely that any deciduous incisors were observed.

Bromehead (1941) advised Wilson (1941) on areas in England in which to expect mottled enamel on geological reasoning. Predictions were confirmed especially in the case of small areas in Buckinghamshire and Bedfordshire where phosphatic deposits had been located. As to caries, Bromehead said: "The greatest immunity was found by King (1940) in the Island of Lewis; apatite is among the chief accessory minerals of the gneisses of which that island is composed. In this connection it may also be noted that Westmorland, mentioned above as a fluorspar district, has the highest immunity to caries in England." He also noted that Sutton (1936) had found fluorine to exceed 1 p.p.m. in only 1 of 43 drinking waters of Derbyshire, "perhaps one of the best known sources of fluorspar," and that Wilson (1941) had found no fluorosis traceable to these wells.

Wilson (1941) examined 1048 children 5 to 16 years old, of Somerset in England, for mottled enamel and caries. She found an inverse relationship. "Thus an increased intake of fluorine is associated with a relative immunity to caries."

Wilson (1941) said: "Among 90 rural housewives in Oxfordshire aged 21–45 years, 19 had false teeth; 13 who showed mottled enamel varying from F2 to F7 on Dean's scale had but small amount of caries, while the remaining 58 gave no evidence of dental fluorosis but showed a considerable amount of decay." This was offered as a commentary on Smith and Smith (1940).

Murray and Wilson (1942) reported on mottled enamel and caries in 589 London children, aged 10 to 15 years. The DMF rate was: 4.1 for 167 children with no mottled enamel; 2.1 for a group of 258 with doubtful signs of mottling; 1.0 for the 83 with very mild mottling; 0.9 for 54 classed "mild;" 0.4 for 7 moderately mottled; and 1.1 for 6 severely mottled cases. The three main London water supplies, however, showed only 0.1, 0.15 and 0.32 p.p.m., though one well source had 1.2 p.p.m. Murray and Wilson associated the low caries rate with preeruptive fluorine, suggesting that "such low levels of fluorine intake in water during tooth development are significant in relation to incidence of caries."

Clawson, Khalifah and Perks (1940) found mottled enamel in every subject born and resident in Palmyra, Syria, where there is 2.0 to 2.5 p.p.m. fluorine in the water. Though they had no further water analyses, on the basis of observation of mottled enamel they declared "the whole of the Arabian Peninsula, Asia Minor, the Near East and Mesopotamia (to be) endemic, in scattered sections at least, to this water-borne mottling of human teeth."

Ockerse (1941b) examined South African children for mottled enamel and caries in relation to the fluorine content of the water supplies. The survey was not limited to children of continuous residence. In Upington, with 0.38 p.p.m. fluorine in the water, 74 per cent of the children had caries. In Kenhardt, with 6.8 p.p.m. fluorine, 26 per cent; and in Pofadder, with 2.5 p.p.m. fluorine, 17 per cent had caries. The mottling of enamel ranged up to "severe" in Kenhardt and Pofadder children. Ockerse gave the opinion with respect to mottled enamel: "In many cases, it is so severe that it is a predisposing cause of dental caries. . . . Twenty-two cases of mottled enamel in deciduous teeth were observed. The majority showed very mild mottling, only one or two teeth being involved, mostly showing opaque patches." In the four cases described specifically, the molars were most strongly affected; in two subjects chalky appearance of the teeth was general.

Badger (1949) reported mottled enamel in the permanent teeth of 30 per cent of the children of Hobbs, New Mexico, where the municipal water supply was reported as containing 0.9–1.0 p.p.m. of fluorine. She did not report any observations on the dental caries rate.

King (1944) found no correlation between caries and mottled enamel in first and second premolars and the first molars of 12- to 14-year-old-children of Maldon (5 p.p.m.) and Burnham-on-Couch, Essex, and of Oxfordshire and Ipswich. The *general* caries rates, however, were found to be low in the fluoride area. A positive association was confirmed between M-hypoplasia (Mellanby's hypoplasia) and caries.

Discussion

The literature of mottled enamel provides much of value in the etiology of caries both as to theory and as to facts. Before the dominant role of fluorine was discovered an array of theories as to the cause of mottled enamel was presented. These theories were based on a supposed: (1) calcium deficiency, either *per se* or as soft water before or after the eruption of the teeth; (2) excess of manganese, iron or magnesium; (3) corrosion by acid at the gum line; (4) hereditary factors; (5) food characteristics; (6) trace elements in excess or deficiency; and (7) malnutrition. Some of these theories were sustained by facts such as Beust's histological evidence and experimental *in vitro* production of mottled enamel and Pierle's production of mottled enamel in rats. Also, some of the theories were disproved by facts such as the absence of manganese from the waters of endemic areas and Fossum's demonstration that a tooth immersed in a suspected water for long periods did not become mottled.

These theories bear strong resemblance to many advanced to explain dental caries. For example, the calcium deficiency idea was apparently based on the facts that: (a) enamel contains calcium; (b) there are hard and soft waters; and (c) there is an association of mottled enamel with water supplies. The inadequate theories of the cause of mottled enamel were quickly forgotten after publication of the work of Churchill, of Smith, Lantz and Smith and of Velu. It is not likely that the false theories of dental caries origin will be so quickly discarded when the true system of causes is revealed, since mottled enamel is a recently recognized condition, not widely spread compared to caries, and since the theories were not thoroughly indoctrinated by repetition, whereas caries stands in diametric opposition on all three points.

It may be noted that the etiology of mottled enamel was clarified by use of experimental lesions in rat incisors, teeth that have no counterpart in man.

The mottled-enamel literature also illustrates how facts are frequently forced to follow theory. The dictum that caries susceptibility is not related to enamel structure was supported by McKay in

pointing out that mottled enamel is not more susceptible to caries than unmottled enamel. This view was sustained by others. This argument is invalidated by the fact that mottled enamel is generally *less* susceptible to decay than "normal" teeth, but this invalidation does not *prove* that structure of enamel is a factor in resistance to decay. Demonstration of the pertinent structures associated with both decay and no decay is essential for the proof. Some of the facts of mottled enamel are of interest in dental caries theory.

The structure of enamel is subject to alteration because of the chemical composition of the diet during formation, but the alterations shown by fluorine and by cadmium are of the variety caused by toxic factors.

The chemical composition of enamel may possibly be subject to alteration because of the chemical composition of the formative ration. However, fluorine, because of its property of combining with calcium phosphate wherever it occurs, may be a false indication that other dietary constituents can alter the chemical composition of enamel.

Teeth with different structures and different fluorine contents can exist in the same mouth, and the distribution of such teeth among several mouths differs, depending on the periods of exposure to varying formative conditions.

When definite data are provided it is shown that the caries rate in children in mottled enamel districts is lower than that in non-endemic regions. The statements that the caries rate is not changed or is increased are generally not accompanied by any data.

The dental caries pattern in mottled-enamel districts is different from that of non-endemic regions, the chief characteristic being the almost complete absence of decay in the *anterior permanent* teeth. *Anterior upper deciduous* teeth are as subject to decay as *upper posterior* teeth.

Mottled enamel may be prevented by change of water supply or treatment of water to lower the fluorine content. The only successful preventions that have been reported are based on change of community water supplies; there are no reports of success of the efforts of individuals, particularly in the day-by-day treatment of water.

There is a seasonal variation in mottled enamel, though this has been related to change of water supply rather than to variation in consumption of water. That a seasonal variation in human mottled enamel may occur is suggested by the irregularity in which flecks and white spots occur in the questionable stage of mottled enamel.

The caries rate in deciduous teeth is not lowered as much as the rate in permanent teeth. This fact may be associated with the observation that the fluorine content of deciduous teeth is lower than that of permanent teeth from the same area, but this does not prove that the caries susceptibility is a function of fluorine content.

Deciduous teeth may be retained longer in mottled enamel areas and permanent tooth eruption delayed. These scattered observations have no confirmation in systematic study. Delayed eruption of permanent teeth would have an effect in lowering caries incidence in teeth of a certain age group because of the reduced posteruptive exposure.

FIELD STUDIES OF WATER-BORNE FLUORIDES AND DENTAL CARIES

Klein and Palmer (1937) found marked differences on a geographical basis in caries rates in American Indians. They said: "The southwestern area, which has the lowest caries attack rates of all the areas studied, is in general an endemic fluorosis area. This fact may have important implications and would seem to justify some discussion. Fluorides are well known as enzyme inhibitors and it may be suggested that perhaps a measure of the responsibility for

low caries attack rates in the southwestern area may be the result of the drinking of fluoride waters. Such water may provide an enzyme inhibitor which will operate to limit the chemical degradation of tooth-impacted carbohydrates to organic acids, so reducing the production of local acidity about the teeth, and so limiting an important vector in caries initiation. It may be mentioned in this connection that support for this concept is discernible in the literature."

Dean (1938a) reviewed the evidence that a low rate of dental caries is associated with mottled enamel. He reported his observations of caries in 236 9-year-old children. Of 122 of these children from 3 cities with 1.7 to 2.5 p.p.m. fluorine in the water, 49 per cent had all permanent teeth, and 31 per cent all deciduous teeth, caries-free. The corresponding figures for children from 0.6–1.5 p.p.m. fluoride-water areas had 26 per cent and 11 per cent, respectively. He pointed out an inverse relation between the caries rate of children of South Dakota (Messner, Gafafer, Cady and Dean, 1936) and mottled enamel prevalence. A similar inverse relation was shown for cities of Colorado and of Wisconsin. For example, 687 children 12 to 14 years old of Green Bay, Wisconsin, showed 275 carious permanent teeth per 100 children (2.3 p.p.m. F); 244 Sheboygan children had a rate of 710 carious teeth per 100 (0.5 p.p.m. F). (Compare Bull. (1943)).

Dean posed four possibilities: (1) the physical structure of a tooth may play a role in either susceptibility to or immunity from dental caries; (2) the higher fluoride content of the enamel of a mottled-enamel tooth may be the immunity-producing factor; (3) the limited immunity, directly or indirectly, may be due to the well known inhibitory action of fluorine on enzymatic processes, and (4) although the evidence points to fluorine as the active agent, "other elements of comparatively rare occurrence in water or ordinary constituents of drinking water present in unusually large concentration, may directly or through synergistic action with the fluoride, produce the observed effects." Dean reported that the low caries rate of Colorado Springs was associated with 2.5 p.p.m. fluoride in the water and the very low hardness of 20 p.p.m.

The subsequent studies of Dean and his coworkers have mainly been concerned with No. 3 of the above questions and have given generally negative answers.

Dean, Jay, Arnold, McClure and Elvove (1939) examined 12- to 14-year-old children of four Illinois cities for caries in relation to the fluorine content of the water supplies. Their data, briefly, for all children with history of continuous residence were: for Galesburg and Monmouth, with 1.8 and 1.7 p.p.m. fluorine in water, there were 194 and 208 carious teeth per 100 children; for Macomb and Quincy, with 0.2 p.p.m. fluorine, there were 368 and 628 carious teeth per 100 children. They noted that there was little decay in the anterior teeth of the Galesburg and Monmouth children, their data indicating 16 times as many approximal surfaces of the upper four incisors decayed in Macomb and Quincy children.

Dean *et al.* observed a caries rate of 200 per 100 children in 114 Galesburg subjects *with* mottled enamel; in 129 children of the same city *without* mottled enamel the rate was 186. They said: "It would appear that the factor responsible for the low amount of caries in this city was operative irrespective of whether the child showed macroscopic evidence of mottled enamel."

The count of *L. acidophilus* in the saliva was positively correlated with the caries rates of the children of Quincy and Galesburg. Dean *et al.* gave the results of observations by McClure (1939b) on salivary amylase, as indicated by production of maltose from starch under closely controlled conditions: "A total of 63 specimens of saliva from children living in Quincy averaged 105.9 ± 5.2 mg. maltose, as

compared with an average of 108.7 ± 3.1 mg. of maltose for 82 specimens from children living in Galesburg." McClure wrote: "It may be said with reasonable assurance that fluoride ingestion, brought about by the use of a domestic water supply containing approximately 1.8 parts per million of fluorine, does not change the final amylolytic activity of the saliva secreted under these conditions."

Dean, Jay, Arnold and Elvove (1941a) studied the incidence of caries in Bauxite, Arkansas, children born before and after a change in water supply from 14 p.p.m. fluorine to a fluoride-free source. Kempf and McKay (1930) had found a 100 per cent incidence of severe mottled enamel among children of Bauxite in 1928, just prior to the change of water. Dean, McKay and Elvove (1938) had shown that about 10 years later enamel formed on the fluoride-free water was normal.

The 82 children with histories of continuous residence in Bauxite were divided into 3 groups. Of these, 26 were old enough to have mottled enamel derived from use of the high fluoride water; 23 were in a group born just after the change of water and had only a trace of mottled enamel; the third group of 26 were born several years after the water change. The numbers in the groups were admittedly low, but caries rates were given. They were, respectively, 27, 43 and 15 per cent caries-free. Of the first molars, 39, 34 and 65 per cent were decayed, respectively. Thus the *youngest* group, that is the one with no fluorine in the water *at the time of formation of the teeth*, showed the highest caries attack. The transitional group showed reduced caries, suggesting placental or mammary transmission of fluorine. The authors pointed out that the order of caries incidence was the reverse of the usual case, that is, lower incidence in an older group. It may be noted that the *L. acidophilus* count in salivas tended to correlate with the extent of caries and with the probable fluorine content of the enamel of the teeth, but its count was obviously not consistent with the current water supply common to all children at the time of sampling.

Dean, Jay, Arnold and Elvove (1941b) studied the caries experience of 2,832 children of 8 northern Illinois cities, 4 with little or no fluorine in the water and 4 with more than 1 p.p.m. fluorine. In the high fluorine cities the carious teeth per 100 children ranged from 252 to 321, while in the low fluorine cities the range was from 444 to 810.

The counts of *L. acidophilus* in the saliva were associated with the caries activity. The authors pointed out the differences in the dental practice which must prevail in the fluoride and non-fluoride regions.

Arnold, Dean and Elvove (1942) counted the *L. acidophilus* of the salivas of 109 children of Garrettsville, Ohio, at 6-month intervals from May 1940 to April 1941. The water supply of Garrettsville had been changed in November 1939 from 0.1 p.p.m. fluorine to 0.7 p.p.m. by inclusion of the water from a well with 1.7 p.p.m. fluorine. No change in the distribution of high and low counts could be found, unless possibly an increase in the frequency of counts over 30,000. At the time of an examination for caries in December 1940, the children ranged in age from 9 to 17, with an average of 13.6 years. Their total caries experience was 708, which the authors compared with that of Waukegan, Ill.

Dean, Arnold and Elvove (1942) examined the 12- to 14-year-old children of 13 cities in 4 states for dental caries in relation to the water supply. A condensation of their data, together with those from the 8 cities of the preceding study, are shown in the accompanying table. The two examiners were the same for all 21 cities; the total number of continuously resident children available and examined was 7,257. No bacteriological studies were made in the 13 cities.

The relation of fluorine in drinking water to caries experience and mottled enamel in 12–14-year-old children
(Dean, Arnold and Elvove, 1942)

Cities	Number of children examined	Mean fluorine in water, p.p.m.	Caries experience per 100 children			% incidence of mottled enamel
			Total	Carious upper incisor surfaces	1st molar mortality	
Galesburg, Ill.	273	1.90	236	0.46	15.0	47.6
Colorado Springs, Colo.	404	2.55	246	0.31	4.7	73.8
Elmhurst, Ill.	170	1.80	252	0.60	11.8	40.0
Maywood, Ill.	171	1.20	258	0.59	11.7	33.3
Aurora, Ill.	633	1.20	281	0.78	14.5	15.0
East Moline, Ill.	152	1.20	303	0.16	15.8	31.6
Joliet, Ill.	447	1.30	321	1.30	19.5	25.3
Kewanee, Ill.	123	0.90	343	1.40	29.3	12.2
Pueblo, Colo.	614	0.60	412	0.47	20.2	6.5
Elgin, Ill.	403	0.50	444	4.10	20.3	4.2
Marion, O.	263	0.43	556	3.30	25.1	6.1
Lima, O.	454	0.32	652	3.20	55.9	2.2
Evanston, Ill.	256	0.00	673	10.70	42.6	1.6
Middletown, O.	370	0.21	703	7.10	65.9	1.1
Quincy, Ill.	330	0.13	706	11.20	71.2	0.3
Oak Park, Ill.	329	0.00	722	9.00	31.0	0.6
Zanesville, O.	459	0.19	733	11.40	99.8	1.5
Portsmouth, O.	469	0.13	772	10.40	73.8	1.3
Waukegan, Ill.	423	0.00	810	17.70	79.9	0.2
Elkhart, Ind.	278	0.11	823	11.20	34.2	0.4
Michigan City, Ind.	236	0.09	1,037	18.10	80.1	0.0
Total	7,257					

McClendon, Foster and Supplee (1942), using data on caries derived by Mills (1937) from Messner, Gafafer, Cady and Dean (1936), stated: "Dental caries in city school children varies inversely with the fluorine content of cows' milk. The correlation coefficient is −0.37."

Taylor (1943a) examined elementary and high school children of Texas for caries correlated with fluorine content of the water and other factors. For Deaf Smith County, with 2.2 to 2.7 p.p.m. F, he found a DMF (decayed, missing, filled teeth) rate of 1.23; Randall County, fluoride not given, DMF rate 1.57; Coryell County, 2.5 p.p.m. F., DMF rate, 1.95; Garza County, 6 to 8 p.p.m. F, DMF rate, 2.42. Taylor believed the lower caries rate for Deaf Smith County to be associated with higher mineral content of the soil, particularly of calcium and phosphorus. He found a caries rate of 1.02 DMF in 76 12- to 14-year-old children "who had lived in Deaf Smith County as long as five years" compared with 1.01 DMF for 54 of comparable age and history of continuous residence. On the basis of this observation and the assertion of a local dentist that numerous cases of arrested caries were found in people moving into the area, Taylor concluded: "Caries immunity is at least partially acquired after eruption of the teeth." Concerning the DMF rate of 2.42 in Garza County, where mottling was severe, he said that caries "was of different appearance from that in non-fluoride areas. It was difficult to determine in most cases whether it was active caries or a progressive fluoridic hypoplasia. The tooth tissue is of a dark, leathery texture and appearance. Eventually, however, the pulp is exposed and destroyed. The initial progress seems to take place more from within the tooth in

many cases." *L. acidophilus* count was positively correlated with the number of caries-active cavities.

Price (1943) said that "activator X," which he believed to be the substance in butter effective in the prevention of dental caries, is particularly abundant in butter from near Hereford, Deaf Smith County, Texas. "It has been shown that dairy products in Hereford vicinity may vary through a range of fiftyfold in a few weeks' time in the vitamin A and activator X content, the range depending directly on the fodder."

McClure (1944a) has tabulated analyses of foods by a number of authors and compared them with the data for calcium and phosphorus of foods of Deaf Smith County, Texas, as reported by Taylor (1942). He found no evidence for the claims of Taylor that the low caries rate in that locality is to be ascribed to high calcium and phosphorus of the foods, but attributed it rather to the fluorine content of the water. The subsoils of Deaf Smith County had lower calcium and phosphorus content than the soils of other areas.

Taylor (1943b), in evaluating dental health measures, recognized lower rates of permanent tooth losses in Texas areas where fluoride was present in the water supplies. Blackerby (1943) dealt mainly with the fact that a difference in caries rate exists in rural and semirural children of East, Middle and West Tennessee. East Tennessee had a significantly higher caries rate for ages compared with Middle and West Tennessee. Average number of clear days was lowest for a 20-year period in East Tennessee. The averages for fluoride content of the waters were 0.123, 0.142 and 0.172 p.p.m., respectively, "and the respective differences by section are so small that variability of the testing methods might well invalidate any significance that could be assigned to them." The number of water sources analyzed were 165, 91 and 98, respectively, but it is not indicated whether or not these were the water supplies of the subjects, and correlation of caries with specific water supplies was not attempted.

Bull (1943) examined 12- to 14-year-old children of Green Bay and Sheboygan, Wisconsin, for mottled enamel and caries. The fluorine content of the Green Bay water supply averaged 2.1 p.p.m., that of Sheboygan (Lake Michigan) was estimated at 0.05 p.p.m. For the 1,647 children born and reared in Green Bay, a DMF rate of 2.62 was found and for 1,877 Sheboygan children, 8.54. In Green Bay, 30 per cent of the continuously resident children had no caries experience, compared with 3 per cent in Sheboygan. Twice as many Sheboygan first molars had decayed as in Green Bay, and there were 15 times as many anterior teeth attacked. As Bull pointed out: "There is not a single breakdown of dental caries experience that does not show a pronounced advantage in dental health to those children who have continuously used fluorine water."

The DMF rate of children residing in Green Bay but born elsewhere was 4.59. It was indicated by an incidence of about 18 per cent of mottled enamel in this group compared with 75 per cent in the continuously resident group that many of these children had received significant amounts of fluorine during the formation of the teeth.

Bull wrote: "Certainly, to date, there is no evidence to prove any of the extravagant claims being made that fluorine holds the secret of all dental decay prevention. On the other hand, nothing has been demonstrated yet that equals fluorine in its ability to lessen dental caries in large groups over a long period of time." No reference was given as a basis for the first of these two statements nor was any procedure mentioned for comparison with prevention of caries by fluoride.

Bull noted that the soil of the country surrounding Green Bay was more deficient in calcium and phosphorus than in the case

of Sheboygan. Green Bay is about 1° of latitude north of Sheboygan.

Deatherage (1942) reported observations showing the total carious teeth per 100 children from fluoride areas to be 274 and from non-fluoride areas to be 481.

Deatherage (1942) obtained records of caries in 6000 white military selectees in Illinois. For 164 men of the age group 21 to 28 with continuous residence in communities with fluoride waters, the total caries-experience rate was 530 per 100. In 164 men from cities near fluoride areas, but using waters with 0.0 to 0.5 p.p.m. F, the rate was 1016. The rate for 77 men who lived the first 8 years, but not their entire lives, in fluoride areas was 581. The rate for 82 men who lived their first 8 years in fluoride-free areas and the remainder elsewhere was 1077.

Deatherage (1943a) extended the preceding studies to include more subjects. He reported the data in the age groups of 21–24, 25–28 and over 28 years. As the trend was generally toward increase of caries with age in all classes, Deatherage's combined caries rates for all ages were: for entire life in fluoride-free areas, 1079; in areas with 0.5 to 0.9 p.p.m. F, 788; in areas with 1.0 plus p.p.m. F, 621; for first 8 years the corresponding rates were 995, 887 and 728.

Deatherage concluded that significant protection against caries is acquired by teeth during calcification with waters containing optimum amounts of fluoride. He considered the 0.5 to 0.9 waters as suboptimal.

Deatherage (1943b) presented data from 263 inductees who had lived the first 8 years of their lives in fluoride-free communities and an additional 1 to 24 years in areas with 1.0 p.p.m. or more of fluorine in the water. Their total caries experience was 838 per 100. Comparison was made with the 286 men of the preceding paper having a caries rate of 1079, and with other pertinent groups. The conditions of exposure to fluorine and total caries experience per 100 men were as follows:

1.0 p.p.m. or more continuously from birth	621
1.0 p.p.m. or more, birth to 8 years; F-free	728
0.5–0.9 p.p.m., continuously from birth	788
F-free birth to 8 years; 1 p.p.m. or more 1–24 years	838
0.5–0.9 p.p.m. birth to 8 years; F-free	887
F-free birth to 8 years	995
F-free continuously from birth	1079

Deatherage concluded: "Other factors being essentially the same, it is reasonable to assume that the marked difference in dental caries experiences by the two groups is attributable to the differences in the fluoride content of the domestic water used and indicates that fluoride (F) has an inhibitory effect on dental caries after the calcification of the permanent teeth."

Senn (1943) reported the incidence of carious, filled and missing teeth in 7,170 white aviation cadets of the Army of the United States between the ages of 18 and 27. All of the 48 states were represented, with a minimum of 10 from Nevada and a maximum of 228 from Indiana. There were 200 or more from each of 23 states and less than 100 from 15 states. The minimum of 9 carious, filled or missing teeth was found in 226 cadets from Texas and in 214 from Oklahoma; the maximum was 21 in 156 cadets of Washington. Maryland with 68 cadets, Massachusetts with 200 and Vermont with 43 were shown as having 20 defective teeth. Senn related the geographic distribution of caries to fluorine areas. His table shows a median of 15 carious, filled or missing teeth in the cadets from Florida, Idaho, Indiana, Louisiana, Missouri, North Dakota and Tennessee. Of the 18 states below this median, 14 have 1 or more mottled enamel areas; of the 23 states above the median, 7 have mottled enamel. Senn indicated an opinion that incidence of

sunlight was not related to the geographic distribution of caries.

Weaver (1944a) examined 500 5- and 12-year-old children for caries incidence in each of the cities of North Shields and South Shields, which are on Tyneside in northeast England. The fluorine content of North Shields, located across the Tyne River from South Shields, was found to be less than 0.25 p.p.m.; that of South Shields was 1.2 to 1.8 p.p.m. The populations were economically alike. Weaver found the DMF rate of *deciduous* teeth in 5-year-old children to be 6.6 and 3.9 for North Shields and South Shields, respectively, and the average caries figure (Mellanby) as 16.0 and 9.5, respectively. The upper central and lateral incisors showed reduced incidence of decay but proportional to that shown by the molars. The DMF rates for 12-year-old children were 4.3 and 2.4, and the average caries figures were 10.0 and 5.1, respectively. The reduction in incidence of caries in the upper central and lateral *permanent* incisors was much greater than in the molars. Weaver said of this difference in distribution of caries in deciduous and permanent teeth as influenced by fluorine: "Some of the explanations put forward to account for it have been rather fantastic, and the true explanation is probably quite a simple one, namely, that F is not sufficiently powerful as a caries-inhibiting factor to produce a great reduction in those teeth, such as molars, which are specially prone to caries, but that it is sufficiently powerful to protect the great majority of those teeth which are not naturally highly susceptible." Weaver presented data on the eruption of the permanent teeth and found no significant difference in those from the two cities.

Boyd (1943a) found no correlation between annual increment of caries in 55 children and either the maximum or minimum regional fluorine content of waters. The negative correlation coefficients between incidence of caries in permanent teeth and regional fluorine values were likewise below significance levels. No data were given relative to the fluorine levels.

Weaver (1944b) concluded from the increase shown in the DMF teeth in children and in adults in North and South Shields, England, that fluorine acts to defer caries 3 to 5 years rather than to prevent its onset. He examined 800 immigrants into a fluoride area in comparison with 800 age- and sex-paired natives. The ages at immigration ranged from under 1 year to 14 years and over. He considered only the data for first permanent molars as being of statistical value. The DMF first molar teeth did not differ as between the natives and those who entered the fluoride area at age 5 or less, but in all the other age groups the rate was lower in the natives. Weaver said: "Those children who arrived at about the ages of 6 and 7 years apparently missed something, and those who arrived at about 8 years of age or later were no worse off than if they had arrived at 7 years of age." Weaver offered two tentative statements: "(a) If F (probably fluorine) produces its caries-inhibiting effect through an influence exerted on the teeth before eruption, it is only during a late stage in the pre-eruptive life of the teeth that such an influence is effective. Exposure to the influence of F before that stage is reached has no effect on caries incidence. (b) If F (probably fluorine) produces its caries-inhibiting effect through an influence exerted after eruption, it is only during an early stage in the post-eruptive life of the teeth that such an influence is effective. If there is no exposure to the influence of F until after that stage is passed the caries incidence is not affected." He concluded: "The evidence obtained is, on the whole, in favour of the view that the influence of F is exerted on the teeth during the pre-eruptive period."

Ockerse (1943) analyzed teeth from children of the Knysna and Calvinia districts of South Africa. Of 673 children from the

Knysna district only 6 had no caries, and no mottled enamel was found. "The teeth and jaws of the children in the Calvinia district are well developed and strong in appearance. The teeth are lustrous and of a light cream colour. The cusps are low and flat. The grooves and fissures are shallow and exhibit no defective enamel structure, and this prevents food particles from lodging in them. Of 556 children examined, 335 never suffered from caries. One hundred and forty-five children had mottled enamel, varying in degree from very mild to moderate." The fluorine contents of the waters were, respectively, 0.19 and 1.5 p.p.m. The average analysis of a total of 40 samples of enamel showed 153 p.p.m. F in carious and sound enamel in the Knysna samples and 445 p.p.m. in the sound enamel from the Calvinia district. The Knysna carious dentin contained 252 p.p.m. F, the sound dentin 268 p.p.m.; the sound dentin from Calvinia contained 755 p.p.m. F. The method for fluorine was not described. Ockerse said: "It appears that an optimum amount of fluorine in the drinking water assists the proper calcification of the teeth, making them more resistant to caries." That fluorine is not the only factor in freedom from caries was indicated by skulls of Bushmen from the Knysna district with no evidence of caries. They drank water from the same sources as the present residents but were apparently heavy meat eaters. No analyses were made of these teeth.

Box and Hodgins (1944) reported the findings of Arkle on the relation of caries to the fluorine content of the waters of three communities of Ontario.

Nizel and Bibby (1944) reported the number of DM ("defective" and "missing") teeth in 22,117 men by states of U. S. A. The highest rate was in men from Delaware (17.05) and the lowest, for men from Texas (6.44). The northeastern and the northwestern states had generally the high rates. The authors speculated on the influence of soil composition as affecting caries rate, with specific mention of fluorine.

McKay (1945) presented, by bargraphs the number of decayed areas found in the teeth of "100 persons who were native to and had maintained continuous residence in Colorado Springs which has had a water supply containing fluorine for upwards of sixty years." They were unselected and ranged in ages from 11 to 60, with an average of 25 years. There were 35 with no decayed teeth and loss of teeth through decay was negligible. The average number of decayed teeth in the 100 persons was 2.8. "The graph shows that decay has been practically limited to the molar teeth and that its occurrence drops to almost nothing in the bicuspids. Aside from this, it is interesting to note that the decay has occurred almost entirely in the pits and fissures. It rarely originates on the proximal surfaces but is limited to the occlusal surfaces. With the exception of a single decayed area in one incisor tooth, the cuspid and incisor group is immune. All fillings were charted as evidence of past decay but it can reasonably be assumed that many of those on occlusal surfaces had been inserted for protective purposes. On this assumption, the amount of actual decay would be materially reduced.

"Many discolored fissures in fluorosed teeth can be observed in persons well into the twenties and even thirties that cannot be classified as being decayed, whereas in the usual environment such fissures would no doubt long since have become carious."

Reference was made by McKay (1945) to a study by Dr. R. A. Downs in which 31 of 71 children, average age 13.7, "all with fluorosed teeth," had an average of 2.07 decayed teeth per child, and 31 were caries-free.

Boyd and Cheyne (1946a) reported the relations of caries and mottled enamel in 85 high school children of Ankeny, Iowa, where the fluorine content of the water was changed in 1936 from more than 6 p.p.m. to 1 p.p.m. Two-thirds of the subjects had

mottled enamel. The average number of DMF teeth for 35 children with no mottling of enamel was 9.6; for 50 with mottled enamel, 6.7; for subjects with mottling and continued use of water with varied concentration of fluorine, 6.1. These investigators found no evidence of protective effect of posteruptive ingestion of water containing fluorides.

Downs and McKay (1946) examined high school students in Montrose, Colorado, where the water supply was reported to contain 1.4 p.p.m. fluorine but the caries rate was high. Of 77 native children only one was free of dental caries and no mottled enamel was found. In 67 children from surrounding areas, with mottled enamel, 27 were caries-free. The natives averaged 10.2 carious areas each; the children with mottled enamel, 1.8. There was an average of 0.818 extracted teeth among the native children and 0.179 in the mottled enamel group. Analysis of the water showed the report of 1.4 p.p.m. fluorine to be in error. The correct content was 0.3.

Klein (1946) reported the increment of decay in the teeth of two groups of Japanese children relocated from Los Angeles, with 0.1 p.p.m. fluorine in its water supply, to an area with the same amount of fluorine and to one with 3 p.p.m. There were 196 children in the first group and 120 in the second. The caries increment was observed after two years of residence. The percentage increases were 58.3 and 35.9 in the first permanent molars of 8- to 10-year-old children, and 38.1 and 35.1 in the 11- to 14-year group. In the second permanent molars, increases were 47.3 and 15.9 in teeth that were erupted at the beginning of the study, and 26.0 and 3.5 carious in teeth that had erupted during the two-year period. In second bicuspids, erupted at the beginning of the study, cavities were found in 6.6 per cent in the fluoride area and 9.5 per cent in the non-fluoride area; in teeth erupted during the period the incidences were 0.7 and 4.5 per cent. Klein concluded that there is some protection by water fluoride to teeth very late in the period of eruption and possibly some protection posteruptively.

Klein (1947) found that the association of caries rates of children with that of the father and mother was not obscured by reduction of caries rates by water fluoride. In three New Jersey towns with water fluoride ranging from 1.3 to 2.2 for the 19 previous years, the children of 123 fathers and 131 mothers showed lower caries rates if their fathers and mothers were in the low caries rate category. The DMF rates among the children of these towns in comparison with non-fluoride areas of New Jersey were: for ages 5 to 9 years, 0.3 and 1.4; for ages 10 to 14 years, 1.5 and 5.9; for ages 15 to 19 years, 3.0 and 11.4.

Klein (1948a) compared the caries rates of the primary and permanent teeth of children in neighboring New Jersey towns, two of which had used since 1927 water with 1.4 to 2.2 p.p.m. fluorine. The caries rate in the towns with the fluoride water was generally less than one-third that of children of the same age from the non-fluoride town up to the age of 18. A similar ratio was found for the primary teeth. Migrants from fluoride to non-fluoride areas and in the reverse direction showed intermediate effects.

Klein (1948b) determined the reduction of caries rates of the various teeth by the use of water containing 1.3 to 2.2 p.p.m. fluorine by persons up to 19 years of age. Expressed as per cent reduction in comparison with non-fluoride but neighboring areas, the results for 15- to 19-year-old children were:

	Maxillary	*Mandibular*
Central incisors	91.4	95.6
Lateral incisors	89.2	100.0
Cuspids	90.9	100.0
First premolars	86.4	79.8
Second premolars	80.4	83.5
First molars	58.4	39.7
Second molars	65.8	47.1

Frisch (1948) examined 414 native, continuous-residence children and 327 migrants with mottled enamel of varying degrees in Salida, Colorado, where water supplies ranged in fluorine content from 0.7 to 4.4 p.p.m., with the main supply having 1.3 p.p.m. Caries rates in all age groups up to 17 years were about one-fifth those of children in Madison, Wisconsin, with a water supply containing 0.05 p.p.m. The caries rate in the deciduous teeth in the age group 6 to 8 was significantly lower than in Madison, though not as widely different as in the case of the permanent teeth. The consistently though slightly higher rate of caries in the permanent teeth of the children of Union Grove, Wisconsin, with 1.0–1.2 p.p.m. fluorine compared with the Salida children on the water supply with 1.3 p.p.m. led Frisch to state: "The increased protection to decay, however, by 1.3 as against 1.0 is significant". Enamel fissures which could be entered with an explorer were frequently found non-carious in the teeth of the 15- to 17-year-old children and of the adults of Salida.

McKay (1948) summarized observations on the caries conditions of 400 native, continuous-residence persons of Colorado Springs and compared them with findings of Frisch on 1746 natives of Madison, Wisconsin, of similar age. Included was a study of 216 migratory persons with mottled enamel, examined in Colorado Springs and Montrose, Colorado. The DMF teeth index and average number of teeth lost were much higher for Madison than for Colorado Springs.

Pelton (1949) reported on the basis of examination of 8,229 school children in four Wyoming cities that the dental caries attack rate in one town with 0.0 to 0.3 p.p.m. fluorine in the water supply was about twice that in cities with 0.3 to 8.0 p.p.m. The loss of teeth was correspondingly high.

Russell (1949a) examined 339 children of Mitchell, South Dakota, who had been exposed for 18 months prior to eruption of their first permanent molars to water containing a mean level of 1.15 p.p.m. About 10 per cent of the girls and 5 per cent of the boys showed very mild to mild degrees of mottled enamel.

Russell (1949b) examined the caries rates of children migrating from various South Dakota fluoride areas into Mitchell, South Dakota, with a water supply at 0.2 p.p.m. Group A children were the continuous-residence subjects of Russell 1949a; Group B comprised 127 children with fluoride exposure prior to age 6; Group C were children with exposure to fluoride water both before and after 6 years of age. Russell concluded that the protection acquired by fluoride exposure in Group B, less than four years on the average, did not persist.

Hardgrove and Bull (1947) reported DMF rates of 1.27 and 4.82, respectively, in the teeth of 557 and 416 5- to 6-year-old continuous-residence children of Green Bay and Sheboygan, Wisconsin. The fluorine content of the waters were, respectively, 2.3 and 0.05 p.p.m. The percentages of caries-free children were 58.1 in Green Bay and 20.4 in Sheboygan.

Ockerse (1947) in a letter stated that he had observed that children born of mothers using water containing 10 p.p.m. fluorine during gestation and lactation had deciduous teeth that "were remarkably free from caries and were not mottled."

Coumoulos (1949) reported on her basis of grading of mottled enamel in deciduous teeth that 52.2 per cent of 1552 teeth of 5-year-old children in Maldon, England, showed some sign of mottling. The "average caries figures" for these children, given for surveys in 1943 and 1945, were 0.20 and 0.21, respectively, compared with 0.63 teeth from urban areas of England and 0.66 for rural areas. She remarked: "Many of the deciduous teeth were unusually white and shiny and aesthetically perfect."

Aisenberg (1946) found from statistics of poliomyelitis cases that "in a total population of 12,427,512 there were reported

9,174 cases of poliomyelitis, or seventy-four cases per 100,000" where the fluorine content of the water was less than 1 p.p.m. Where the fluorine was 1 p.p.m. or higher, "In a total population of 927,905 there were reported 404 cases of poliomyelitis, or forty-four per 100,000". His hypotheses were that poliomyelitis virus found a portal of entry in exposed dental pulp and that fewer such exposed pulps would be present in fluoride areas because of the lower caries rates.

Drum (1949) analyzed water from 42 different sources in Ireland and found no fluorine in 32 and a level of the order of 0.2 p.p.m. in the other 10.

Harrison (1949) found a mean fluorine content of the enamel of 89 New Zealand-born subjects to be 42 p.p.m., with no significant difference between the sound enamel of carious and non-carious teeth. New Zealand waters generally have less than 0.5 p.p.m., with a maximum of 0.6 p.p.m. in one water source. Irregularly high fluorine contents were found in European-born subjects and in the teeth of natives of several Pacific island groups. Low values were indicated for teeth of Australians.

Shourie, Hein, Leung, Simmons and Marshall-Day (1949) stated, "Fluoride content of drinking water in Puerto Rico usually is either negative or 0.1 p.p.m. However, the town of Carolina, where children have the lowest D.M.F., has 0.4 p.p.m. of fluoride".

Discussion

Dean has given further evidence that the deciduous teeth, on a whole-mouth basis, are less protected by fluorine than the permanent teeth. Deatherage's data, which show by the declining caries rate with advancing age that deciduous teeth are included, indicate, however, that fluorine does partially protect the deciduous teeth.

It has been shown that a condition of mottled enamel is not necessary for a reduced caries rate. Rather it appears that protection against caries by fluorine is operative in spite of the defective structure in mottled enamel.

The amylolytic activity of saliva is unaffected by fluorides in community water supplies up to 1.8 p.p.m.

L. acidophilus counts are correlated with caries activity rather than with the fluorine content of the current water supply, indicating that the action of fluorides in protecting teeth from caries is not one of the direct inhibition of *L. acidophilus* by the fluorine of the water or by fluoride adsorbed on tooth surfaces from the currently imbibed water. *L. acidophilus* counts may be negatively correlated with the fluorine content of the enamel but evidence from field studies is lacking, since no pertinent analyses of enamel are available.

As Dean and his coworkers examined children 12 to 14 years of age, with a history of continuous unchanged residence from birth, they have given no evidence as to whether protection is pre- or post-eruptively effective or both. Cox (1940) has deduced from the data on 76 children, reported by Dean, Jay, Arnold, McClure and Elvove (1939) as living in Galesburg during the first 6 years of their lives but not born there or with interruptions of residence, "that the oral conditions presented by drinking water or by modification of the saliva during the posteruptive period were not the factors in the protection of the teeth of the children born in Galesburg." The intermediate caries rate "of children residing in Green Bay but born elsewhere" shown by Bull and the evidence of some mottled enamel in this group might be considered presumptive evidence that only teeth formed with fluorine are protected from caries.

Deatherage, by examination of the dental records of men who had lived the first 8 years of their lives in areas high (or low) in fluorine and subsequently lived in areas with low (or high) fluorine, has shown that preeruptive fluorine is protective.

On the other hand, men who moved from a high- to a low-fluorine district had more caries than those who lived continuously in a fluoride area. This suggests a post-eruptive protection by fluoride.

Deatherage's (1942) instructions to selective service clerks were to record *"Where lived the first 8 years of life."* This implies that the data are concerned with an age centering on 8 years and ranging from 7½ to 8½, rather than centered on 8½ years. The crown formation tables of Schour and Massler (1940), cited by Deatherage to prove that "the enamel of the permanent teeth (excluding the third molars) has completely calcified at 8 years of age," are unsupported by any data. Even if it were true that calcification of enamel is complete at 8 years, there would be considerable variation, and many children at less than 8 years of age would have considerable increments of enamel on second molars yet to be acquired. If this enamel were formed in low-fluoride areas, it would presumably have the characteristics of low-fluoride enamel with respect to dental caries.

There have been statements made that eruption of the permanent teeth is delayed in mottled-enamel areas, though no actual data support the statements. A delay of eruption suggests that completion of crowns is also late in areas with high fluorine. If such were actually the case there would be more second-molar enamel finished in low-fluoride zones, in the case of migrants at 8 years mean age, than in high-fluoride areas.

The data of Deatherage (1943b) indicate that fluorine has failed to protect about two teeth per man after 8 years of age. It is suggested by the reviewer that the second molars are responsible for the differences in the rates. (Third molars were excluded in all of Deatherage's studies.) The difference between fluorine-free until 8 years and fluorine-free continuously is 0.84 tooth per man. However, in this case the possibility of delayed crown formation operates in favor of the view that there is posteruptive protection from fluorine, but the general statistical facts of age-of-moving and completion-of-crowns are unchanged in their import.

It may be noted that Akeroyd (1923) said: "In going over the results of a school survey at Columbus, New Mexico, it was found that several cases, with histories of having been born or having lived a number of years in this district, were recorded as 'not mottled', 'not stained'. Generally, these cases were children who came to live in this district when they were about nine years old. Upon reexamination of these cases the affection was seen to exist in all cases. The stained and mottled condition showed only upon twelve-year molars."

The significance of slight differences in caries rates representing whole-mouth conditions may be questioned. For example, Dean (1938a) gives a rate of 275 carious permanent teeth for Green Bay children; Bull (1943) reports 262. Dean gives 710 for Sheboygan and Bull shows 854. The data for Green Bay are in excellent agreement, but those for Sheboygan indicate an increment of 1½ decayed teeth per child.

There is no evidence from field studies of fluorine and caries that enamel structure is a factor in resistance to caries. Similarly, there is no direct evidence from these studies that the chemical composition of the teeth is related to caries rates.

Conclusions

1. Fluorine, present in the drinking water during all of the first 12 to 14 years of life in such amount that little or no mottling of enamel is produced, effects a reduction in caries rate of permanent teeth to about one-third of that found in comparable non-fluoride areas.

2. Deciduous teeth are also protected from caries but to a lesser degree than permanent teeth.

3. The mechanism of prevention of caries by the fluorides of drinking water has not been shown by field studies.

4. Most of the protection from caries appears to be from the fluorine present during the formation of the enamel.

THE MINIMA OF FLUORINE FOR CERTAIN TOXIC EFFECTS

Lethal Dose

Rabuteau (1867) observed the effects of the fluorides of sodium, potassium, ammonia and others on cats, rabbits, dogs and frogs. In particular he noted vomiting and intense salivation. Considering the effects not as drastic as he had anticipated, Rabuteau took by mouth one-quarter gm. of sodium fluoride in 25 cc. of water. (This concentration is approximately 4500 p.p.m. as fluorine.) He noted slight nausea and epigastric disturbance, which disappeared in about five hours. Salivation was especially intense one-quarter to one-half hour after taking the salt but ceased 1½ hours later. He noted an itching sensation that lasted about a week in the trunk, hands and feet.

Hewelke (1889, 1890) observed that a dog, given 20 to 50 mg. NaF daily for 47 days, died. Another heavier animal given doses of 20, 100, 150 and finally 400 mg. of NaF daily, survived the experiment of 100 days' duration. In a third dog there was no effect on nitrogen output with a daily administration of 200 mg. of NaF.

Gettler and Ellerbrook (1939) estimated the fluorine content of human tissues by the Armstrong (1936) method. The range of values they reported as p.p.m. F for normal wet tissues and for 5 cases of fatal fluorine poisoning were as follows:

	Normal	*Poisoned*
Liver	0.36–0.70	4.4–15.0
Kidney	0.56–0.78	4.6–11.6
Blood	0.20–0.64	3.5–12.1
Brain	0.40–0.68	1.6– 3.4
Lung	0.16–0.42	12.4–15.6
Heart	0.44–0.60	10.6
Spleen	0.25–0.30	11.8
Femur	112, 119, 141	
Teeth	275, 310	
Enamel	190	
Urine	0.40–0.56	

The fluorine content of the fatal-case tissues was variable but was consistent in that the fluorine was increased about the same for all tissues analyzed. The tissues of 5 dogs killed by fluoride poisoning had fluorine of the same order as that of human tissues, but chronic poisoning by 18 and 32 mg. of NaF per kg. per day in two dogs caused no increase in the fluorine content of the soft tissues.

On the basis of their data and excluding the fluorine of the gastrointestinal tract, bones, teeth, fat, skin, hair and nails, Gettler and Ellerbrook calculated for a man of 63 kg. weight: "The smallest lethal dose of fluorine absorbed into the internal organs in our cases was 104.66 mg." The dosage used by Rabuteau (1867) as fluoride was approximately 110 mg.

Chronic Fatal Intoxication

Cristiani and Chausse (1927) fed guinea pigs one-fiftieth, one-twentieth and one-tenth the daily dose of sodium fluoride necessary to cause acute intoxication and death. They found cachexia bordering on death with the one-tenth dosage in 2 to 3 months.

Weaver (1944b) compared the crude death rates for Tynemouth and South Shields, England, for the 10-year period 1930–1939. In every year the death rate was higher in South Shields, with 1.4 p.p.m. fluoride in the water, than in Tynemouth, with less than 0.25 p.p.m., the rates ranging from 12.6 to 15.6 for South Shields and 11.4 to 13.3 for Tynemouth per 1000 population. Weaver could not link any component of the death rate statistics with the fluoride of the water.

Retardation of Growth

McClure and Mitchell (1931a) studied the relative effects of sodium and calcium fluorides in paired feeding of rats. Fluorine at 313 p.p.m. from either salt inhibited growth.

Lamb, Phillips, Hart and Bohstedt (1933)

concluded: "Daily intakes of approximately 20 mgm. fluorine per kgm. of body weight per day in the form of sodium fluoride, and 40 mgm. fluorine per kgm. body weight per day in the form of rock phosphate, appear to be the upper limits of safety for growth in the rat."

Phillips, Hart and Bohstedt (1934b) found the critical margin of fluorine intake to affect growth by dairy cows to be 2 to 3 mg. per kg. body weight per day.

Smith and Leverton (1933, 1934) found the growth rate of rats depressed 15 per cent by 226 p.p.m. fluorine added as sodium fluoride in the Sherman Diet B.

Sharpless (1936a, b) found growth unimpaired in rats fed 250 p.p.m. NaF but slowed to two-thirds by 1000 p.p.m.

Reproduction and Lactation

Lamb, Phillips, Hart and Bohstedt (1933) found that 190 p.p.m. of sodium fluoride in the diet of mothers lowered birth weights of rats slightly, reduced weaning weights significantly (34.9 gm. at 4 weeks compared with 43.4 gm. for controls) but was without effect on mature weights of the animals.

Phillips, Lamb, Hart and Bohstedt (1933) found that such interference by sodium fluoride as occurs in reproduction and lactation in the rat can be ascribed to inanition. The oestrus cycle was not disturbed until a threshold value of 25 mg. F per kg. body weight was reached, and parallel effects were obtained in controls by restricting food intake to a comparable level. Lactation was affected, apparently, by the demand for increased food resulting in an intake of fluorine over 25 mg. per kg. body weight per day.

Phillips, Hart and Bohstedt (1934b) observed that milk production was reduced as to volume but unaffected in composition by 2 to 3 mg. per kg. intake of fluorine by dairy cows. Higher intakes of fluorine failed to interfere with reproduction.

Smith and Leverton (1934) raised 4 generations of rats on rations with 226 p.p.m. added soluble fluoride. The young were smaller at birth and fewer were weaned on control rations. Reproduction failed on 452 p.p.m. fluoride addition.

Calcium Balance

McClure and Mitchell (1931a) concluded that at the 623 p.p.m. level sodium fluoride lowered food consumption. The calcium balance was not affected by either sodium or calcium fluoride up to 313 p.p.m., but both salts lowered calcium retention at 623 p.p.m. The ash content of the bones was increased at the 313 p.p.m. level for both salts.

McClure and Mitchell (1931b) found no effect on calcium balance in 5 pigs fed 170 and 260 p.p.m. fluorine (3.5 and 5.5 mg. per kg. per day) as calcium fluoride mixed with tricalcium phosphate.

Blood Pressure

Greenwood, Hewitt and Nelson (1933–34) found injection of 2.9 mg. of fluorine (as sodium fluoride) per kg. was required to produce a perceptible increase in respiration rate in dogs, 22.9 mg. to affect blood pressure, 47.0 mg. per kg. to cause death. The data are for 5 dogs. An oral dosage of 22.6 mg. produced a detectable increase in respiration; a higher dosage caused vomiting. The authors said: "Dr. A. E. Merkel of Ankeny, Iowa," where Ostrem, Nelson, Greenwood and Wilhelm (1932) had reported mottled enamel associated with 10 to 15 p.p.m. fluorine in water, "has made blood pressure studies of 65 school children who received fluorine water and exhibited mottled enamel, and he has observed that there is no significant variation in blood pressure from the normal."

Ears

Lewy (1928) reported the treatment of 8 cases of human otosclerosis with 0.12 gm. of calcium fluoride given 3 times per day in alternate months. The results were inconclusive. He found the cochleae of mice

affected by 6 to 9 months' feeding of calcium fluoride. He found no dental effects other than slight discoloration. All of his experimental animals (6) died.

Lewy (1944) reported the examination for deafness of 132,572 children of 68 of the 102 counties of Illinois. Of 109, 869 children from low fluoride areas, 5,406 had hearing defects, a percentage of 4.9. "In four counties near Chicago, whose drinking water contains fluorine not to exceed 1.4 parts per million, from which statistics were available, 20,488 children were examined; the number with defective hearing was 574; the percentage defective, 2.8. In Chicago alone, 21,200 children were examined, 922 of whom were found defective, a percentage of 4.3. Chicago drinking water comes from Lake Michigan and is fluorine-free." He said, "One cannot know how many potential otosclerotic or fibrotic ears are in the group."

Enzymes

Kastle and Loevenhart (1900), in studying hydrolysis of ethyl butyrate by animal lipases, found the fluoride ion was the most potent inhibitor. Loevenhart and Pierce (1906–1907) extended the lipase-fluoride studies to find inhibition of 50 per cent of the hydrolysis of ethyl acetate by a clear liver extract by 1–5,000,000 NaF. However, the action of a turbid pancreas lipase on olive oil was accelerated 13 per cent by 1–5,000,000 NaF and was inhibited only by a concentration of 1–5000. In general the point of change of inhibitor to accelerator varied with the enzyme preparation and with the substrates. Amberg and Loevenhart (1908) concluded that the inhibition of lipase action diminishes as the fatty acid chain of the substrate lengthens. They studied the determination of fluoride in milk, meat and beer by means of lipase inhibition. Inhibition of 25.9 per cent was observed in milk with 5 p.p.m. of NaF added. In most instances 1 to 20 per cent acceleration of action was noted in milk. In meat 100 p.p.m. NaF could be detected. The test failed when applied directly to beer.

Clifford (1936) found that chlorides, bromides and iodides generally accelerated the hydrolysis of starch by pancreatic and salivary amylases. No acceleration by fluorides was found at any concentration. Ammonium fluoride was inhibitory at 0.0005 molar, potassium fluoride at 0.03 molar, but sodium fluoride failed to inhibit at concentrations as high as 0.5 molar. (The 0.0005 molar ammonium fluoride corresponds to 9.5 p.p.m. F.)

Rockwood (1919) tested a series of 0.01 molar salt solutions for their action on digestion of starch by ptyalin. Sodium and ammonium fluorides decreased amylolytic activity. The fluoride ion concentration in these tests was 190 p.p.m.

McClure (1939b), in tests with careful pH control, could find no effect on salivary amylase of various fluorides ranging in concentration up to 8550 p.p.m. He found the mean formation of maltose by the salivas of 82 children of Galesburg, Illinois, (1.8 p.p.m. F in water) to be 108.7 $\pm$ 3.1 mg.; for 63 Quincy, Illinois, children ("fluorine-free" water) the maltose formed was 105.9 $\pm$ 5.2 mg.

Roholm, Gutman and Gutman (1937–1938) found normal serum phosphatase in 14 of 20 subjects who had worked in contact with cryolite for 8 to 34 years. There was a slight elevation of phosphatase in the other 6 cases.

Stiff Backs

Brandl and Tappeiner (1891) fed a large dog 402.9 gm. of sodium fluoride over a period of 21 months. They recovered fluorine equivalent to 330.5 gm. of sodium fluoride from the urine and feces, about one-fifth being in the latter. In the tissues they found 64.6 gm. (NaF) and, in view of tissues not analyzed, considered they had accounted for all fluorine. They noted that the dog developed a stiffness of the lower spine. The bones were whiter than normal and more

resistant to pulverizing. They found crystals, tentatively identified as calcium fluoride, in the Haversian canals.

Shortt, Pandit and Raghavachari (1937) investigated a condition of lumbar stiffness of natives of districts near Madras, India. This stiffness of the back was found in adults over 30 years of age; in some adults over 40, in the extreme condition there was complete rigidity of the spine and joints of the limbs. Shortt *et al.* found a concurrent high incidence of mottled enamel with fluorine (indicated by preliminary studies of the water) at 0 to 10 p.p.m. The natives associated the condition with the water supply.

Shortt, McRobert, Barnard and Nayar (1937) examined 10 subjects with stiff backs. The radiological findings showed an increased density of the bones in the affected parts. They found renal impairment in most of their cases but did not associate it as cause or effect. Pandit, Raghavachari, Rao and Krishnamurti (1940) estimated the daily fluorine intake in the stiff-back districts of India as: 5.4 mg. in areas with slight incidence; 9.4 mg. in an area with moderate incidence; and 13.2 to 24.0 mg. in districts with the severest conditions. These estimates were based on a daily use of 6 pints of water. They believed there was a "definite relation to the economic and nutritional status of the communities" and implied a vitamin C deficit was contributory. They reported "bone affections were fairly prevalent" in a town "with a fluoride content of only 2 parts per million in their water supply."

Pandit and Rao (1940) gave 10 mg. of sodium fluoride per day to four monkeys (*M. radiata*), two of them being on a scorbutic diet. Two other monkeys served as controls. During the first 5 days, 20 mg. NaF was given. The dosage was halved because of diarrhea. "After four months when the animals were becoming asthenic, the dose was further reduced to 5 mg." In one monkey, in an experiment for 16 weeks, 71.9 per cent of the fluorine was retained; in another, on the scorbutic diet for 12 weeks, 78.4 per cent was retained. The excretion of fluorine was about equally divided between urine and feces. Blood glycolysis was not affected. After four weeks it was noticed that the urine of two of the monkeys, one on the scorbutic diet, became dark on standing and homogentisic acid was identified. The bones, particularly the mandibles and to a greater extent in the animals with a low vitamin C intake, showed exostoses. Ribs, lumbar vertebrae, pelvis, humeri, femora, radii and ulnae showed radiographically increased density.

Capizzano, Toledo, Megy and Valotto (1939) reported nine cases of osteopetrosis in the endemic mottled enamel districts of Argentina. Capizzano, Valotto and Megy (1940) extended the above observations to include 5 of 6 persons (in one family) as affected, 2 of 18 examined in another locality, and 14 in 154 in a third. Numerous radiographs were shown but no pertinent water analyses. Apparently the fluorine content of the waters exceeded 1.5 p.p.m.

Mascheroni, Munos and Reussi (1939) described a case of general osteoporosis in a 23-year-old woman from La Pampa province in Argentina. No water analyses were given. The fluorine in the ash of a sample of bone was 9750 p.p.m.

Ockerse (1941a) reported 8 cases of suspected fluoride osteosclerosis in South Africa, 6 of which he observed personally. Exposure was to drinking water containing about 12 p.p.m., which produced mild to severe mottling of the teeth of children. The 6 adults examined showed stiffness of the spine and other symptoms as described by Shortt *et al.*, (1937) and Roholm (1937b).

Kemp, Murray and Wilson (1942) made a radiological examination of 5 adults and 22 children in England to determine association of bone changes with mottled enamel. They found 2 cases of severe skeletal changes, associated with severe dental effects.

Linsman and McMurray (1943) described fluoride osteosclerosis in a man aged 22. His exposure to fluoride-containing water

was: 12 p.p.m. for 7 years; 5.7 p.p.m., 2 years; 4.4 p.p.m., 7 years; low F, 2 years; 4.4 p.p.m., 3 years. At 15 he suffered an injury to the right kidney, and autopsy showed a cystic condition. The left kidney was atrophied. Tooth enamel was severely mottled. Roentgenograms showed greatly increased density of the pelvic and spinal bones; other bones were less affected. The fluorine content of the bone ash of the sternum was 0.69 per cent and of the skull bones, 0.75 per cent. The lower bicuspids had 0.45 per cent fluorine in ash. Control bones and teeth showed no fluorine. The authors indicated belief that the greater effects were present in vertebrae because of higher activity of these bones. They said: "The chronic fluoremia may have aggravated existing kidney lesions. Or it is entirely possible that, because renal impairment was present, the osteosclerosis developed as a result of fluorine retention." They recommended radiographic examination of patients with dental fluorosis and anemia with or without signs of renal impairment. They also recommended study in regions where the fluorine content of the water exceeds 3 p.p.m.

Hodges, Fareed, Ruggy and Chudnoff (1941) examined radiologically 86 residents of Kempton, Illinois, (1.2 to 3.0 p.p.m. F) and Bureau, Illinois, (2.5 p.p.m. F). The age range was from 7½ to 71 years. They found no evidence of bone injury and said: "The use of drinking water containing up to 3 parts per million of sodium fluoride apparently does not cause radiologically demonstrable sclerosis of the skeleton even though the water is taken for a long time."[6]

Mottled Enamel

Smith and Leverton (1934) were able to detect, with certainty, pigment striation on rat incisors on a ration with 14 p.p.m. added fluoride from any source and, in some cases, with 7 p.p.m. from sodium fluoride or barium fluosilicate. Intermediate additions were not tested. They concluded: "The amount of fluorine required to cause initial damage to the rat incisors was so small that differences in solubility of the compounds were not a factor, and no differences in toxicity of fluorine from the various compounds studied could be noted."

Marcovitch, Shuey and Stanley (1937) stated from studies on rats: "Faint striations were produced in 50 per cent of the animals by sodium fluoride given in the drinking water at the rate of 4 parts of fluorine per million. As the animals drank about 10 or 11 cc. of water, this is equivalent to 0.4 mg. per kilogram of body weight. According to these figures, a 30-pound child will require only 6 mg. of fluorine, in water used for drinking only, to produce mottling."

H. V. Smith (1935) found that the results for fluorine by the Foster (1933), Willard and Winter (1933) and Sanchis (1934) methods as applied to Arizona waters were substantially in agreement, but that the Fairchild (1930) method gave generally too high results. The earlier analyses of Smith and Smith had been by the Fairchild method. Smith listed Arizona and non-mottled enamel and stated: "The evidence indicates strongly that any water with a fluorine content of 0.9 p.p.m. (when analyzed by the Foster, Willard or Sanchis methods) or over is dangerous from the standpoint of probable damage to the teeth."

Summary

Below are listed some estimates of minimum levels of fluoride that produce certain toxic effects together with the species upon which observations were made. Future studies may lower some of these values, but as most of them are far above the levels of fluoride found most useful and safe in the prevention of dental caries such new values will probably be of little significance.

[6] The report of Weart and Klassen (1937), from whom Hodges *et al.* derived their data for water fluorides, indicates that the fluoride of these water supplies is stated on a fluoride ion basis and not on sodium fluoride.

Lethal dosage (absorbed)	100 mg.	man
Chronic fatal intoxication	10 mg./day	guinea pig
Retardation of growth	20 mg./kg./day	rat
Retardation of growth	2–3 mg./kg./day	cow
Retardation of growth	about 200 p.p.m. in food	rat
Birth weight reduction	190 p.p.m. in food	rat
Oestrus cycle interference	25 mg./kg./day	rat
Milk volume decrease	2–3 mg./kg./day	cow
Reproduction interference	450 p.p.m. in food	rat
Food consumption reduction	623 p.p.m. in food	rat
Calcium imbalance	623 p.p.m. in food	rat
Blood pressure, no change	10 p.p.m. in water	man
Lipase inhibition*	0.2 p.p.m.	
Amylase inhibition	Probably 200 p.p.m.	
Amylase of saliva, inhibition	1.8 p.p.m.	man
Phosphatase interference	Severe mottled enamel dosage	man
Stiff backs	2.5 p.p.m. in water	man
Mottled enamel	4 p.p.m. in water	rat
Mottled enamel	0.9 p.p.m. in water	man

* Variable action, stimulation under some circumstances.

SOME CONSIDERATIONS OF FLUORINE AS A BENEFICIAL ELEMENT

Essentiality in Animal Life

Wilson (1846b) wrote: "In conclusion, I would observe, that physiologists will doubtless now be tempted to speculate on the possibility of fluorine performing some essential function in living animals. Its occasional absence from their bones would not disprove that it may be necessary for the perfection of certain organs, though not for all. Quantitative analyses appear already to have indicated that the enamel of teeth contains more fluorine than any other part of the body. If that result shall be confirmed, we may suppose that, if fluorine be furnished when the development of the teeth is proceeding, it may be wanting at other periods, without injury to the animal; just as chloride of sodium must be considered as essential to the healthy life of most creatures, though they may be deprived of it for long intervals without death ensuing.

"The small quantity of fluorine found in living structures can be counted no argument against its occasional or constant importance. Quantity is at best but a rude measure of the value of an ingredient, in relation to the necessities of an organism. The law of final causes, in truth, would indicate that *only* a minute proportion of fluorine should occur in any organ; for it would be perilous to an animal to introduce into its system a large quantity of fluorides, which can so readily be changed into the deadly hydrofluoric acid. Such speculations, however, are premature. It will be time enough, when many qualitative, but especially quantitative, researches have been prosecuted, as to the presence of fluorine in animal structures, to consider of what service it is to them, if it be of any."

Osborne and Mendel (1913), finding that an "artificial protein-free milk" made from analytical grade chemicals failed to give the growth in rats that a salt mixture of ordinary commercial chemicals gave, added traces of iodine, manganese, fluorine and aluminum "without a guide as to the proper amount. . . ." The sodium fluoride was 0.0062 gm. per kg. of the food mixture into which it was incorporated. Better growth was obtained with these additions, but no conclusions were reached with specific reference to any one of them. In 1918 a salt mix-

ture containing four times as much sodium fluoride, but with no changes in the other trace elements, was used.

Mazé (1925) found that rats could not reproduce on skim milk powder but could do so on whole milk powder or on skim milk powder to which had been added 1 part $CaHPO_4$, 0.1 part NaF and 0.05 part KI per 1000 parts skim milk powder. The $CaHPO_4$ was found to have no effect. Fourteen litters with 75 young were produced with the supplemented milk powder but none on the unsupplemented. Experiments were initiated to determine the relative effectiveness of iodine and fluorine, but at the time of publication only one litter had been obtained. It was from a ration with fluoride addition.

Price (1932a) reported that very high dosage of fluorine to rats (1 per cent of NaF in the ration) depressed iron, calcium, phosphorus and potassium of blood and increased magnesium.

Krasnow and Serle (1933) stated of rats, in groups of 25: "Diets containing traces of F and 0.0005 per cent of viosterol (half minimal dose) often caused greater percentage gain in weight than the same diets having more F. On diets containing 0.0001 per cent of NaF and 0.0008 per cent of viosterol, rats while younger than fifteen weeks grew more rapidly than those on diets containing traces of F. On diets containing 0.0025 per cent of NaF, *female* rats showed, after twelve weeks, greater increase in weight than female rats on rations containing no added F, whereas all *males* on this diet reacted like males on diets containing 0.0001 per cent of NaF."

McClure (1939b) has recorded in rat growth studies: "There was the suggestion of a stimulating effect of 22.6 p.p.m. of fluorine in the diet on appetite and daily body gain. . . . Fluorine levels of 45.2 p.p.m. or more in water and food gave evidence of a reduced rate of gain. . . ."

Sharpless and McCollum (1933) studied the indispensability of fluorine in the diet of the rat. The basal ration was:

	%
Casein	18.0
Butterfat	8.0
Salts 51 A	6.5
Yeast	10.0
Starch	57.5
Viosterol	15 drops/1 kg.

The composition of Salts 51 A was:

$CaCO_3$	150
KCl	100
NaCl	50
$NaHCO_3$	70
Ferric citrate	50
KH_2PO_4	170
$MgSO_4$	60

All food constituents were analyzed for fluorine, which was found absent except in some samples of starch and of commercial casein. The fluorine of the starch and casein could be removed by washing with dilute acids.

The experimental ration was prepared from the basal by the addition of 0.003 per cent sodium fluoride. Five males and five females were mated on the low fluorine ration and three of each sex on the experimental diet.

No effects were observed in reproduction, but there was largely a failure to rear the young, an effect not ascribed to either the absence or presence of fluorine. The fluorine content of the ash of bones and teeth of stock rats increased with age.

Sharpless and McCollum found that "young rats (16–18 days) contain little, if any, fluorine." The fluorine content of bones could be reduced to "between six and twenty-five parts per million and can be eliminated from the teeth, insofar as can be determined, without showing any gross deleterious effect."

Evans and Phillips (1939) bred rats through 5 generations on milk mineralized with traces of iron, copper and manganese and containing naturally 0.1 to 0.2 p.p.m.

fluorine or 1.6 p.p.m. on basis of dry matter. They found no deleterious effects on the animals and no improvement on the addition of 0.1, 1.0, 10 or 20 p.p.m. of fluorine as sodium fluoride.

Shils and McCollum (1942) reviewed the literature on fluorine with especial reference to mottled enamel and dental caries, through May 1942. Concerning the essential nature of fluorine they said: "More refined nutritional experiments or the elucidation of its role in tooth and bone structure may show that it is necessary."

Essentiality in Plant Life

Salm-Horstmar (1861), in studies of the growth of barley in solutions of pure salts on quartz sand, found traces of potassium fluoride necessary for development of the plant.

Mazé (1915) grew maize in nutrient solutions containing N, P, K, Ca, Mg, S, Fe, Mn, Zn, Si and Ce. These were sufficient if tap water were used but not distilled water. Addition of Al, B, F and I produced maize equal to the controls. Arsenic was toxic. The concentration of fluorine as NaF was 2 p.p.m. Mazé (1919) published the full details of the foregoing. Gautier (1915) found that fluorine in the soil favored the growth of cabbage, hemp and others but was of doubtful value for wheat, rye, oats, etc.

Gautier and Clausmann (1919), on the basis of the elective localization of fluorine in animals, expressed their opinion that fluorine is essential in the animal economy. Of 13 plants grown in pots they found 7 (cress, cabbage, California poppy, spinach, adder-wort, spurry and hemp) were favored by added fluoride, 3 (morning glory, onion and rye) were unaffected, and 3 (sweetpea, chick pea and centaury) gave inferior yields.

Voelcker (1921) reported that in pot culture of wheat, potassium fluoride applied to give 0.1 per cent fluorine in the soil produced 4.53 times as much grain as the control and 2.66 times as much straw. For 0.05 per cent the yields were 3.40 and 2.02, respectively. But sodium fluoride at 0.1 per cent in the soil killed all the seed; at 0.05 per cent level, it killed some of the seed but increased the yields of grain and straw 4.60 and 2.78 times, respectively. Calcium fluoride at 5 cwt. per acre gave 0.43 and 0.94 yields; calcium silicofluoride at 5 cwt. per acre, 1.91 and 1.41.

Price (1932a) discussed the relations of naturally occurring fluorides to plant and animal life. He reported: "F as NaF, added in progressive amounts to sprouting corn—when the plant is utilizing only the stored minerals of the kernel, while growing in distilled water—had a progressive stimulating effect up to 10 parts per million of the water; 20 parts, or more, were very toxic and produced only a stunted growth with a bronzed appearance." Addition of calcium and phosphate to such solutions reduced toxicity.

Early Speculations and Studies on Fluorine as Beneficial to Teeth

It is interesting to note that Morichini (1805) remarked on a possible relation of fluorine to dental disease, but the intent seems to have been of a diagnostic nature.

Erhardt (1874) recommended fluorine for internal use "since it is fluorine which gives hardness and lasting quality to the enamel of teeth and so protects against caries" (translation). He said that fluorine pastilles had been recommended in England several years earlier. He extracted a molar from a dog and then fed the animal small doses of potassium fluoride for a period of four months. He then extracted the opposite molar and stated that the enamel was thicker and harder and that this was evidence that the fluoride had been deposited there. He stated that fluoride was especially suitable for children during the period of tooth development and for women during pregnancy, when the teeth so frequently suffer. In a footnote, he indicated that the

fluoride pastilles, with directions for use, were available through pharmacists in Freiburg in Breslau at one mark per box. The fluoride content of the pastilles was not indicated.

Crichton-Browne (1892) remarked: "The late Dr. George Wilson showed that fluorine is more widely distributed in nature than was before his time supposed, but still, as he pointed out, it is but sparingly present where it does occur and the only channels by which it can apparently find its way into the animal economy are through the siliceous stems of grasses and the outer husks of grain, in which it exists in comparative abundance. Analysis has proved that the enamel of the teeth contains more fluorine, in the form of fluoride of calcium, than any other part of body and fluorine might, indeed, be regarded as the characteristic chemical constituent of this structure, the hardest of all animal tissue and containing 95.5 per cent of salts, against 72 per cent in the dentine.... I think it well worthy of consideration whether the reintroduction into our diet, and especially into the diet of childbearing women and of children, of a supply of fluorine in some suitable natural form—and what form can be more suitable than that in which it exists in the pellicles of our grain stuffs?—might not do something to fortify the teeth of the next generation."

Wrampelmeyer (1893) investigated the fluorine content of teeth with the idea of adding an appropriate amount of fluorine to the diet to improve the resistance of the teeth. He found from 0.65 to 1.55 per cent fluorine in 7 whole teeth from adults and children. Molars, incisors and cuspids were represented, 4 being decayed and 3 being sound. He could not conclude that there was any relation of fluorine content to the condition of the teeth.

Michel (1897) cited the contradictory evidence of Magitot and of Röse concerning caries incidence in calcium-rich and calcium-poor areas and suggested that, whatever the supply of calcium may be, a caries-resistant tooth cannot be constructed without some other ingredient. He suggests that calcium fluoride in enamel may be that ingredient acting in a two-fold manner in hardening the enamel and conferring antibacterial properties. Michel ashed sound and carious crowns that had been approximately equalized in dentin and enamel content by boring out holes in the sound teeth similar to those in the decayed specimens. This procedure suggests that homologous teeth were used, but the weights of the teeth indicate this was not the case. He found in 5 carious teeth 0.63 to 0.67 per cent fluorine, and in 4 sound teeth 0.56 to 0.63 per cent, and concluded no difference was shown.

Kobert (1901), in remarks on Siegfried's work, which had to do with fluorides as antiseptics, said: "Whether it is possible through giving very small amounts of sodium silicofluoride to children with milk to promote the development of normal teeth, especially in rickets with its frequently defective enamel, is still an open question, which child and dental specialists might take in hand for a cautious test" (translation).

Hempel and Scheffler (1899) found 0.2, 0.39 and 0.31 per cent fluorine in the ash of horse teeth. Analyses of the ash from human teeth showed 0.19 per cent from decayed teeth and 0.33 and 0.52 per cent from sound teeth. They indicated a belief that fluorine in the enamel had an anticaries action.

Gassmann (1908) found no positive etching of glass from 2.02 and 4.78 gm. of carefully ashed tooth substance. He found the gases evolved from tooth ash in the Hempel gasometric method to consist mainly of hydrogen chloride. Gassmann (1909, 1910), using the same data, renewed his attacks on the findings of Hempel and Scheffler (1899) that sound teeth have a higher content of fluorine than decayed teeth. He stated that he could detect 1.5 mg. of NaF with the etch test, which would correspond to about 35 p.p.m. of fluorine

in a 2 gm. sample of ash. Modern results suggest that Gassman should not have failed to find fluorine in the amounts of tooth ash that he used. He considered the fluorine-caries hypothesis of Hempel and Scheffler (1899) as demolished.

McClendon (1922) wrote: "As to the special nutrition of the teeth, it has been found that the teeth require all those food elements needed by the body as a whole and in addition the elements giving it its hardness, chiefly calcium, fluorin, and the phosphate ion. In other words, as Gautier has shown, the enamel of the teeth has nearly the same composition as crystalline phosphate rock or apatite, $CaFCa_4(PO_4)_3$."

McClendon (1923) pointed out that the question of the indispensability of fluorine had never been settled. He said: "Certain persons have, since 1850, held the view that the fluorin is necessary for sound bones and teeth and that its absence caused them to have weak or decayed teeth." He said he had fed "apatite containing fluorin to white rats, with very beneficial results. In no case was there any indication of fluorin poisoning when all the calcium phosphate necessary in their diet for good health was derived from fluorin apatite."

McCollum, Simmonds, Becker and Bunting (1925), in their work in which they found mottled enamel in rat teeth, were testing the hypothesis that "perhaps a deficiency of fluorine in the food might lead to the formation of teeth which had poor structure, and consequently possess little power to resist the agencies which lead to decay."

The evidence of the dental protective effect of fluorine, in a dosage which is expressible with physiological rather than toxicological implications, has already been given in detail in this review.

Conclusions

1. There is no convincing evidence that fluorine is essential to animal life in general, though a greater refinement of "fluorine-free" diets may possibly produce acceptable evidence.

2. Fluorine may be necessary for certain plants. At least plant life seems able to tolerate or even be benefited by such amounts of fluorine in water as seem beneficial to human enamel.

FLUORIDIZATION OF WATER

Proposals

Cox (1939) has discussed the addition of fluorides to water for the prevention of dental caries. The proposal involved water prepared by direct addition of fluoride or by blending two available waters so that the year-round fluorine intake from all sources would be a statistical constant. The level of fluorine in water was to be in all cases below that which causes mottling of enamel. It was not proposed, as has been erroneously implied, to produce mottled enamel to prevent decay. (Smith and Smith, 1940; Editorial, 1940b). The objective was the formation of teeth under the conditions of optimum supply of fluorine on the theory that such teeth would remain resistant to caries throughout life.

The bases of the theory of permanent resistance to caries were: (a) The observation by Cox, Matuschak, Dixon, Dodds and Walker (1939) that rat teeth, formed with a sub-mottling dosage of fluorine derived from placental and mammary transmission, have increased resistance to coarse cereal caries. (b) The analyses of Armstrong (1937) and of Armstrong and Brekhus (1938b) that showed teeth which had not decayed had a higher content of fluorine than those experiencing caries. (c) The observations by Dean, Jay, Arnold, McClure and Elvove (1939) that children of Galesburg, Illinois, with 1.8 p.p.m. fluorine in the water supply had about one-third the caries rate of children of Quincy, Illinois, with a low fluoride water.

The fluoridization of water was also discussed by Cox, Matuschak, Dixon, Dodds and Walker (1939) who said: "Regulation

of fluorine should be directed at an optimum intake of the element. In particular, the fluorine content of the water supply can be varied seasonally to compensate for varying water consumption. Climatic differences will make it necessary for each locality to find its own standards for addition of fluorides to the water supply."

Cox (1940) has examined the possible explanations of the observed reduced incidence of caries in fluoride areas and concluded that a theory of resistance through enamel structure, derived either indirectly by the influence of fluorine in the formation of the tooth or directly by fluorine as a part of the enamel, best fits the facts. He discarded a chemical explanation, that is, anti-bacterial action of fluorine *per se*, because severely mottled enamel, known to have two to three times as much fluorine as "normal" enamel (Armstrong and Brekhus, 1938a, b), is subject to decay. McKay (1939, 1941) has said: "The points or areas of carious attack, on mottled teeth, correspond with those on teeth of which the enamel is normal; namely, pits, fissures and other stagnation areas. . . . After caries has begun in a mottled tooth, it progresses in the same manner as in a tooth that is not mottled."

The possibility that fluorine acts through the saliva was excluded on the basis that the fluorine content of saliva is only of the order of 0.1 p.p.m., a level only one-third that of the control medium used by Bibby and Van Kesteren (1940) in the best demonstration of the inhibition by fluorides of acid formation by bacteria.

Cox and Matuschak (Cox, 1940) found 0.095 p.p.m. fluorine in the pooled salivas from three children of Galesburg, Illinois, (1.8 p.p.m. F in water supply) and 0.12 p.p.m. in similar salivas from Quincy, Illinois, (0.2 p.p.m. F in water supply); and, with adequate data from children of these two cities, McClure (1941a) has shown that the fluorine levels of salivas of the two communities (of the order of 0.1 p.p.m.) are not essentially different.

Cox (1940) further showed that it was probable, on the basis of data of 76 children of Galesburg (Dean *et al.* 1939) who had not been continuous residents from birth, that protection was derived from fluorine present during the formation of the teeth.

In default of a satisfactory explanation of a low caries rate by antibacterial action (a) of fluorine in the enamel or (b) of fluorine in the saliva, and in view of (a) the structural changes in mottled enamel, (b) the evidence of continued protection of rat teeth on a low-fluorine ration and (c) the higher caries rate of children without a continuous history of exposure to sub-mottling fluoride during formation of the teeth, Cox proposed a structural explanation of resistance to caries because of fluorine.

The above arguments in support of a structural explanation of caries-resistance derived from fluorine in optimum amount during the formation of enamel were repeated by Cox and Levin (1942).

Cox (1939) pointed out that much study was necessary before fluoridization of community water supplies could begin. It would be necessary to learn the water consumption by children in relation to environment and, in particular, to seasonal change. It would be necessary to determine the fluorine requirements for children in order to arrive at the levels to be supplied in the water. He wrote: "Use of water to which a part per million of fluorine has been added for other than drinking can conceivably lead to undesirable effects. Plant and animal life may be injured. There may be interference with industrial uses. Every use of water must be examined as well as current water treatment practices before fluoridization can be begun."

Chapin and Mills (1942), in view of the high rate of caries in children in the Panama Canal Zone, said: "It would seem wise to consider increasing the fluorine content or

total hardness (or both) of the Canal Zone water supply."

Arnold (1943) has considered the evidence mainly of field studies by Dean and coworkers and concluded: "The results of both epidemiologic, chemical and experimental studies suggest that the addition of small amounts of fluoride, not to exceed 1 part per million, to fluoride-free public water supplies may be a practical and efficient method of markedly inhibiting dental caries in large group populations."

Arnold has considered differences of *climate* as influencing the amount of fluorine to be added to water but has not discussed the effects of *seasonal* variations. He wrote: "It cannot be too strongly stressed that the fluorine-dental caries theory rests basically upon the question of the amount of fluoride ingested by the population. For example, a water containing 0.5–0.7 parts per million of fluoride in southwestern United States may have an effect equal to that of a water of 1.0–1.5 parts per million of fluoride in the north central section of this country. For this reason, it may be desirable to base precise estimates upon the more acceptable biologic measurement of fluoride intake in a population, the index of dental fluorosis." (Dean 1942).

It may be pointed out that Dean (1934) experienced difficulty in making estimates of mottled enamel in its mildest manifestations because mottled enamel appeared in an irregular manner. It is possible that these irregularities may be found, on correlation of their occurrence with the specific time of their formation, to be due to excessive consumption of fluorine in water during hot summers. Conversely, absence of mottling of other teeth in the same mouth may prove to have resulted from these teeth being completed, with respect to the pertinent areas, in the winter, spring or fall or in cooler summers.

Ast (1943a) proposed fluoridization of water with 0.8 p.p.m., or 1.77 p.p.m. in terms of sodium fluoride. His statement, "By deliberately treating public water supplies with effective yet nontoxic doses of fluoride salts, daily protection would be afforded without the public being aware of it," implies protection to adults. None of his citations to the literature shows justification for any *posteruptive* effects of fluorine at a level of 0.8 p.p.m. However, in his proposal of a practical large-scale test of the plan, using two cities of population of 25,000 or over, he said: "The period of investigation should extend over 10 to 12 years because the current theory is that the effective action of the fluorine takes place during the years of tooth development."

Ast (1943b) described the water supplies and populations of two New York cities, designated A and B, which he has selected as suitable for trial of the addition of 0.8 to 1.0 p.p.m. of fluorine to the water supplies. The cities, 30 miles apart, were judged to have adequate population (30,000 with 4,200 children of the proper age groups) to provide sufficient data from the test. The New York State Department of Health has approved the study. The next step, presentation of the idea to "key physicians, dentists, public spirited citizens and city officials" had not yet been undertaken. It is interesting to note that Ast estimated the cost of the sodium fluoride to be $1240 per annum or about 30 cents per child per year. He suggested a lower dosage for cities further south but considered no seasonal variation of the dosage for the New York cities.

Ast (1944a) stated it was planned to add NaF to the water supply of Newburgh, N. Y., to raise the fluorine content from 0.12 p.p.m. F to 0.8 to 1.0 p.p.m. He estimated it would require 43.8 pounds of NaF per day for 3,000,000 gallons of water for this city of 30,000 population. The dental results of this addition were to be evaluated by comparison with children of Kingston, N. Y., where a fluorine-free water was used.

Dean (1943), in a symposium including Ast (1943b), has commented on various

factors that are to be considered in the use of fluorides in the prevention of caries.

Dean (1936) recommended, for the prevention of mottled enamel, the provision of distilled or rain water for children in high fluoride areas. It is very significant that Dean and Arnold (1943) wrote, "the continuous use throughout the formative period of the tooth of water containing about 1 part per million of fluorine will result in an incidence of approximately 10 per cent of the mildest form of dental fluorosis," but they recommended: "For the purpose, therefore, of preventing fluorosis, children under 9 years of age should use both for drinking and cooking: (1) a natural water, otherwise safe, the fluoride content of which does not exceed one part per million; or (2) a treated water the fluorine content of which is about one part per million; or (3) distilled water, cistern water or other fluoride water so that the final fluoride concentration of the mixture is approximately 1 part per million."

Dean (1944b) has briefly reviewed the fluorine-caries relationship and said: "The mechanism by which fluoride inhibits dental caries still provides a fruitful field for discussion and investigation." He urged, in reference to the observations of himself and coworkers: "*Before attempting to convert this observed natural phenomenon into one of general usefulness, specifically planned epidemiological studies must clearly demonstrate the safety of low fluorination as it might relate to other aspects of the community's general health.*" He recommended, after such a demonstration, the use of two cities of 40,000 to 50,000 population with the fluoride of one of them brought up to 1 p.p.m. and observation of the results over a period of years.

Faust (1944) has discussed the cost of treatment of water supplies with sodium fluoride and estimated a per capita cost of 7½ cents for chemicals and equipment but excluding labor. He calculated the cost on the basis of the whole population and said, concerning the cost of caries in children and the possibility of benefit to adults from a fluoride program, "Of course, the fluoride treatment of water supplies means much more than this since all age groups will be benefited, but to what extent must first be determined before a complete cost analysis can be prepared."

Faust cited the opinion of the Attorney General of Michigan concerning certain legal aspects of the fluoridization of water. Concerning negligence, part of the opinion was: "However, it is possible that liability could be established were it shown that through some negligence in treatment quantities in excess of one part per million were introduced from which damage resulted." Another opinion was: "Assuming the authority of a municipality to add fluorine to its public water supplies, there would be no method by which a citizen not injured by, but not sympathetic to, the program could enjoin such treatment."

Finn and Ast (1947a) reported "a consistent drop in counts of 20,000 or over and a rise in counts of less than 100" lactobacilli in salivas of Newburgh, New York, after fluoridization of the water for two years. Comparison was made with salivas of children from Kingston, New York, with no fluoride added to the water.

Finn and Ast (1947b) reported that after two years of the artificial fluoridization of the water supply of Newburgh, New York, the percentage of children with low or null counts of *L. acidophilus* in saliva samples rose from 11.9 to 20.0, and the samples with counts over 20,000 dropped from 63.5 to 47.3 per cent. In the control city of Kingston, without fluoridization, the low counts remained constant at 16.2 per cent of the children and the high counts at 54 per cent.

Davies (1950) counted the lactobacilli of one-tenth of the children of Evanston, Illinois, in 1946 and again in 1948 after fluoride had been added to the water to 1 p.p.m. He wrote: "It would seem from the data that there has been a shift of about

5.0 per cent of the cases from the higher count groups (those over 1,000 lactobacilli per ml. of saliva) to the lower count groups, (those under 1,000 per ml.) in Evanston in 1948. This would seem to indicate that there was a decrease in the lactobacillus counts of the children from 6 to 8 years of age, after one year of fluoride in the water. There also seems to be a decrease of 4.7 in the average number of carious surfaces, and 4.2 in the average DMF surfaces per child. These decreases, however, are not sufficiently large to be considered as an indication of trends. They may fall within the range of statistical error, due to accident or chance sampling. It should be remembered that these are only preliminary findings, and by no means indicate that fluoride in the community water will definitely have this effect."

Hill, Jelinek and Blayney (1948, 1949) tabulated the data on the fluorine content of community water supplies of 30 states. They obtained their data by correspondence with Chief Sanitary Engineers. On the basis of the 1940 census they reported 5,646,987 or 4.3 per cent of the total population of the United States used waters with 0.5 p.p.m. or more. Of these 2,195,115 used water with 0.9 to 2.0 p.p.m.; 626,177, 2.1 to 3.0; 127,243, 3.1 to 5.0 and 40,151 over 5.1 p.p.m. fluorine. The numbers of people using waters in the above catogories were shown on maps by counties for Illinois, Indiana, Ohio, South Dakota and Texas.

Martin (1947, 1948) reported the fluorine content of whole teeth obtained in Evanston, Illinois, prior to fluoridization of the water supply, as ranging from 104 to 287 p.p.m. for sound teeth, with an average of 202 p.p.m., and for carious teeth from 45 to 270 p.p.m., with an average of 133 p.p.m. The 16 sound teeth reported were from 5 individuals with no history of places of residence, and the carious teeth from 10 persons. The fluorine content of femurs of 9 human fetuses averaged 20 p.p.m.; mandibles and maxillar, 19 p.p.m.; and of tooth buds, 12.5 p.p.m. on fat-free, dry basis. Bone analyses, made on crests of ilium taken from adults ranging in age from 32 to 84, averaged 374 p.p.m. from 13 specimens.

Martin and Hill (1949) analyzed stimulated saliva samples from 10 per cent of the children being studied in Evanston, Illinois, where sodium fluoride had been added to the water supply. In the salivas of children, 6 to 8 years of age, exposed to the fluoridized water for 20 to 25 months, they found 0.14 to 0.35 p.p.m. fluorine, with an average of 0.25 p.p.m.; in a 12- to 14-year-old group exposed 25 to 29 months, the values were 0.11 to 0.29, with a mean of 0.20 p.p.m. There was no apparent relationship between the fluorine content of the saliva and the *L. acidophilus* counts or the prevalence of dental caries.

Blayney and Tucker (1948) outlined plans for the study of the effects on dental health of the addition of fluorine to the water supply of Evanston, Illinois. The children of Oak Park, Illinois, were to be used as control subjects. Plans included observations of general oral conditions, bacteriological studies, inquiry into family food habits, and analyses of food, teeth and urine for fluorine. The observations were planned for a period of 15 years.

Bull (1949) was the first to report the effect on caries of artificial fluoridization of public water supplies. After 3 years of the addition of 1 p.p.m. of fluorine to the water supply of Sheboygan, Wisconsin, the caries rate of 5- and 6-year-old children was reduced from 480 to 346 carious teeth per 100 children, from 303 to 246 in the permanent teeth of 9-year-old children and from 854 to 692 in 12- to 14-year-old children. The numbers of children examined in these groups were 525, 483 and 1442, respectively. Bull also gave data on caries rates of 8 Wisconsin cities and pointed out that the lowest decay rate in primary teeth is found associated with about 2 p.p.m.

An important feature of the regulation

of the fluorine content of community water supplies, stressed by advocates of the practice, is that it does not depend upon individual initiative. Fluorine in a community water supply would be ingested daily without thought or effort and with no danger from faulty practices if the necessary studies on dosage and methods are effectively conducted.

The Opposition

Opponents of the proposal to fluoridize water for the partial prevention of dental caries have generally been motivated by a fear of toxic consequences rather than by doubts of effectiveness. In many cases casual remarks have been made against the procedure that indicate the authors have not examined the proposals in their entirety. For example, McClendon, Foster and Supplee (1942) have written: "Because the *careless* addition of fluoride to drinking water is dangerous . . . ," but they gave no citation to anyone who had proposed careless fluoridization of water.

The paper in which Cox (1940) discussed the mechanism of fluorine prevention of caries and urged study of fluoridization of water to effect mass prevention of caries was followed immediately by an editorial (1940b). The title of the editorial, "Endemic Fluorosis and Dental Caries," takes it entirely out of the category of effective criticism, as no one has yet proposed the production of mottled enamel to prevent dental caries. Of the papers of Smith (1940) and of Smith and Smith (1940), dealt with in detail elsewhere (Cox, 1944), it would seem only necessary to point out that, in addition to a number of other errors of misinterpretation, they too have implied that it was proposed by Cox (1939) to produce mottled enamel to prevent dental caries. It may be pointed out here that, concerning the procedure of Cox, Matuschak, Dixon, Dodds and Walker (1939), King (1944) has fallen into error in writing: "The fluorine concentration required to increase the rats' resistance to the disease was, however, of the order of 41.6 p.p.m., which is far above the minimal toxic dose for man." The fluoride was fed to the mothers and reached the young only by placental and mammary transmission in a dosage that did not mottle the teeth. (Cox and Levin, 1942). Cox *et al.* tested caries resistance in the young, not in the mothers.

Discussion

The suggestion of Cox (1939) to regulate the fluorine content of water supplies according to season was based on the idea that enamel formed under optimum conditions of fluorine supply would permanently increase caries resistance regardless of lack of continued exposure to fluorine. The method proposed by Arnold (1943) and Ast (1943a) of a constant level of fluorine is based on the observation that an unvarying level of fluorine in drinking water results in a lowered caries rate and no objectionable mottling of teeth.

Since the mechanism by which fluorine prevents caries is not yet known, it would seem premature to set a limit by ukase to the amount of fluorine that may be put into water. An editorial (1943) in the September 18, 1943, Journal of the American Medical Association used the term "permissible maximum, i.e. 1 part per million" in reference to prevention of mottled enamel, and the legal opinions cited by Faust both infer that introduction of quantities of fluorine in excess of 1 p.p.m. into water may constitute negligence. If it later develops that greater joint freedom from caries and mottled enamel can be obtained in some given locality by 0.7 p.p.m. in the summer and 1.4 p.p.m. in the winter, it may be difficult to attain this latter treatment because of these arbitrary and premature opinions. It would be better to use, until more knowledge is available, a less definite but more significant expression "non-mottling level" rather than 1 p.p.m.

There are no data yet available for dosage of drinking water on a seasonal basis to

ensure a statistically constant intake of fluorine from water for the entire year. McClure (1943b) has searched the literature in vain for any data on water consumption by children in relation to environment. He used in his estimates of water consumption the formula of Adolph (1933) of "1 cc. per calory of energy in the daily diet." This formula is obviously inadequate for day by day water consumption, as it would not show an increase on very hot days.

The proposals of Arnold (1943) and of Ast (1943a) contain varying shades of implication that adults will benefit from the fluoridization of water. If a community is "sold" such water treatment with the idea that everyone will benefit immediately, considerable harm may come to what may be a very valuable public health measure should adults subsequently find that they have no diminution of dental caries.

Volker and Bibby (1941) and Bibby (1944a) have concluded that most of the protection from caries by fluoride drinking water is acquired posteruptively. The principal fact offered in proof is the very low incidence of caries in the upper anterior teeth. This suggestion first appears in the paper by Volker (1939). The conclusion that the anterior teeth do not decay because of fluorine adsorbed on their surfaces is possible, in part, because of lack of data on decay in the smooth surface of the posterior teeth. The papers of Dean and coworkers, cited for example by Arnold (1943), state only that first-molar mortality is decreased about 75 per cent. They do not state what parts of the teeth are decayed. Bunting, Crowley, Hard and Keller (1928) indicate that there may be very little decay of molars on lingual, buccal and proximal surfaces. This would not be explainable on the basis of Volker's adsorption-from-drinking-water theory.

The data of Anderson (1932b) on caries in *deciduous* teeth in a mottled enamel district, are not in accord with the suggestion of Volker (1939) that upper, anterior teeth do not decay because of fluorine acquired posteruptively in the act of drinking.

POSTERUPTIVE USE OF FLUORIDES

Some Evidence of Inhibition of Bacterial Action by Fluorides

Gottbrecht (1889) found that putrefaction of meat under water was arrested by hydrofluoric acid in concentration of 0.1 per cent or greater. Growth of bacteria was stopped in the region of 0.05 to 0.1 per cent HF.

Hewelke (1889, 1890) found *Torula cerevisae* development prevented by NaF in 1–100 to 1–300 dilution, fermentation very much reduced at 1–100 to 1–3000 and still affected at 1–4000. One part NaF in 2000 of urine delayed decomposition to alkaline reaction for 14 days compared with 3 to 4 days in a control. Decomposition of blood was prevented by dilutions of NaF as low as 1–640. A variety of bacteria could not flourish in pure culture in 1–200 NaF and some cultures, particularly of pathogenic forms, remained sterile at 1–300.

Tappeiner (1890) observed suppression of growth by 0.5 per cent NaF of the cholera bacillus, *B. coli*, *S. pyogenes aureus*, and a butyric acid bacillus. *B. coli* and the butyric acid bacillus showed weak growth in 0.25 per cent concentration of NaF; 0.1 per cent had only slight retarding effect on any of the organisms. The bacteria were slowly killed by 2 per cent NaF over a period of 6 days. Spores were not affected. Tappeiner considered that fluoride poisoning of warm-blooded animals was through respiratory failure.

Vaughan (1890) reported the successful use of sodium silicofluoride as an antiseptic in root canals.

Arthus and Huber (1892) prevented putrefaction of milk, blood, transudates, urine, bile, eggs, fruit, various organs, saliva and gelatin by means of 1 per cent sodium fluoride. Lactic acid fermentation of whey was prevented by 0.4 per cent NaF but not by lower concentrations. The critical level to arrest alcoholic fermentation was 0.3 per cent NaF.

Digestive enzymes were unaffected by 1 or 2 per cent of NaF.

Fosdick and Hansen (1936) in considering the relation of oral carbohydrate degradation to dental caries wrote: "When fermentation was prevented by the presence of sodium fluoride, there was no solution of dental enamel. In this connection, it is interesting to speculate on the possible relationship of fluorides in fermentation and the reduced susceptibility of mottled enamel to decay." They did not discuss concentration of the fluoride ion that would be effective.

Wright (1937) found that acid formation from sucrose by a streptococcus was inhibited by sodium fluoride, but his minimum concentration was 1–50,000, or about 10 p.p.m. fluorine. The final pH at this minimum concentration was 4.9 compared with 4.7 for the control.

Bibby and Van Kesteren (1940) found that heavily fluorosed rat dentin reduced the acid production by as much as 20 per cent but "dentin from animals fed a low fluorine diet (20 p.p.m.) did not influence acid formation differently from normal rat dentin." Artificially fluorosed enamel and dentin reduced acid formation. Growth of the organisms was not affected by the dental tissues *per se*. "Differences between the acid production in the fluorine-containing and fluorine-free dental tissues did not appear until after the bacteria had ceased to multiply rapidly."

Bibby and Van Kesteren (1940) tested (a) acid production from glucose and (b) viability of mouth organisms in media with 2, 10, 20, 50, 100, 250 and 1000 p.p.m. added NaF (0.9 to 452 p.p.m. F). The organisms included 14 strains of streptococci and 4 strains of lactobacilli as well as other acidogenic mouth forms. The authors concluded that fluorine concentrations of less than 1 p.p.m. of added fluoride "limit acid production by bacteria but concentrations in excess of 250 p.p.m. are needed to affect bacterial growth."

Solubility of Enamel as Influenced by Fluorine

Robb, Medes, McClendon, Graham and Murphy (1921), considering enamel as devoid of organic matter and fearing contamination of enamel by other substances, sought a pure mineral to simulate enamel. "Since enamel contains fluorine, we selected fluor-apatite as the mineral that most closely resembles enamel. . . ." They found that fluor-apatite lost weight appreciably in synthetic salivas "only in distinctly acid solutions (pH 5)." Brekhus and Armstrong (1934) found a negligible loss of weight in two pieces of apatite worn in the mouth for one year in a removable bridge at an upper first molar site.

Volker (1939) wrote: "Human enamel and dentin powdered to pass a 100-mesh screen were separated and purified by the centrifugal-flotation method. Five hundred milligram samples of enamel were shaken for periods of one hour in 250 cc. of solutions containing one part of sodium fluoride in 25, 100, 1000 and 10,000 parts of water, and then thoroughly washed in distilled water. Samples of dentin were similarly treated with 1/1000 sodium fluoride solutions. Solubilities of these and untreated control samples were compared by measuring the respective weight losses of 50 mg. samples after one hour in 20 cc. of a 0.2 M acetic acid sodium acetate buffer at pH 4.0."

All treated samples showed reduced solubility, the amount dissolved being 13.2, 14.1, 15.6, 18.5 mg., respectively, for the above series and 27.3 mg. for the untreated enamel. Confirmatory values on solubility were obtained in "decalcification periods of 5 and 30 minutes and 1½, 2 and 2½ hours. Five- to 10-minute applications of the fluoride were almost as effective in reducing the solubility as the 1-hour treatments. The diminished solubility of the enamel was not lost after washing in water or saliva for periods up to 70 hours. It was also found that the natural surfaces of whole teeth treated with sodium fluoride were much less affected by acid than those of untreated

teeth." (No data were supplied regarding this latter statement. The tests were probably qualitative.)

Volker interpreted the reduced solubility of fluoride-treated enamel as "change to a fluorapatite, an adsorption of fluorine or a combination of both." In view of the rapidity of the action he suggested that "during the act of drinking, the fluorine from fluorine-containing waters could combine to some extent with the teeth. Since such an effect would be most marked on the upper anterior teeth which would have the greatest contact with the water and on which there is a minimal amount of saliva, it is probably significant that Dean found that the resistance to decay produced by fluorine-containing water was definitely the most marked in these teeth." Volker suggested "controlled applications of fluorine-containing compounds as a means of preventing dental caries."

Volker (1940) tested the solubility of normal and fluorosed human and rat enamel by the technic of Volker (1939). He found lower solubility of the fluorosed enamels. He concluded: "The presence of fluorine in large amounts may decrease the solubility of the dental hard tissues. Small amounts of fluorine show no demonstrable reduction in enamel solubility. It seems doubtful that the amounts of fluorine present in slightly fluorosed teeth are sufficient to alter their acid solubility."

Volker (1943) exposed powdered enamel to solutions of sodium or calcium fluoride at a concentration of 8 p.p.m. fluorine for periods of as little as 5 minutes and found a detectable and comparable decrease in solubility in acid.

Volker, Bonner and Brudevold (1943) extended observations to the absorption of fluoride from aqueous alkyl sulfate solutions by enamel. They found some interference by the alkyl sulfate but concluded: "These findings indicate the possibility of attempting to reduce caries susceptibility by means of a dentifrice containing soluble fluoride and alkyl sulfate."

Bibby, Buoncore and Diener (1945) stated, "The effect of lead is much greater than that of fluorine" in reducing the solubility of enamel in "a variety of acid buffers."

Bibby and McKinnon (1946) treated powdered enamel at pH 4 with the nitrates, chlorides, sulfates and acetates of various metals and tested the solubility of the treated enamels in sodium acetate-acetic acid buffer at pH 4. Lead salts gave the lowest solubility, 9.4 per cent, and lithium next at 20.5 per cent, compared with 32.7 per cent for water treatment alone. In a series of fluorides, lead fluoride was the most effective, with a solubility of 3.9 per cent.

Bibby (1947) found that sodium fluoride solutions at pH 4 or lower were much more effective in reducing the solubility of powdered enamel in dilute organic acids than were unmodified aqueous solutions. He also observed that a 0.06 per cent solution of lead fluoride at pH 4.0 was more effective than sodium fluoride and solutions of eight other inorganic fluorides in reducing enamel solubility.

Manly and Bibby (1949) tested the effects on enamel solubility of 147 substances. Of these, 27 were more active in reducing enamel solubility than 0.1 per cent sodium fluoride and 38 were less active, though effective. Of the fluorides, lead fluoride was most effective; silver fluoride was less effective than sodium fluoride.

McClure and Likins (1949) tested the solubility of non-fluorosed, fluorosed and sodium-fluoride-treated human enamel in eight 0.01 normal acid solutions. The fluorosed and fluoride-treated enamels were less soluble than the non-fluorosed enamel in formic, lactic, acetic, tartaric and citric acids, but differences were not observed in hydrochloric, nitric and phosphoric acids. The fluorine contents of the ashes of the enamels were non-fluorosed, 0.0111; fluorosed, mild, 0.0618 p.p.m.; moderate,

0.0909; severe, 0.0758; sodium-fluoride-treated, 0.0460 p.p.m.

Boyd (1949) examined the solubility in buffered solutions at pH 2.5 and 4.0 of powdered calcium fluoride, calcium fluorapatite, powdered enamel and tricalcium phosphate. The solubility increased in the above order.

Phillips and Muhler (1947) determined the effect of sodium fluoride solutions at varying pH values in reducing the solubility of dental enamel powder and of enamel in sections. They tested the solubility of the treated enamel in a 0.2 molar acetic acid-sodium acetate buffer. They found in general with both powder and sections that the lower the pH of the sodium fluoride, in the range 2.6 to 7.5, the greater was the reduction of solubility of the enamel.

Muhler and Van Huysen (1947) tested the solubility-reducing effect of 12 inorganic salts, iodoacetic acid and saliva on powdered human enamel. They found irregular differences between filtered and unfiltered solutions of the reagents, with 9 of the 13 being more effective when filtered. Eight of the reagents were more effective in reducing enamel solubility in 0.2 molar acetic acid at pH 4.0 than was sodium fluoride.

Van Huysen and Muhler (1948) treated powdered human enamel with 1/500 and 1/20,000 concentrations of sodium and stannous fluorides in flavored solutions designed as mouthwashes. The solubility of the enamel in 0.2 molar acetic acid-sodium acetate buffer at pH 4.0 was reduced more by the stannous fluoride. The dilute solutions were effective, being equivalent to 22 p.p.m. fluorine for the sodium fluoride and 12 p.p.m. for stannous fluoride. The authors found no effect on solubility of prior treatment of the enamel powder by alcohol.

Muhler and Van Huysen (1948), in a series of tests of reduction of solubility of powdered human enamel by buffered and unbuffered solutions of sodium fluoride, ranging in concentration from 0.2 to 4.0 per cent and in pH from 3.70 to 7.25, found the greatest reduction of solubility by a 2.0 per cent solution at pH 4.50.

Muhler, Boyd and Van Huysen (1950) determined the protective value of 32 reagents against solution of enamel, dentin and tricalcium phosphate in 0.2 molar acetic acid buffered to pH 4.0. They used as a criterion the calcium and phosphorus in the filtrates from the material after a standard procedure of treatment. The most effective agent was found to be stannous fluoride, but samples obtained from different sources varied in the protective effect. Lead fluoride was more effective than sodium fluoride in protection of enamel and dentin.

Phillips and Swartz (1948) determined the increase in hardness of enamel of extracted human teeth produced by treatment with solutions of various salts. Hardness was measured before and after treatment on an artificially flattened area of the enamel by means of the Tukon Tester. Stannous fluoride was the most effective agent in increasing hardness of enamel. Comparison of hardness after softening in 0.2 molar sodium acetate-acetic acid buffered solution, followed by treatment with the salts and re-immersion in the acid solution, showed 19.2 per cent difference in hardness due to stannous fluoride, 15.4 per cent for lead fluoride, 12.1 per cent for uranyl nitrate and 7.9 per cent for sodium fluoride. Maximum effect was attained by 25 minutes' treatment with the solutions. Hardness was not conferred by deposition of a film. Swabbing the teeth was more effective than spraying or immersion.

Phillips and Swartz (1950) found enamel hardness increased by stannous fluoride at 1:10,000 concentration. When stannous fluoride was used as a polishing agent in a mixture with hydrogen peroxide and insoluble sodium metaphosphate, the increase in hardness of the enamel was less than that produced by a solution of the fluoride. They found no difference in increase in hardness

of enamel by stannous fluoride in human teeth from subjects aged 10 to 19, 20 to 39 and 50 to 59. For the testing it was necessary to prepare flattened surfaces and the authors believed this may have removed age characteristic of the surface.

Hord and Ellis (1949) applied a 2 per cent solution of sodium fluoride to dog teeth *in vivo* for 10 minutes once a week for 8 weeks. Opposite teeth were untreated. They found the Knoop hardness number increased by sodium fluoride treatment in every one of 12 such pairs of teeth.

Topical Application of Fluorides

Cheyne and Rice (1942) and Cheyne (1942) reported: "Effectiveness of locally applied fluorine was tested on deciduous canine and molar teeth of an experimental group of 46 boys and girls divided into control and fluorine-treated. The children were born in the city (with the water low in fluorine content) and had lived there continuously since birth. Average age of children at beginning of experiment was 5.52 years; at time of last treatment, 6.09 years. Average interval between treatments was approximately 0.28 years. All except 1 child evidenced active caries at the beginning of study. Fluorine, applied as potassium fluoride in aqueous solution (1 cc. applied from 4 to 6 minutes per treatment) had concentrations from 60,000 p.p.m. to 500 p.p.m." Cheyne (1942) indicated that the treatment was "with approximately 1 cc. of a 500 parts per million aqueous fluoride solution." In one year, new surfaces with carious lesions increased 3.09 in the treated group and 6.04 in the control group. "Data indicate that application of fluorine in manner described is efficient in suppressing carious activity in existing lesions as well as in preventing development of new lesions in deciduous canines and molars. *L. acidophilus* counts taken from saliva samples were low in number examined, but showed no tendency toward correlation." (Cheyne and Rice, 1942).

Bibby (1942a, b) initiated a study of the effects of topically applied fluoride solutions with 100 children between the ages of 10 and 12. "The fluoride treatment consisted of cleaning the teeth with hydrogen peroxide, isolating the quadrant with cotton rolls, dehydrating with alcohol and air, and keeping all surfaces of the teeth wet with 1/1000 sodium fluoride for seven or eight minutes by means of repeated applications with cotton wool. Various quadrants (upper right, lower left, etc.) were treated in different mouths and to minimize possible differences from patient to patient, variations in susceptibility, etc. the corresponding quadrant (upper left, lower right, etc.) on the opposite side of the mouth was used as a control .... During the course of the year of study three fluoride applications were made on the teeth at intervals of approximately four months." In the 90 subjects remaining at the end of a year the control quadrants showed 61 new carious lesions compared with 33 in the treated quadrants.

Bibby (1943a) reported that, after 2 years of the treatments in 78 subjects remaining, 123 new cavities had appeared in the control and 82 in the treated quadrants. "Caries was reduced in the incisors and canines by 32 per cent and in the premolars by 34 per cent and in the molars by 40 per cent. Although the extent of caries reduction during the second year was not as large as during the first, there was a distinct reduction in caries activity in the treated quadrants."

Bibby (1944) reported the details of his continuation of topical fluoride applications during the second year, with 80 subjects under the continued observation. In the test quadrants there were 83 definite new caries and 90 surfaces with questionable changes, or a total of 173 "total caries increases" compared with 124, 115 and a total of 239 in the control quadrants. Bibby concluded: "Thus support is given to our previously stated conclusion that fluorine acts to prevent caries principally by combining directly with the enamel surface to

increase its resistance to the action of acid."

Bibby, Zander, McKelleget and Labunsky (1946a, b) cleaned the teeth of one side of the mouths of 47 girls, 6 to 14 years old, with a paste prepared by adding 1 volume of 4 per cent sodium fluoride to 2 volumes of commercial hydrogen peroxide and pumice. The teeth of the other side of each mouth were cleaned with a similar paste devoid of the sodium fluoride. The pastes had a pH of 4.0. After 3 such prophylactic cleanings, 42 per cent less new decay was found in the teeth of the treated sides of the mouths. In a second group of 95 children, aged 6 to 15 years, a 25 per cent reduction in new decay was found in a year after 2 treatments. A greater reduction was found in upper than in lower teeth. The authors also reported: "The use, thrice weekly, of an acid (pH 4) mouthwash containing 0.1 per cent sodium fluoride for 1 year did not reduce caries activity in 31 dental students below that of 15 using a control fluoride-free mouthwash, or 39 using no mouthwash."

Bibby, De Roche and Wilkins (1947) applied a "saturated lead fluoride solution in pH 4 sodium acetate-acetic acid buffer" topically to quadrants of teeth of 120 children, aged 11 to 13, at 4-month intervals for a year. No reduction of the incidence of new caries was observed, whereas "0.1 per cent sodium fluoride produced a 46 per cent reduction in new dental caries."

Klinkenberg and Bibby (1950) applied a 1 per cent solution of sodium fluoride to one quadrant of teeth of 139 students and alternately 0.06 lead fluoride to the upper or lower quadrant. The opposite quadrants were left untreated. Treatment was preceded by dehydration with alcohol and air, and apparently this treatment was not given to the teeth of the control quadrants. All teeth were cleaned before the treatments. The treatments were repeated at intervals of 3 months, with 4 being given to 129 subjects and 3 to the remaining 10. The subjects ranged in age from 18 to 40, with an average of 25. After 14 months the quadrants treated with sodium fluoride showed 97 new DMF surfaces compared with 173 in the untreated quadrants, a reduction of 44.5 per cent. The values for the lead fluoride treatment were 121 and 167 and 27.5 per cent reduction.

Fosdick (1942) wrote: "When it is considered that the teeth absorb large quantities of the fluoride ion, it appears possible that the concentration in the teeth is sufficient to inhibit the local formation of lactic acid to such extent that the natural neutralizing or immunizing influences of the mouth will cause immunity to caries."

Knutson and Armstrong (1943b) followed the principle of Bibby in treating certain quadrants of the mouth and using the other quadrants for control. All children "in the treated group received a dental prophylaxis treatment consisting of scaling and polishing of the teeth. . . . The treatment consisted of isolation of the teeth with cotton rolls, drying the teeth with compressed air, and wetting the crown surfaces of the teeth with 2 per cent sodium fluoride solution. The applied solution was allowed to dry in air for approximately 4 minutes. After the cotton rolls had been removed, the child was instructed to expectorate." For 8 weeks one group of children was given 2 such treatments weekly to a maximum of 15 and a minimum of 8 treatments; in another group there was one treatment weekly for a maximum of 8 and minimum of 7. The children were re-examined after a year. The authors concluded that the differences in new teeth and new surfaces decayed were significant in the upper quadrants and possibly so in the lower teeth. The effects on new surfaces in teeth previously decayed was not judged significant. There was considered to be no evidence of arrest of caries. The increase in caries in the untreated teeth was comparable to that in the group of 326 children of comparable age who received no treatments. Hence it was concluded that the effects were strictly through local rather than systemic means.

Knutson and Armstrong (1945) reported the caries incidence after 2 years in 270 of the children remaining from the group whose teeth were treated topically with sodium fluoride solution in 1942 (Knutson and Armstrong, 1934b). They found 41.3 per cent fewer teeth became newly carious following fluoride treatment compared with 39.8 per cent in the first year, with 46.6 per cent less caries during the second year. "The number of additional surfaces that became decayed in previously carious teeth was 25.2 per cent less in treated than in untreated carious teeth."

Knutson and Armstrong (1946) reported the percentage reduction in caries of 242 children remaining for observation after 3 years (Knutson and Armstrong, 1943). There were no further applications of sodium fluoride. There were 40 per cent fewer carious teeth in 1942 and 22 per cent in 1945; there were 12 per cent fewer new carious surfaces in 1942 and 33 per cent in 1945.

Knutson, Armstrong and Feldman (1947) made 2, 4 and 6 applications of a 2 per cent sodium fluoride solution to the teeth of one side of the mouths of 2016 children, aged 7 to 15. The applications were not preceded by "prophylaxis". In the 1458 children remaining for examination after 2 years, the incidences of newly carious teeth were reduced 9.3, 20.1 and 21.3 per cent by 2, 4 and 6 applications; newly carious surfaces in previously carious teeth were reduced 16.2, 9.6 and 22.2 per cent. The conclusion was that omission of prior cleaning of the teeth materially reduced the effectiveness of topically applied sodium fluoride solution in reducing carious attack.

Galagan and Knutson (1947) showed 21.7, 40.7 and 41.0 per cent reduction of carious attack in 1 year in teeth of 301, 247 and 259 7- to 15-year-old children given 2, 4 and 6 asymmetric applications of 2 per cent sodium fluoride solution, preceded by "a dental prophylaxis, consisting of necessary scaling and polishing". Similar application of 0.06 per cent lead fluoride solution to 272, 214 and 262 children gave 5.9, 1.6 and 0.8 per cent reduction of carious attack. They concluded, "Two, four and six topical applications of a 0.06 per cent solution of lead fluoride are not associated with a significant reduction in the incidence of dental caries."

Galagan and Knutson (1948), using 7 groups of children ranging in number from 208 to 371, concluded, a year after various types of application of sodium fluoride solutions to the teeth of one side of the mouth,

"1. The use of calcium chloride as a supplemental treatment to applications with a 2 per cent solution of sodium fluoride does not enhance the caries-inhibitive action of sodium fluoride alone.

"2. An increase in the spacing between applications of a 2 per cent solution of sodium fluoride from one or two weekly, to 3-month or to 6-month time intervals, decreases the observed caries-inhibiting action and apparently postpones the time when the full effectiveness of four applications is operative.

"3. Apparently a 1 per cent solution of sodium fluoride is as effective as a 2 per cent solution. However, clinical experience with the caries-prophylactic effect of a 2 per cent solution is at present far more extensive than with solutions of lower concentration.

"4. Application of the fluoride solution to the teeth by means of a spray appears to be as effective as when application is made by cotton applicator."

Arnold, Dean and Singleton (1944) made a single topical application of a fluoride solution of 500 p.p.m. fluorine to the teeth of 129 U. S. Coast Guard Academy Cadets. An equal number paired as to "past caries experience, *L. acidophilus* counts, exposure to fluoride intake first 8 years of life, and age" with the experimental group, served as controls and received treatment with a

sodium chloride solution. The controls in 1 year developed 58 new carious teeth and 206 new carious surfaces; the experimental group showed 64 new carious teeth and 213 new surfaces.

Volker, Mellilo and Belkakis (1944) found no reoccurrence of caries underneath 80 per cent of more than 100 silicate restorations but continuation of caries underneath 75 per cent of about 150 amalgam fillings. "It is suggested that the fluoride content of the silicate cements may account for the decreased caries susceptibility."

Atkins (1944) reported data of change of *L. acidophilus* and yeast counts in the saliva of 20 subjects after 4 weeks' use of a mouth wash containing 5 p.p.m. of fluoride. Three of the subjects showed increased counts, the remainder decreases. No data were given on controls. He said: "The amount of improvement due to such other conditions as general dental service, faithfulness to instructions, and the change in sugar intake cannot be ignored."

East, Ziskin, Stowe, Karshan and Richardson (1945) treated one of a pair of caries-free teeth of 48 children with a solution of 500 p.p.m. potassium fluoride at intervals of 4 months. The management of the study was such that the identity of the teeth was not revealed to the final examiner nor to the statistician. In 45 pairs of teeth, 13 developed cavities in one side and 11 in the other, a difference of no statistical significance.

McCauley and Dale (1945) gave 3 to 11 topical applications of 0.1 per cent sodium fluoride solutions to the teeth in one side of the mouths of 21 children, aged 2 to 13 at the beginning of the study. During the next year no treatment was given. They found the caries rate increased during the second year.

Dale and McCauley (1947) examined 35 men exposed from 2 to 33 years to hydrofluoric acid fumes for oral conditions in comparison with 11 control subjects. Their results were as follows: Total cavities, 2.6 ± 3.2 and 4.8 ± 2.6; surfaces, cavities and restorations, 8.4 and 26.0; arrested lesions, 1.5 ± 1.8 and 0.3 ± 0.5.

Jordan, Wood, Allison and Irwin (1946) after prophylactic cleaning gave 1, 2 and 3 topical applications of 2 per cent sodium fluoride to the teeth of one side of the mouths of 241, 575 and 161 Minnesota children ranging in age from 6 to 12 years. After 1 year they found new carious lesions reduced 4.9, 14.5 and 40.0 per cent in the deciduous teeth and 4.9, 10.0 and 21.0 per cent in the permanent teeth.

Lukomski (1946) reported, as abstracted by J. F. Volker, "One hundred eighty-eight school children were used as test subjects. The right molars were treated with 75 per cent sodium fluoride in a glycerine base. The length of treatment was 1–2 minutes. The treatment was repeated three times. The left untreated molars served as a control. Progressive caries was noted in the control teeth. Of the 376 teeth studies only one tooth in the treated group was found to be carious. However, thirty-five carious lesions were found in the control group."

Stones, Lawton, Bransby and Hartley (1949) studied the effects of topically applied potassium fluoride and ingested sodium fluoride on dental caries in about 250 6- to 14-year-old children over a 2-year period. They used groups of 6, selected as to age, sex and extent of caries. The treatments were: control group, 1 child; 1.5 mg. of fluorine ingested daily, 1 child; 2 per cent potassium fluoride adjusted to pH 4.0 with approximately 0.6 per cent potassium bifluoride, 2 children; both ingested and topically applied fluoride, 2 children. They found reduction of caries only in the boys from ingestion of sodium fluoride. They considered failure to obtain protection from caries by the topical application as possibly due to (1) use of an acid solution (2) single applications at 3-month intervals (3) not drying the solution on the teeth, though a

7-minute period of contact was allowed. The general low rate of caries in their group was ascribed to the "institutional factor" commonly observed in children resident in children's homes. The reduction in caries in the boys' teeth by ingested fluoride was shown by the deciduous molars and was "considered as tentative and inconclusive."

Wittich (1950) applied a 2 per cent sodium fluoride solution to the deciduous cuspids and molars of 40 children aged 3 to 6. The successive weekly applications with prophylaxis were given after all carious lesions detectable by explorer and radiographs were filled. The teeth were examined every 5 months for an average period of 2 years and 3 months, with new carious lesions being filled at each 10-month examination. In the treated quadrants 70 fillings were required; in the untreated sides, 90. The reduction in requirement for fillings was 22 per cent.

Bibby (1945) reported the results of a 2-year study of the effects of fluoride-containing dentifrices on dental caries in children aged 4 to 16 years and in men 18 to 23 years of age. Initially 223 children and 163 men were studied, but the number of children available at the end of the study was 126, while 143 of the men completed the course. Liquid and paste dentifrices were used with 0.01 per cent sodium fluoride for approximately the first year and then 0.1 per cent. Counts of *L. acidophilus* after 4 months on the 0.1 per cent sodium fluoride dentifrices in 243 of the experimental and control subjects indicated no differences. At the end of the study no differences in new carious lesions were found. The failure to affect the course of caries in comparison with topical applications of sodium fluoride was explained on the basis of the relatively poorer efficiency of tooth brushing in bringing the fluoride into contact with the tooth surfaces.

McClendon and Carpousis (1945) reported that 40 men who brushed their teeth with powdered fluorapatite developed 0.5 new cavities compared with an average of 1.5 in 80 men used as controls. Continuation of the study (McClendon, 1942) of brushing the teeth of rats with fluorapatite powder showed 0.16 carious teeth per rat in 60 to 100 days and 0.6 in 100 to 150 days. Control rats had 3 and 4 carious teeth, respectively.

McClendon and Carpousis (1946) reported 0.57 new cavities per year in 30 adults using a dentifrice containing synthetic fluorapatite, 0.5 cavities in 40 using rock phosphate, and 80 controls developed an average of 1.5 cavities.

Shaner and Reed (1946) studied the effects on *L. acidophilus* counts of the use of a dentifrice containing 0.5 per cent sodium fluoride in the presence of calcium carbonate. Fifteen men used the fluoride dentifrice after an initial dental "prophylaxis" and 15 controls used the dentifrice without the fluoride. An additional group of 17 used the fluoride dentifrice without the initial prophylaxis. Ten of the 32 using fluoride showed a decrease in *L. acidophilus* counts and one of the 15 in the control group.

Armstrong and Knutson (1945) analyzed the enamel of teeth extracted from the mouths of children who had received two to six applications of 2 per cent sodium fluoride to half their teeth. They found no evidence of increased fluorine in the *whole* enamel.

Rovelstad (1947) and Rovelstad and St. John (1948, 1949) studied the effects of sodium fluoride on freshly cut dentin in 51 sound teeth of children; these teeth were to be extracted for orthodontic reasons. Cavities were prepared in the teeth and the dentin was treated for 5 minutes with (a) 4 per cent sodium fluoride, (b) a paste of equal parts glycerin, kaolin and sodium fluoride and (c) crystals of sodium fluoride moistened with water. The teeth, extracted immediately or at intervals up to 80 days, showed pulpal injuries of greater severity and of different nature from that produced by the dental operations alone.

Straub and Adler (1949) found the fluoride ion content of 200 ml. of solution containing 10 p.p.m. fluorine as sodium fluoride diminished to a concentration of about 0.34 p.p.m. in 60 minutes on contact with 5 grams of dental enamel and remained unchanged thereafter.

Likins and McClure (1949) found significant differences in the amounts of fluoride adsorbed from ten different salts by powdered human enamel. Each solution contained 8 p.p.m. fluorine. No statement was made of the salts studied.

Scott, Picard and Wycoff (1949) immersed over 500 slabs of human enamel in 2 to 4 per cent sodium fluoride solutions, with pH varying from 4.5 to 8.0 and for periods from 4 minutes to 30 days. Control slabs were treated with water and sodium chloride solutions; washing with distilled water after treatment was for periods of 1 minute to 6 days. An inhomogeneous crystalline deposit was observed by electron microscopy after immersion in all fluoride solutions after 15 days; traces were seen after 40 hours; under 40 hours, treatment yielded largely negative results. All deposits were removed by washing for 6 days and many in less time. The deposit was found to be calcium fluoride by studies of 75 slabs by electron diffraction, which is more sensitive than electron microscopy. The normal apatite pattern was replaced by the typical pattern of calcium fluoride after only 3 minutes' treatment with a 2 per cent solution of sodium fluoride and in all longer treatments. The original apatite pattern reappeared on washing for 90 minutes after a 3-minute sodium fluoride treatment and after prolonged washing for the longer treatments.

Bone Meal

Discussion of the use of bone meal in the prevention of caries, because of its fluorine content, is appropriate here since (a) the one report of its use concerned a posteruptive application and (b) proposals have been made in favor of such usage.

Harootian (1943) gave 5 grains of bone flour three times a day to nine male "psychotic patients living in the stable environment of a mental hospital" for a period of nine months. Only one new cavity developed. No controls were used but comparison was made with the Bodecker (1939) life-caries index. The small number of subjects, the wide variation in their ages (22 to 46 years) and in number of teeth remaining to be attacked by caries (2 to 22 missing teeth with an average of 10.3) invalidates such a control method. The daily dosage of bone flour provided an average 267 mg. of calcium, 135 mg. of phosphorus and 0.93 mg. of fluorine. Whether or not fluorine is the effective agent cannot be deduced from this study; only use of a pure fluoride would illuminate this aspect.

Gies (Editorial, 1943b) related how he had given bone ash daily to dogs over long periods without any evidence of injury. The dosage was 1 gram per kg. body weight. One dog remained on such a regimen from weaning until death, a total of 11 years. Gies accordingly regarded the fluorine of bone as innocuous, as being "balanced" physiologically, and suggested inclusion of bone ash in the diet or in dentifrices according to the final proved mechanisms of fluorine prevention of caries.

Branson (1944) has discussed edible bone as a source of calcium, phosphorus and fluorine, mainly in the control of caries. He implied that bone would supply mineral-starved teeth through the medium of saliva. He gave no evidence that such therapy would be effective or that the fluorine of bone would not act in a manner identical to fluoride from any other source in toxic as well as therapeutic manifestations.

Discussion

None of the reports of the successful tests of topical application of fluorides in the reduction of the incidence of dental caries can be regarded as illuminating the etiological relationship of fluorides in drink-

ing water and reduced caries rate. Fluorides in concentration of 500 p.p.m., or more, especially when dried down on a tooth surface to much higher concentrations, are not at all comparable to fluorine in concentration of 1 p.p.m. The concentration of fluorides in which bactericidal power is exhibited is approached in these tests and in no case has a comparable bactericidal agent been used in a control series. The conclusion is justified that the fluoride applications have reduced the incidence of new caries, but there is no proof that this is a specific effect attainable only with fluorides and not with other bactericidal agents applied with equal diligence.

There is no doubt that topical application of fluoride is an entirely safe measure, since the amounts employed in the infrequent treatments are of the order consumed by an individual on a hot day in the drinking water in the Northern Illinois fluoride areas. The demonstration by Largent and Moses (1943) that topically applied fluorides *washed out of the mouth* could not be recovered in the urine was not necessary to show the safety of topical procedures and in no way can be interpreted as proof of efficacy.

The cost of topical application of fluorides has not been estimated. It might be negligible if the fluoride were incorporated in a dentifrice but not so if individually applied by a dentist or dental hygienist.

The topical application of fluorides or the use of bone meal depend upon individual initiative. This would constitute a very erratic factor in comparison with the highly reliable day-by-day presence of fluorine in drinking water. The latter method requires no thought or effort on the part of the subject or those immediately responsible for the welfare of the subject. It may be pointed out that prevention of *mottled enamel* has been proposed (a) by change or treatment of the general water supply or (b) by treatment of the individual water supplies. Collection of data on the prevention of mottled enamel by the first method is simple and reports of prevention have been made. Collection of data on prevention by the second method would be difficult and no reports have been made.

As to bone meal, it is difficult to understand how it can act posteruptively. The evidence of the presence of fluorine in the saliva at a constant level and unaltered by dietary fluorine seems to indicate that saliva does not act to transfer fluorine from alimentary bone meal to the teeth. It would not be expected that fluorine in bone meal, if brought into contact with the teeth, would be transferred to their surfaces, since the form of combination is the same in both cases.

It is possible that bone can serve as a source of fluorine for preeruptive use. However, there is no evidence to show that it would be more effective than the same amount of fluorine given as sodium fluoride. In fact, bone meal may be at a disadvantage. Machle and Largent (1943) have said of a 5-week experiment in which 4 gm. of bone meal was fed 3 times a day to a single subject to provide 6 mg. of fluorine: "Some difficulty was encountered in the first few days of the experiment because of the constipating effect of the bone meal. It did not dissolve completely in its passage through the enteric tract and was quite abrasive during evacuation." They found 37 per cent of the fluorine was absorbed from bone meal. It is likely absorption would be a function of the fineness of the bone meal, though no data are available on that point. Machle and Largent found practically complete absorption of sodium fluoride. It may be noted that the probable error of the mean of urinary fluoride concentration from bone meal was about 3 times that of the probable errors from other forms of fluoride, suggesting considerable variation in absorption of fluorine from bone meal. These considerations indicate that administration of fluorine as bone meal may result in irregular absorptions due both to the particle size and to individual variations.

The activity of fluorine with respect to both its adsorption on bone and its availability from bone has been reviewed in detail.

FLUORINE AND MISCELLANEOUS REGIONS WITH LOW CARIES RATES

Cox, Matuschak, Dixon, Dodds and Walker (1939) wrote: "Present evidence on fluorine and dental caries is sufficient to require that all future studies of dental caries must consider the influence of fluorine and that past investigations be scanned for the possible contribution of this element."

Marshall (1926a, b) examined 54 inhabitants of the South Atlantic island, Tristan da Cunha. He found 14 mouths with caries in all age groups. He noted that there was a low consumption of sugar. Potatoes were the staple food, and fish were plentiful but did not appear to be popular. Milk was nearly always plentiful.

Sampson (1932) examined 156 of the 163 inhabitants of Tristan da Cunha. He found 130 subjects with no caries. Only 1 of 879 temporary teeth was carious and 74 of 3181 (1.82 per cent) of the permanent teeth. The water supply was obtained from a spring in the mountain side. In the diet, fish and potatoes were staples. Milk was plentifully used and all children were breast fed. Hen and penguin eggs were consumed to some extent. Cereals were not grown, and flour and sugar were available only on the rare occasion of ship calls.

Barnes (1937) re-examined the people of Tristan da Cunha 5 years after Sampson's observations (1932). He was assisted by J. R. Moore of Capetown, who had aided in the earlier studies. Barnes found 92 caries-free mouths in 183 persons, with the 4.6 per cent of permanent teeth carious compared with 1.82 per cent in 1932. The rate of caries in diciduous teeth was 2.6 per cent compared to 0.113 per cent earlier. Barnes records that 10 vessels had called during the preceding 3½ years and that the inhabitants had enjoyed a higher "luxury index." There was an appreciable increase in consumption of sugar and of flour, but "they are used very sparingly if compared" to the English standard.

Sognnaes (1939) confirmed previous observers on the dental conditions on Tristan da Cunha and noted that "developmental enamel defects" were common in the last generation.

Sognnaes (1941) reported that of 3907 permanent teeth of the inhabitants of Tristan da Cunha, 15.8 per cent had "white spots" and 10 per cent of 765 deciduous teeth were similarly affected. He considered the white spots were mottled enamel. The suspicion that the teeth were fluorosed was confirmed by Sognnaes and Armstrong (1941) by finding an average of 140 p.p.m. fluorine in both the deciduous and the permanent enamel. There were 196 and 270 p.p.m. in the deciduous and permanent dentins, respectively. As the water was found to contain only 0.2 p.p.m. fluorine, Sognnaes attributed the slightly mottled condition of the teeth to the fluorine of fish, which constituted a principal item of diet, as well as to the water. Sognnaes said, "lesions were slight and diffuse in the deciduous teeth, while in the permanent teeth they were well demarcated from the unaffected portions of the tooth surfaces"; also, "of 1145 permanent incisors, 17.7 per cent were affected with white spots in the enamel, while no carious lesions were present, except in some of the adult people."

Sprawson (1932b) pointed out that the main difference between the intake of Pitcairn Islanders and the inhabitants of Tristan da Cunha was that the latter had an abundance of raw milk in the dietary. He ascribed the rampant caries of Pitcairn Islanders to the absence of raw milk other than human milk.

Hanke *et al.* (1933) observed 20 children with "obviously hypoplastic teeth" for a period of 2 years and said such teeth "appear to have been no more susceptible to dental caries than were the normal-appearing

teeth of the rest of the group" of 323 children. Five of the 20 children developed no caries in the 2-year period. The observations were in an orphanage at Mooseheart, in Northeastern Illinois.

Mills (1937) has cited a personal communication from R. A. Kehoe "that, during a series of physical examinations on a considerable number of Mexicans of all ages in an isolated region of central Mexico, he saw practically no caries."[7]

King (1940) suggested from a survey of 1,530 children in the Island of Lewis "that the superiority in tooth structure and in freedom from caries of the Lewis rural children was to a large extent due to the relatively high fat-soluble vitamin, calcium and phosphorus content of the diet." Price (1933) found: "In the interior of the Isle of Lewis the teeth of the growing boys and girls have a high degree of perfection with only 1.3 teeth out of every hundred examined that had even been attacked by dental caries." He ascribed the low rate of caries to the use of primitive foods, since in Stornaway, the chief port of the island where modern foods were used, caries was rampant. Bromehead (1941) said: "The greatest immunity was found by King (1940) in the Island of Lewis: apatite is among the chief accessory minerals of the gneisses of which that island is composed."

GENERAL DISCUSSION AND CONCLUSIONS

Fluorine affords a significant degree of protection against caries, the direct evidence being: (a) incidence of decay is reduced in districts in which fluorine-caused mottled enamel is endemic; (b) incidence of caries is reduced in areas where fluorine is present in the community water supplies in amounts of the order of 1 p.p.m.; (c) the molars of rats formed on low levels of fluorine that are probably transmitted placentally or by lactation show increased resistance to coarse cereal caries; (d) fluorine fed posteruptively to rats in amounts substantially in excess of those which mottle rat incisors, or applied directly, protects rat molars from coarse cereal caries. The indirect evidence that fluorine protects against caries is: (a) fluorine is found in unmottled caries-free enamel; (b) fluorine in concentrations as low as 1 p.p.m. appreciably reduces the formation of acid from carbohydrates by bacteria; (c) the solution rate in dilute acid of powdered enamel fluorosed by brief contact with fluoride solutions in concentration of 8 p.p.m. or more, is less than that of the same untreated enamel.

Fluoride will deposit on calcium phosphate, bone or enamel in a purely non-vital way to form fluorapatite. The presence of fluorine in very slightly elevated amount causes mottled enamel, and this latter phenomenon appears to be an interference with cellular activity because (a) posteruptive increase of the fluorine content of enamel does not cause mottling and (b) an apparently identical effect can be caused by feeding of cadmium in toxic dosage during enamel formation. So far as enamel is concerned these vital and non-vital activities of fluorine seem fairly well indicated, but there is as yet no clear-cut evidence as to how the fluorine acts as a protective agent against caries.

The fluorine of enamel probably represents the amount deposited at the time of formation, but that of dentin may reflect fluorine added after formation as well. There has been no demonstration of an increment of fluorine in the intact enamel of human teeth by contact with food and water containing fluorine at the borderline mottling level; however, there is evidence of an increment of fluorine in the enamel after topical exposure to higher concentrations of fluorine for short periods.

The most sensitive test of injury from fluorine is mottled enamel. It is probable

[7] See Kühns (1888) and Armstrong and Brekhus (1938a).

that mottled enamel is related in its threshold appearance to the absolute amount ingested daily during formation of the areas of enamel affected and not to the level of fluorine in the water or the food. If this be true, then mottled enamel would not necessarily appear in enamel formed with waters containing up to 1.8 p.p.m. in winter and 0.9 p.p.m. in summer. (This assumes a doubled consumption of water in the summer.) The relative caries resistance of teeth formed with such seasonal variation of the fluorine content cannot be predicted with certainty, but the fluorine content of the enamel would undoubtedly be higher than that of enamel formed on a year-round level of 0.9 p.p.m.

There have been suggestions that fluoridization of water to submottling levels may cause obscure maladies as yet unknown. In addition to alertness for the appearance of mottled enamel or of stiff backs, it may be well to observe blood-clotting time and also to determine hearing acuity. These studies may be made in advance in fluoride areas such as those in northern Illinois. Rejections from military service because of defective hearing, correlated geographically, may be found linked with either a deficiency or an excess of fluorine resulting in deposition in the ear bones.

The minimum level at which fluorine has been shown to affect acid formation by bacteria is about 1 p.p.m. This is about 10 times as high as the normal fluorine level in saliva. The growth of bacteria is affected at about 250 p.p.m., which is about twice the concentration of fluorine in enamel that resists decay. Consequently it is not possible to explain the anti-caries property of fluorine on an anti-bacteria basis based on the fluorine as it exists in the mouth.

Furthermore the fluorine of enamel, either combined or adsorbed, would be expected to act only at atomic distances. If these distances are exceeded by fluoride ions being detached, it would appear that the fluoride content of the enamel could not be maintained. It is difficult to see how the fluoride of enamel can affect the action of enzymes within bacterial cells.

The counts of *L. acidophilus* are correlated with caries activity but probably not with the fluorine content of the current water supply. The counts may also be correlated with the fluorine content of the enamel, but this remains to be shown.

Enamel may show a reduction in solubility rate in acids proportional to its fluorine content, either acquired in formation or artificially increased, but the enamel of anterior teeth in mottled-enamel areas shows *no* disintegration, presumably in caries, by solution in acids. Also bacterial action is not affected by dentin until late in the contact, that is, presumably until some fluorine-containing tooth material is in solution. These considerations suggest that fluorine of teeth may affect rate of caries after initiation by chemical action but that resistance to initiation of decay in enamel results from a different mechanism.

If fluorine is the most potent factor yet found in the protection of teeth from caries, and bacteria are the agents most destructive, it does not necessarily follow that the two are directly related. The direct relation between fluorine and bacteria is increased inhibition of bacterial action by increased fluoride concentration; yet, on present evidence, severely mottled teeth with 350 p.p.m. of fluorine are subject to dental caries but homologous nonmottled teeth with 100 p.p.m. do not decay. It is possible that a study of the flora of carious-like lesions in teeth with severely mottled enamel and comparison with the microorganisms of unmottled, decayed teeth could be decisive in establishing whether or not caries-producing bacteria can thrive in enamel and dentin with increased levels of fluorine.

In his 1943 review Dean listed among "four cardinal points" contributing to

the clarity of the epidemiological picture of fluorine and caries, elimination of the "circulation factor," that is, confining observations to subjects of continuous residence. Deatherage, by breaking with this rule, has been able to show that those who migrate from regions with fluoride in the water after their teeth are formed retain protection from caries. With better controlled observations, that is, by showing which teeth were affected and in what sites, he could have produced acceptable evidence for or against protection acquired posteruptively from fluorides in natural waters. Data are urgently needed on the incidence of decay *in specific areas of teeth, rather than in whole teeth*, in children who have migrated from high to low (and low to high) fluoride districts at a variety of ages. These data would show to what extent fluorine protects definite areas of teeth from decay and how much of that protection is posteruptive.

If fluorine acts only during the formation of enamel, the fluorine level of plant and animal tissues (other than bone) commonly used as food is probably too low to provide sufficient fluorine for the formation of caries-resistant teeth, and additional fluorine must be added to the dietary to obtain the optimum conditions. If there is a common water supply, the simplest, safest, and most effective way to provide the extra requirement of fluorine is through the addition of fluorides to the water in amount adjusted to the climatic and seasonal needs.

Bone can serve as a source of fluoride, but insofar as the fluorine is absorbed it is just as toxic as fluorine from any other source. Bone may not be as reliable a source of fluorine as a soluble salt because there may be (a) wide differences in absorption resulting from individual variations and (b) variation in fluorine content and in physical state of bone. An attractive feature of bone is that, as in the case of a community water supply, it cannot provide enough fluorine for an acute toxic dosage. The efficacy of bone fluoride in the prevention of caries merits careful study.

Fluorine is transmissible by the placenta and by the lactating breast, though it is unlikely that sufficient fluorine for the formation of caries-resistant enamel normally reaches infants by these routes, since (a) the fluorine content of deciduous tooth enamel is probably lower than that of permanent enamel in the same regions, (b) deciduous enamel is less frequently mottled than permanent enamel and (c) deciduous teeth are not as effectively protected from decay in mottled-enamel districts.

There is as yet no conclusive demonstration that fluorine is essential to animal life. The possibility remains, of course, that a ration may be devised with a lower fluorine content than any thus far used, and studies conducted with it may reveal that, for the best welfare of animal life, some fluorine is required.

(Author's Note: The foregoing section includes literature published prior to July 1950)

NUTRITION AND DENTAL CARIES

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Contents

NUTRITION AND DENTAL CARIES

The development of methods for the reduction of the present high incidence of tooth decay without question constitutes the most important problem in preventive dental medicine. Certainly this problem is of major importance in the broad field of public health. On a theoretical basis, one could postulate that the susceptibility to dental caries in man might be altered by disturbances in the oral and systemic environments of the tooth, by the structure and resistance of the tooth or by a combination of these and other agents. On the basis of our present fundamental and clinical research knowledge, it appears to be impossible to determine with any degree of accuracy how much the development of carious lesions is dependent upon any one or any combination of the above situations or to predict how the development of carious lesions can be prevented or arrested by the alteration of any of the above situations. At present, in view of the high incidence and insidious nature of dental caries, it seems to be more important to find methods for the prevention of carious lesions than to understand the causes for the development of the lesions. However, if the causes of the varying susceptibility and resistance to tooth decay were known precisely, the ability to predict methods of control would be infinitely enhanced. The development of preventive measures for numerous other human ailments has been possible through scientific calculations and controlled investigations based on the fundamental knowledge of their causes. Undoubtedly the greatest hope for the prevention of dental caries lies in increasing our fundamental knowledge of the various factors which influence development of the lesions. And yet this survey shows all too clearly that there is an almost complete absence, on an institutional, national or world level, of a coordinated program to provide the needed answers.

It is conceivable to postulate that the food consumed by an individual throughout his entire life could directly or indirectly affect the susceptibility of the teeth to decay through the following channels: by alterations in the differentiation, development and maturation of the teeth and their supporting structures (the gingival margin, epithelial attachment, cementum, periodontal membrane and alveolar bone), by changes in the metabolic processes in the mature hard dental structures and in their supporting structures, by alterations in the composition and quantity of saliva, by differences in the functional ability of the teeth, their supporting structures, and the salivary glands resulting from diets of varying physical characteristics, and finally, the most obvious and most extensively investigated potential effect, but not necessarily the most important, by mixture of food constituents with oral secretions to form the debris in the oral cavity and the materia alba on the tooth surfaces. Since the total effect of the diet may involve at least these and possibly additional, unrecognized modes of action, any accurate evaluation of the direct and latent role of the diet in the causation and prevention of dental caries must eventually consider each of these possibilities individually, and collectively in their proper relation to each other. Unfortunately the answers to many of these problems are not available for human beings or for experimental animals. How frequently these unexplored problems occur and how badly the precise answers are needed will become evident in the progress of this report. Despite these omissions, a considerable amount of data has been collected on the relation of diet to dental caries. That portion of the data concerned chiefly with the relation of diet to the oral environment and to the saliva has been discussed in the section, Oral Environment and Dental Caries. That portion of the

data which was concerned primarily with the nutritional relationships of the diet to dental caries will be discussed in this section in terms of the possible pathways through which the experimental efforts might have operated. Wherever a question arose of the diet affecting the oral environment in addition to or simultaneously with any effect on the metabolic processes of the individual, an effort has been made to discuss the data presented in terms of oral environmental as well as systemic effects. In all cases, it has been the desire to discuss all results impartially in terms of the data presented and the experimental procedures used to collect those data. Wherever criticism or suggestions were made for improvement of the collection, presentation or interpretation of data, they have been accompanied by the reasons why the reviewer believed that such changes would have made more acceptable scientific investigations and presentations. In all cases, the conclusions in the summary appear to be entirely reasonable on the basis of present experimental findings under the conditions of the investigations. The many gaps in present knowledge indicate all too clearly that these conclusions should be considered as working hypotheses to be tested by critical experiments which will substantiate, strengthen, weaken or negate current conclusions.

Early research in nutrition clearly related the nutritional value of the diet consumed during the developmental period to tooth structure. These findings prompted much speculation and research on the relation of nutrition, both during tooth development and after eruption, to tooth decay. Much of the research on the role of individual nutritional factors in the prevention of dental caries was performed in the period between 1920 and 1935. At that time, nutritional knowledge was insufficient to permit any accurate evaluation of what constituted an optimum diet for normal growth and development for children in any age group. Indeed the exact specifications of what constitutes an adequate diet under all circumstances are not known completely today, nor do we know whether the maintenance of normal growth, physical and mental development, reproduction and lactation are complete diagnostic criteria of an adequate diet. The maintenance of intact teeth might in the future be shown to be just as essential a criterion of the adequacy of a diet. Frequently in these early studies a dietary regimen could not be devised which was deficient in only one essential nutrient nor could the deficient factor or factors be supplied to the children of the experimental groups in pure form. Because of these handicaps, early investigations on the relation of essential nutritional factors to the susceptibility to dental caries were not performed as precisely as the investigators believed. The results of these handicaps are not to be interpreted in terms of the lack of ability of these investigators but rather in terms of the early stages of nutritional knowledge in which they were experimenting.

The difficulties encountered in the accurate diagnosis, recording and evaluation of the extent and progress of tooth decay are numerous. Deatherage, Wilson and Ledgerwood (1939) reported the results of independent examinations of 63 children in an Illinois orphanage by 5 staff dentists. A great variation in evaluation was observed and a statistical formula was devised for treatment of such data. Radusch (1941) observed that when each subject was examined by 3 dentists, the average variability in the oral diagnoses of dental caries was 4.2 carious teeth and 5.8 carious surfaces. When the diagnoses were made with the aid of bitewing radiographs, Radusch reported that the variation between examiners was substantially reduced. Sognnaes (1940b) has drawn attention to the number of carious lesions which could be detected by drying the teeth, by cleaning and then drying the teeth, and by radiographs in addition to an ordinary inspection of the teeth with mirror and explorer. The necessity of such pro-

cedures for a complete evaluation of the lifetime dental caries experience of an individual, and especially for the detection and study of early carious processes, was emphasized. Generally, it would be excellent if all examinations could be made with this painstaking thoroughness. In most experimental regimens and especially in clinical surveys, the amount of time and equipment necessary to make such a diagnosis is prohibitive. As a general rule, less exacting examinations may serve a useful purpose in the problem at hand if the provision is made that absolutely identical criteria are used throughout all subjects examined and throughout repeat examinations during the entire experimental period.

Burket (1941) has made a survey of the accuracy of clinical and roentgenologic diagnosis of dental caries as determined by microscopic studies on 460 teeth, of which 347 interproximal and 221 occlusal surfaces were judged carious by one or more methods of examination. All teeth in this survey were obtained at necropsy and were examined after portions of the jaws had been removed. The gross examination was made with a sharp needle and a #17 explorer; dental floss and separators were not used to detect lesions on the interproximal surfaces. Roentgenograms were made, employing a technic similar to that used routinely in the mouth. Preparations for the microscopic study were cut after decalcification at 12 microns; every 10th section was routinely mounted and stained with hematoxylin and eosin. In all three examinations only lesions on the mesial, distal and occlusal surfaces were considered. The criteria used for caries diagnosis was "a tooth was considered carious when there were necrosis and debris associated with cavitation on that surface of the tooth which is exposed to the oral cavity."

Two hundred and twenty-one (48 per cent) of the 460 teeth were diagnosed as having carious lesions of the occlusal surface by one or more methods of examination. In the histological preparations, a lesion was observed on the occlusal surfaces of 137 (62 per cent) of these 221 cases. Of the remaining 84 teeth, the positive diagnosis of caries had been made by gross examination in 78 cases and by gross and roentgenologic examination in 6 cases.

Three hundred and forty-seven interproximal surfaces were found to be carious by one or more methods of diagnosis. A lesion was demonstrated in the histologic preparations of 286 (82 per cent) of these 347 cases. Of the remaining 63 surfaces, the positive diagnosis of caries was made on 30 surfaces by gross examination only, on 11 surfaces by roentgenologic examination and on the remaining 22 surfaces by both gross and roentgenologic methods.

When the data were combined, 61 per cent of the teeth were shown to have been diagnosed carious by gross examination, 35.5 per cent were diagnosed carious by roentgenologic examination and only 29.8 per cent were diagnosed carious by both gross and roentgenologic methods. The clinical methods of detecting caries paralleled more closely the results of the microscopic studies than did the radiographic method.

Most of the lesions diagnosed only by microscopic examination occurred on the interproximal surfaces, while the majority of cavities found only on clinical examination were located on the occlusal surface. Some of the fissures on the occlusal surfaces may have been so formed that by the criteria designated for the definition of caries they would be judged carious but on histologic examination were found to be normal. In other cases, carious lesions diagnosed by gross examination which had not penetrated into the dentin would be lost in the procedure of decalcification. For these various reasons many lesions diagnosed as carious by gross and roentgenologic procedures would not be found in microscopic preparations. This would account for the surprisingly higher incidence of grossly diagnosed lesions.

The data indicate clearly some of the difficulties inherent in obtaining an accurate diagnosis of carious lesions. Obviously, the methods used in preparing the histologic sections did not provide a perfect material by reason of the complete loss of enamel during decalcification. The use of ground sections instead of decalcified ones would not have been an adequate substitute by reason of the inability to prepare sufficient sections from each tooth without loss of appreciable tooth substance during grinding.

As an example of how the evaluation of dental caries experience varied in a given area with the methods, training and opinions of the examiner, the sampling of the population, etc., the results of Messner, Gafafer, Cady and Dean (1936) may be compared with those of Dean, Arnold and Elvove (1942). The dental caries incidence (DMF) per 100 boys, 12 to 14 years of age, for the 7 cities which were recorded in both surveys are presented below:

	Messner *et al.* (1936)	Dean *et al.* (1942)
Colorado Springs, Colo.	205	246
Pueblo, Colo.	223	412
Lima, Ohio	253	652
Zanesville, Ohio	417	733
Portsmouth, Ohio	631	772
Elkhart, Ind.	217	823
Michigan City, Ind.	450	1,037

It is realized that the data presented by Messner *et al.* of the United States Public Health Service were compiled from the oral observations of approximately 8000 practicing dentists and less skilled observers, namely physicians and school nurses, who had been requested to submit their evaluation of the dental caries experience of the school children in their community. The results obtained by this method of sampling probably might be expected to vary considerably from community to community by reason of the varied training, experience and personal opinion of these dentists. In addition the sampling of the school children may not have been representative in some cities where only certain schools or certain children within schools were selected from the total child population. In view of the serious discrepancy between these two series, the question must be raised as to whether statistical surveys on the relation of such factors as latitude, sunshine, winter temperature and hardness of the communal water supply to dental caries incidence based on indirect data of the type presented by Messner *et al.* would be valid.

To test the validity of the data of Messner *et al.* (1936) for the purposes of comparing the caries prevalence among various age groups, East (1941c) evaluated these data by statistical methods and concluded: "When dental data have been collected by different examiners, it is a matter of concern whether sufficient bias has been introduced to invalidate them. One may properly inquire whether the examiners of one city were more skillful or meticulous in locating and recording cavities in the teeth than those of another city and whether differences in the technic and criteria of the examinations were distributed in the same manner as were the climatic factors. To answer this fundamental question, I have determined the closeness with which the frequencies of the various caries rates follow the distributions represented by the bell-shaped probability curve. I found that the frequency distributions of the caries rates for the age-sex groups of children in these 156 cities followed closely the normal law of distribution, except as they may have been influenced by variables which are in themselves distributed according to the same law. The data appear, therefore, suitable for comparing the caries rate of one group with that of another. They also seem adequate for determining the correlation of the caries rates of the groups with the other variables."

Thus the "errors" or "bias" introduced by examinations made by some 8000 different examiners were distributed as represented by the bell-shaped probability curve. It

would seem from this statistical analysis that there were relatively as many examiners in one region who counted or did not count pits and fissures as "cavities" as there were in any other region; likewise there were as many examiners in one community who used or did not use sharp explorer points as in any other community. If this had not been the case, it is highly improbable that the distributions would have resulted in anything but skewed curves.

The data of Messner *et al.* would appear from these considerations to be valid for purposes of comparing the caries prevalence among various age and population groups but would not necessarily be valid as measures of the actual number of carious teeth among these population groups.

These examples indicate very clearly the necessity for a careful standardization of the procedure and criteria used in the dental examination, in order that the successive inspections of the control and experimental subjects will be reliable for statistical comparison of the dental conditions at the respective experimental periods. Undoubtedly the best results can be achieved when the successive dental examinations of the subjects are made by the same examiner throughout the experimental period. When two or more examiners are used during the course of an experiment, an even stricter standardization of the dental examination is necessary along with a training period to establish the ability of the examiners to give as nearly identical examinations as is humanly possible. Such a training process for the development of a correct and simple procedure for examining and recording the actual and complete dental condition present at the time of the examination has been described by Hyatt (1929).

The variety of methods for the presentation of data on lifetime dental caries experience and dental caries increment is exceedingly confusing to investigators who are interested in attempts to correlate data from various experiments or surveys, especially since the criteria used in making the original diagnoses from which the data were obtained were not recorded. A valuable contribution could be made by the universal adoption of standardized methods for recording the lifetime dental caries experience and especially for the dental caries increment in terms of specific teeth, the available number of unattacked surfaces, and the length of time that specific teeth were exposed to the diet under investigation.

When relating dental findings to the diet of children in the period during which new permanent teeth are developing and erupting, it is especially valuable to know the distribution of the teeth affected by the new carious lesions and how long these teeth could have been affected by the diet both systemically and orally. An example of the difference in interpretation which may arise on the basis of such a consideration of the duration of exposure developmentally and orally is demonstrated in the discussion of wartime caries increments by Sognnaes (1947, 1948a). Discussions of correlations between present diet and the lifetime dental caries experience instead of the dental caries increment during the period on the diet evaluated are common in dental investigation. This error of judgment is of sufficient frequency and sufficient seriousness to merit comment. If it can be adequately demonstrated that the present diet is typical of the diet throughout life, then the discussion of a correlation can be justified but not otherwise. Many investigators question the ability to determine with any great degree of accuracy the composition of the current diet of any individual by the most efficient of interview procedures and feel that the estimation of the past diet by this procedure is at best only a very rough procedure. When no attempt was stated to have been made to even obtain this rough gauge of the composition of past food consumption, no comparison of present diet to lifetime dental caries experience can be tolerated.

Statistical analysis of the data obtained

in experiments on the relation of nutrition to dental caries experience has been uncommon, despite the widely divergent dental caries susceptibilities observed in any group of human subjects. Obviously statistical inspection of experimental data cannot remove errors introduced by poorly standardized examination technics. However, statistical analyses of data which were obtained through carefully planned and executed experiments can aid materially in the accurate interpretation of those data by the determination of whether or not the difference between two groups attributed to the experimental variant is significantly larger than would be expected by chance and other unrecognized causes of variation within the groups under observation. Throughout this review of Nutrition and Dental Caries the word "significant" is used only when statistical methods have been applied and have indicated that the observed difference was sufficiently large that it could have occurred through chance and other causes less than 5 times in 100 experiments of these characteristics.

Any experimental study to investigate changes in dental caries incidence obviously must be made with the utmost thoroughness. The selection of highly comparable experimental and control groups, the standardized evaluation of the incidence and progress of carious lesions and the absolute regimentation of dietary control to permit only one dietary variant with no environmental variants are factors which must be meticulously regulated. In the present review an attempt has been made to evaluate critically the data which have been published on the relation of nutrition to dental caries. For each experiment discussed, the validity of the procedure and of the data presented was considered.

The topic of nutrition and dental caries has been divided for discussion purposes into three general headings: the evidence obtained from overall dietary surveys in various populations, the data obtained from experiments during the growth and development of the child when nutritional requirements are at their maximum, and the surveys of the dental caries incidence during pregnancy and lactation when nutritional requirements are again increased.

SURVEYS OF THE RELATION OF DIET TO DENTAL CARIES INCIDENCE IN VARIOUS POPULATIONS

The following studies have been chosen for discussion purposes as examples of data collected in surveys of various populations on the relation of diet to the dental caries incidence. Those papers have been omitted in which the actual data, number of subjects, dental caries experience, type of oral examination and descriptions of the composition of the diet were not presented by the author to substantiate his discussion and conclusions. An earnest attempt has been made to include and evaluate those surveys in which dietary differences were observed and in which the accompanying dental caries data appeared to have been determined in a reliable manner.

Orr and Gilks (1931) made a survey of the physique and health of two African tribes, the Masai and the Kikuyu. The diet of the Masai consisted to a large extent of milk, meat and raw blood, while that of the Kikuyu consisted mainly of cereals, with some roots and fruits. Physical measurements showed that the adult Masai male was on the average 5 inches taller and 23 pounds heavier than the adult Kikuyu male, and his muscular strength, as determined by the dynamometer, was 50 per cent greater. Of 254 Masai studied, only 1.6 per cent of the boys and 3.6 per cent of the girls had dental caries. Among the 2500 Kikuyus, the incidence of dental caries was 13.7 for the boys and 13.1 for the girls. Rickets was observed in 63 per cent of the Kikuyu children but was said to be rare among the Masai. Mention should be made of the fact that even though the incidence of carious lesions was much higher in the Kikuyus

than in the Masai, the incidence in the Kikuyus is still far below the extremely high incidence in the United States, the Northern European countries, New Zealand, etc. Schwartz (1946) has drawn attention to the fact that the Masai apparently have a low resistance to some infectious diseases, especially venereal, since they are rapidly dying out despite their fine-appearing physiques and handsome features.

Price (1936a) made a dental survey of a variety of South African tribes. Among 88 members of the Masai tribe, the percentage of carious teeth was 0.4 in comparison with 5.5 per cent in the 33 Kikuyus examined. The data further indicated that those other groups of natives who, like the Masai, consumed diets containing large amounts of meat and milk had a low caries experience. However, those native groups consuming diets high in cereals and those at mission schools had a higher dental caries incidence.

Oranje, Noriskin and Osborn (1935) surveyed the dental caries incidence in South African Bantus to determine if there was a difference between the primitive and civilized branches of the tribe. Among 465 primitive Xosas who had little contact with civilized communities, 36 per cent had dental caries, with an average of 2.3 carious teeth per carious mouth. Of 90 Xosas who were working in the mines and living in the mining community, 56 per cent had dental caries, with an average of 4.3 carious teeth per carious mouth. A similar comparison was made in the Bapidi tribe; 22 per cent of the 50 Bapidi females who had never worked in town had carious teeth. However, of the 32 males who had worked in town for various periods, 44 per cent had carious teeth.

Staz (1938) examined the teeth of 300 primitive Bantus and similar numbers of urban Bantus and Europeans living in South Africa. A relatively high percentage of the primitive Bantus, considerably more of the urban Bantus, and still more of the Europeans, had dental caries. However, the primitive Bantus had the same number of "potential pits" as the other groups, but Staz believed that the severe attrition produced by the primitive diets prevented the development of carious lesions in the fissures.

Further studies on the dental caries incidence in various groups of the Bantu tribe have been reported by Jones (1940). It was observed that the children living in native locations or attending native schools had a much lower dental caries experience than those attending schools in the cities and towns.

Price (1936b) reported observations on 800 North American Eskimos and Indians who were classified in accordance with their contact with civilization. The percentage of carious teeth among the most primitive groups of Eskimos and Indians was about 0.09. At the point of contact with civilization, the percentage of carious teeth among the Eskimos was 13 and among the Indians 21.5.

Rosebury and Waugh (1939) made a survey of the dental caries experience of 124 Eskimos from Bethel and neighboring settlements, from villages on the lower part of the Kuskokwim River and on the coast of southwestern Alaska. A careful examination was made with the aid of mouth mirror, explorer, chip blower and dental floss. From these examinations it was concluded that dental caries was more prevalent among natives from settlements in which contact with the white man was relatively unrestricted and least prevalent among more primitive groups with a minimum of such contact. Since the incidence of dental caries was progressively less with advancing average age, the authors concluded that dental caries as a whole was of recent origin among these people. Deposits of salivary calculus were observed with a frequency in inverse ratio to the incidence of dental caries and to an extent only partially accounted for by differences in age.

Rosebury and Karshan (1939) compared the dental caries experience with the diets

and dietary habits of the inhabitants in the Alaskan settlements of Kepnuk and Eek and in a Moravian orphanage. Kepnuk is primitive and isolated and its inhabitants have little tooth decay. Eek is primitive but has a resident white trader and its people have much dental caries. The children in the Moravian orphanage had a more extensive influence of the white man's diet than is present at either of the other settlements but had an intermediate dental caries experience. These investigators believed that there was no correlation between dietary carbohydrate in general, cereal and grain foods, dietary protein and fat, calcium and phosphorus and probably vitamin D and the potential reaction of the diets. It was stated that "these conditions consequently either lack influence on, or play secondary roles in, the causation of dental caries among those people." Consumption of sugar was correlated to some extent with the occurrence of dental caries at the three settlements but was considered to be of doubtful importance, since the largest amount of sugar was consumed at Eek where it was ingested in dissolved form in tea. The only food to which significance was attached was the pilot bread or ship biscuit which was consumed in highest amounts at Eek where the incidence of dental caries was highest, in intermediate amounts at the Moravian orphanage where the dental caries experience was moderate, and in smallest amounts at Kepnuk where tooth decay was infrequent.

Höye (1938) reported the results of a Norwegian survey made by Toverud in a comparison of the dental caries incidence in the children, 6 to 15 years of age, of Oslo and Valle. In Oslo only 0.2 per cent were caries-free in comparison with 35 per cent at Valle. The diet of the latter community was largely made up of sour milk, butter, whole grains, berries, with little meat, sugar and delicacies. In Oslo, however, the typical diet contained less milk, butter and whole grains and more refined flour and sugar.

Pedersen (1938, 1939) investigated the relation of nutrition to dental caries incidence in about 3,000 primitive and civilized Greenlanders. The percentage of Eskimos with dental caries in East Greenland varied from 4.3 for the males and 4.6 for the females at the isolated native Angmagssalik settlements to 43.2 and 51.1, respectively, at the trading station. On the west side of Greenland, however, the dental caries incidence was much higher, varying from 31.8 per cent for the males and 44.4 per cent for the females in the native settlements to 83.3 and 90.5 per cent, respectively, for those Eskimos who lived in the immediate neighborhood of the trading post at Julianehaab. In West Greenland it was estimated that 63 per cent of the total caloric intake in 1930 was made up of imported foods, especially sugar and cereals, instead of the 17 per cent in 1901. In East Greenland, however, the native settlements received essentially no imported food and the trading station relatively little, due to its isolated position.

Shourie (1941) examined the teeth of 6,866 children, aged 6 to 18 years, in various parts of India. Carious lesions were observed in only 55.5 per cent of the children. He observed that those children whose diet was based on whole wheat had slightly fewer carious teeth than those whose diet consisted largely of rice. The highest incidence of dental caries was observed in an Anglo-Indian orphanage where Shourie considered the diet to be nutritionally more adequate than that consumed by the average Indian children.

Laband (1941) examined 1,455 Malayan children during a 2-year period. A distinct difference was observed between the children from the town and those from the villages and country districts. One hundred and thirty town boys and 290 village boys were examined twice, with a year between examinations. Of the town boys, 25 per cent were observed to be free of carious teeth at both examinations, in contrast to 52 per

cent of the village boys. Laband suggested that the lower dental caries incidence in the country districts might be associated with the use of home-grown and unrefined carbohydrates.

Arkle (1944) classified the Indians at James Bay in Northeastern Canada in accordance with their contact with the white man's civilization. Absolute immunity from dental caries among the juvenile Indians, under 21 years of age, farthest from civilization, was 63.4 per cent for the males and 66.6 per cent for the females. The chief dietary constituents of this group of Indians were fish, small game and birds, berries, flour and lard. Among the modernized Indians who lived at or near trading posts and mission schools, freedom from dental caries was observed in 17.1 per cent of the males and 3.7 per cent of the females. The diet of the latter group consisted of large quantities of bread, biscuits, pie, cake, jams and jellies, with little meat, berries or vegetables. The latter diet was in distinct contrast to the former in regard to its low content of fat, of good quality protein, and of fruits and vegetables, and its carbohydrate content. Supragingival and subgingival calculus was seldom absent in the mouths of the more remote Indian bands. Dental calculus was often completely absent in more modernized groups, or, if present, it was much less extensive than in the remote groups. There appeared to be a high inverse correlation between dental caries activity and calculus formation.

After a survey of the dental caries experience of 2,894 British children between the ages of 6 and 13 years, Read and Knowles (1938) selected 12 caries-free children and 12 with rampant caries. A detailed inquiry was made of the past and present dietary history of these selected subjects, who ranged from 3 to 14 years of age. It was concluded from the dietary data that the caries-free group had received diets which were good with respect to fats and proteins, while the diet of the rampant caries group was generally poor in these constituents. At the same time, the carbohydrate consumption of the rampant caries group was considered to be excessive, frequently with sweets eaten in large amounts daily, while the carbohydrate consumption of the caries-free group was low and candies were eaten only occasionally.

Collins, Jensen and Becks (1942) classified 366 students in California into 3 groups: (a) caries-free, (b) clinically caries-free but roentgenograms showing 4 to 8 carious lesions and (c) 5 or more open carious lesions. After the food habits of each student were determined by individual interviews, the diet of each was evaluated roughly for adequacy of protein, calcium, phosphorus, carotene, thiamine, ascorbic acid and vitamin D. The recommendations of the Food and Nutrition Board in 1941 were used as the standards of adequacy. The authors determined that there was no correlation between any of these factors and the dental caries incidence of the 3 groups. When the food records were evaluated for the average daily refined sugar consumption, it was found that the 3 groups ingested daily 10.4, 11.6 and 17.9 teaspoonfuls of sugar, respectively. The significance of these data was not determined by statistical methods.

Henriksen and Oeding (1937–38) determined the food consumption of 3 families on Tristan da Cunha over a period of 341 days. The average caloric intake was calculated to be 775, 1,237 and 1,670 per individual, respectively, in the 3 families. In a survey of dental conditions made during the same expedition to the island, Sognnaes (1945) observed dental caries by oral examination and radiographs in about 50 per cent of the island's 188 inhabitants. Potatoes made up 40 to 65 per cent of the caloric intake. The remainder of the diet consisted of fish, crawfish, meat, birds and very small amounts of eggs and milk. Mottling of the teeth was observed (Sognnaes 1941) and the fluorine content of the teeth was higher than that usually observed in teeth from

low fluorine regions (Sognnaes and Armstrong 1941). The local water supply, however, contained little or no fluorine. The total fluorine content of the diet may have been supplied largely by the relatively high fish consumption.

Malherbe and Ockerse (1944) studied the possible factors which might contribute to a high or a low dental caries experience. The George area of South Africa was chosen as a high-caries-incidence region for study purposes; 97 per cent of the 1,515 children examined had dental caries. The Williston area was selected as a representative low-caries-incidence district; 40 per cent of the 1,360 children examined had dental caries. Many differences were noted between the environments of these two populations. The diet in the George area was characterized by a predominance of bread, porridge and the other cereal foods, the frequent use of sweet potatoes, fresh vegetables and small amounts of milk, and the infrequent use of eggs, fish, meat and cheese. The diet of the Williston district was characterized by large amounts of meat, wholemeal bread at every meal, little milk, no butter, cheese or eggs, and small quantities of fresh fruits and vegetables. From their evaluation of the composition of these diets and the total food consumption, Malherbe and Ockerse concluded that the George diet was deficient in almost every essential nutrient and in total caloric intake. Because of the shortage of dairy products, the Williston diet was slightly deficient in vitamin A and calcium. From chemical analysis of locally grown foods, it was suggested that at George the calcium content was slightly low and the phosphorus content distinctly low. The chief differences in the water supply were fluorine (George—0.19 p.p.m., Williston—1.5 p.p.m.), total hardness as $CaCO_3$ (George—27 p.p.m., Williston—594 p.p.m.) and pH values (George—6.1, Williston—7.5). The approximate average hours of sunshine per day at George were 7.30 and at Williston 9.73. There were also considerable differences in the altitude, rainfall, topography, vegetation and soil analyses. Malherbe and Ockerse's data drew attention very clearly to the necessity for the consideration of the possible effect of each variant when any comparison is made to determine the reason for the high or low dental caries incidence observed in a population survey. The infrequency with which this precept is applied in dental investigations becomes evident in the discussions to follow.

Roos (1944) has made a very extensive survey of the incidence of tooth decay in the deciduous teeth of a group of 2,129 children in southern Sweden. In the course of this survey, he compared the incidence of tooth decay in city and country children between the ages of 3 and 6 years. For each age group, the children living in the country had a slightly but statistically insignificantly lower incidence of tooth decay than their city counterparts.

In a survey of the nutritional status at Norris Point, Newfoundland, Metcoff *et al.* (1945) observed a high incidence of multiple deficiency signs. Evidence of vitamin A, riboflavin and iron deficiencies was observed in the women and children, and of vitamin A and riboflavin deficiencies in the men. Dental caries was found in over 80 per cent of the persons examined and was very prevalent even in children 3 to 6 years of age. No exact comparison with the dental caries experience of the United States could be made since there was no dental care available to the residents of Norris Point. If ample dental assistance had been available, one would expect a reduced number of open cavities and missing teeth but not necessarily any reduction in the number of carious lesions.

A similar survey was made in Newfoundland at St. John's and several neighboring outports by Adamson *et al.* (1945). Signs of nutritional deficiency of vitamin A, riboflavin and ascorbic acid were seen very frequently. Thiamine deficiency and mild and chronic niacin deficiency were of frequent occurrence. Signs of rickets were seen

in relatively few subjects. Dental caries was severe and very widespread. Of 376 persons, 16 years of age or over, 41 per cent had lost all or nearly all their teeth. This loss of teeth, however, was not due solely to dental caries but also to the frequent occurrence of gingivitis and periodontitis and to the lack of dental aid.

Mellanby and Coumoulos (1944) reported the comparison between two large dental surveys of five-year-old children in the same or comparable schools of the London County Council in 1929 and in 1943. During the intervening period a great improvement was observed to have taken place, both in the structure of the deciduous teeth and in their resistance to tooth decay. The teeth of 19 per cent of the children were of perfect or nearly perfect structure in 1943, in comparison with only 8 per cent in 1929. In 1943, 22 per cent of the children were caries-free, in comparison with 5 per cent in 1929. At both surveys the dental diagnosis was made by oral examination without the aid of radiographs. Mellanby and Coumoulos attributed the improvement in the dentition and the reduction in the rate of tooth decay to the measures which had been taken to improve the nutritional status of these children. Inexpensive milk had been made available at the schools. Margarine had been fortified with vitamins A and D, and bread with calcium carbonate. Increased allowances of milk, cod liver oil and fruit juices had been made available to pregnant and lactating women and to young children. No discussion was presented on the possible effects of wartime reductions in carbohydrates nor on the possible effects of the use of higher extraction flours, higher vegetable consumption, etc. It will be interesting to know if this improvement will continue when wartime shortages of carbohydrate foods are relieved. In fact, in future years this public nutrition program may help to answer many questions on the relation of diet to dental caries.

During World War I, there was a suggestion of a reduced dental caries incidence among the children of Norway. Since then detailed records of the dental caries incidence have been kept. During World War II, a 50 per cent reduction in the incidence of dental caries was observed (Toverud 1945). The consumption of sugar, refined flour, candy and soft drinks was reduced considerably. A large part of the carbohydrate reduction was replaced, however, by more nutritious, although in some cases less palatable, foods. Toverud stated (in translation), "It can not be questioned that the decrease in caries percentage is caused by the particular nutrition regimen and mode of living during the war time. It is, however, too early to point out one or more factors which caused the reduction. A thorough study is necessary before we can do so. We will here only state what was the characteristic of the diet and mode of living during the 2 to 3 last years of the war." Despite the above unbiased summary, there appears to be throughout the paper a subtle inference that the author believes in the preeminent importance of the restricted carbohydrate intake in the reduction of tooth decay. The possible effect through the improvement of the nutritional value of the diet must be considered, however, even though the caloric intake was suboptimal. Even the reduced caloric intake must be considered as a possible related agent, since restriction of food consumption has been demonstrated to reduce the incidence of tooth decay in the cotton rat (Shaw 1948).

Sognnaes (1947, 1948a) has collected and analyzed available data on trends in dental caries incidence collected by investigators in 11 European countries from the beginning of World War I to the end of World War II. In these studies about 750,000 children were examined. During both world wars a definite reduction in dental caries experience was observed by all investigators. However, Sognnaes draws attention clearly to the fact that the reduction in refined foods and sugar was definitely not concurrent with the reduction in dental caries experience even with

comparable exposure of teeth to the oral environment. In the World War I period, the maximum reduction in dental caries incidence was not observed until 1922 and 1923, that is, several years after the cessation of hostilities and the partial return of refined foods and sugar to the European dietary. In the World War II period, the highest reduction in dental caries incidence yet reported was in 1945, which is the last year for which dental caries data are available. Yearly examination of dental caries experience in European children will be necessary to determine if the maximum freedom from carious lesions had been attained in 1945 and how long the reduced caries incidence will continue. The long time lag between the reduction in sugar intake and the maximum caries incidence could not be caused entirely by the oral environmental changes resulting from reduced sugar intake. On the contrary, the reduction observed in the later years of the two periods would appear to have been due to some role of the wartime diet during the development of the teeth which resulted in teeth with a lower susceptibility to tooth decay. The importance of continued clinical investigation of this type cannot be overemphasized, especially with specific attention to the attack rate of individual teeth.

Knowles (1948) reported the results of a series of surveys on the dental caries incidence of English children in wartime nurseries. The children were divided into two groups, depending on whether they were observed in "day" nurseries where they were kept during working hours or in "residential" nurseries where they lived completely. In both cases, the same dietary recommendations were made to provide adequate nourishment, with provision for supplying appropriate amounts and types of foods despite wartime conditions. The data indicated that the "residential" nursery children had less tooth decay than "day" nursery children. The fact that not all the meals of the "day" nursery children were supplied at the nursery was interpreted to indicate the attainment of a less adequate overall nutritional status for this group. Furthermore, the data seemed to indicate that the caries incidence was higher in "day" nursery children living in poor environmental circumstances than in those from well-adjusted homes. Among the children in "residential" nurseries there seemed to be a definite indication that the earlier their stay in the nursery was begun the lower was their dental caries experience at any given age.

Sognnaes (1947, 1948b) has conducted a series of experiments with three species of rodents, white rats, hamsters and mice, in which the effect of diet during tooth development upon the susceptibility to tooth decay has been studied. For these investigations, the purified ration was used which has been demonstrated by Shaw, Schweigert, McIntire, Elvehjem and Phillips (1944) to result in a high incidence of carious lesions when fed to cotton rats after weaning. When this purified ration was fed for prolonged periods to weanling white rats, hamsters and mice that had been born to mothers fed stock rations, the incidence of carious lesions was very low. However, when the mothers were changed from the stock ration to the purified ration at the time the litters were born, and then the weanling rodents maintained on the purified ration, a greatly increased susceptibility to dental caries was observed in the offspring. When the mothers were maintained on the purified ration throughout gestation and lactation and their weanlings were fed the purified ration, the susceptibility to carious lesions was still further increased. Thus the ration fed during tooth development appeared to be closely related to the susceptibility of those teeth after eruption.

Résumé: The comparative surveys of the dental caries incidence in the Masai and Kikuyu tribes dealt with two groups which lived entirely on foods which were produced locally. The meat-eating Masai had a larger and stronger physique and a lower dental

caries experience than the vegetarian Kikuyu. The poorer physiques and the high incidence of rickets among the Kikuyus were evidences of the inadequacy of their diet. However, environmental and constitutional factors other than the diet were not considered as possible factors in the production of the observed differences. It is to be noted that the dental caries incidence among the Kikuyus was still not nearly as high as among civilized races.

An increase in the dental caries incidence with increasing contact with civilization has been reported in the South African Bantus by Oranje, Noriskin and Osborn (1935), Staz (1938) and Jones (1940), in the Eskimos by Price (1936b) and in North American Indians by Price (1936b) and Arkle (1944), in the native Greenlanders by Pedersen (1938, 1939) and in the native Malayans by Laband (1941). These authors postulated that the association of civilization with increased caries experience was at least partially due to a change in the dietary habits.

The survey by Read and Knowles (1938) indicated that the 12 caries-free children consumed more fat and protein but less carbohydrates and candy than the 12 children with rampant caries. Collins, Jensen and Becks (1942) observed no difference in the adequacy of the diet of 366 students who were classified according to their dental caries experience. The survey of Collins *et al.* grouped all students examined instead of the selection of small groups which were caries-free and which had rampant caries, as Read and Knowles had done. The only difference observed by Collins *et al.* was a small increase in the amount of sugar consumed by their high caries group; the significance of this increase was not determined.

The low dental caries incidence reported by Sognnaes (1945) for the inhabitants of the island of Tristan da Cunha was of special interest because of the very low caloric intake and the high starch content of the diet. The possible effect of fluorides must be considered in this survey. Toverud (1945) seemed to imply that the low dental caries experience in the children of Norway during World War II was more likely to be due to the reduction in refined foods than to an improvement in the nutritive value of the restricted diet. However, the possibility that the nutritive value of the diet has been improved by the increase in unrefined cereals and fish at the expense of refined foods cannot be overlooked. An analysis of wartime caries reduction by Sognnaes (1947, 1948a) clearly established that the decrease in sugar consumption and decrease in dental caries incidence were not concurrent and that a lag of several years was necessary before the maximum dental caries reduction was observed. This relationship has been further studied in rodents, with the definite demonstration that the diet during tooth development has an appreciable effect on the susceptibility of those teeth to decay (Sognnaes 1947, 1948b).

During the period between 1929 and 1943, Mellanby and Coumoulos (1944) observed a substantial reduction in the dental caries experience of 5-year-old London children. These authors felt that this decrease was due to an improvement of the diet and not to a change in the oral environment, even though there must have been a reduction in the refined carbohydrate consumption of these children due to wartime shortages. In this case there seemed to be an overemphasis on nutritional factors without any recognition of the possible involvement of reduction in the refined carbohydrate intake of the children.

The high incidence of dental caries and edentulous mouths in Newfoundland (Metcoff *et al.*, 1945 and Adamson *et al.*, 1945) could not be compared directly with the dental caries incidence in a country where any dental care was available. At present the relation of the inadequate diet of Newfoundland to the high caries incidence cannot be evaluated.

One of the most significant papers reported

in this section was that of Malherbe and Ockerse (1944). The necessity for recognition of all known variants in surveys of diet and dental caries incidence must not be underestimated. This is true not only in surveys but also in experimental procedures when it is necessary to know how many variables were produced in any experimental treatment and also whether the result was achieved directly or was indirectly mediated through some influence of the treatment on interrelated systems.

These varied types of survey data indicate that there is a definite association between the diet and the dental caries incidence. On the basis of the data presented by these surveys, however, it is not possible to conclude that the association observed was due to an oral environmental effect directly related to refined carbohydrate consumption or to a nutritional, systemic effect.

NUTRITION AND DENTAL CARIES IN THE GROWING CHILD

Two major classes of theories have been formulated in the attempt to explain the cause of dental caries: the oral environment theories and the nutritional, metabolic theories.

The oral environment theories have been discussed in the section on Oral Environment and Dental Caries. As mentioned there, the first oral environment theory to be founded on experimental investigation, the chemico-parasitic theory of Miller (1890), resulted from studies which he interpreted as indicating that bacterial fermentation of carbohydrates resulted in acid formation and that the progress of the carious lesion consisted of continued decalcification of the enamel. The popular concept of this theory has involved a tremendous and deceiving oversimplification of Miller's exhaustive and detailed investigations. The most active proponents have come to consider that *Lactobacillus acidophilus* and other acid-producing microorganisms are responsible for the direct production of the caries lesion and that the control of these microorganisms in the oral cavity through the reduction of the dietary sugar and carbohydrate content is the means of controlling dental caries, with the exception that there are certain immune factors which act against the degradation of carbohydrates in the oral cavity.

Later theorists have postulated that there was a simultaneous proteolysis of the organic matrix and decalcification of the inorganic component. This theory has been elaborated by some investigators and dissociated from Miller's theory by the hypothesis that the progress of the carious lesion is fundamentally a degradation of the organic matrix through the enzymatic action of microorganisms and is followed by a physical disintegration of the inorganic complex. However, little evidence has yet been accumulated to support this hypothesis.

These theories and their supporting evidence are based chiefly on the concept that the outer regions of the enamel, where carious lesions begin, are almost completely inaccessible to internal body fluids and that the composition of saliva is not affected by metabolic changes in ways which would influence the susceptibility of the enamel to caries development. In the *Lactobacillus acidophilus* theory, the complex inorganic component of enamel has been suggested as the vulnerable point of the tooth surface, independent of the organic component, whereas in the proteolysis theory, the minute organic matrix has been postulated as the entrance of the carious process, independent of the presence or composition of the inorganic constituent. In both cases little or no ability of the body as a living organism to combat the carious process is recognized, and indeed the fate of the dental structures is suggested to be dependent almost solely upon whether the composition of the diet consumed is such that it will or will not act in the oral cavity for the development of the microorganisms that are postulated to be active in the carious process.

Several versions of the nutritional, metabolic theory arose simultaneously in widely separated laboratories as the result of numer-

ous investigations on the relation of various essential nutrients to the initiation, progress and control of dental caries. The basic premise of all versions is that both the enamel and dentin are not static structures but are vitally influenced by the internal body fluids and by saliva. The premise that enamel can be influenced by body fluids after its formation differentiates the nutritional, metabolic theories from the oral environment theories unequivocally. Thus when the retention of the various known and unknown nutrients involved in the maintenance of the hard dental structures equals or exceeds the total demand of the body for those nutrients, it is postulated that the hard dental structures would be better able to maintain an active defense against the activity of the carious process than if the retention was less than the demand.

The development of new carious lesions is not a constant throughout the human life span nor is it necessarily in direct progressive relationship to the length of time that the teeth have been exposed to the oral environment. The increase in number of carious lesions is high during the years of childhood, increasing to a maximum during adolescence, then decreasing to a relatively constant, low rate in early adult life and so continuing through the life span. No thorough survey has been made by any investigator on the annual dental caries increment throughout the life span. The best available data are to be found in two American surveys: Knutson and Klein (1938) and Klein and Palmer (1938) have determined the annual caries increment in 4,416 school children between the ages of 6 and 14, while Hollander and Dunning (1939) have analyzed the accumulated data of 12,753 subjects from 17 to over 65 years of age. These sets of data were collected in a routine manner and had the great merit of having been made irrespective of the individual desire or need for dental treatment. In the Hollander and Dunning survey the average increment of carious surfaces was about 1.75 surfaces per person up to age 34, beyond which the increment of new carious lesions was found to be about 0.5 carious surfaces per person per year of age. This definite, although gradual, change in caries susceptibility is most pronounced in the interval 25 to 35 years, far beyond the age of adolescence. The decrease is considerably more gradual than might be anticipated from the personal opinions of these investigators unsubstantiated by extensive surveys (Pickerill 1912, Bunting 1930 and Bodecker 1934). However, neither of the above surveys contains data from which can be estimated the extent to which the decrease in dental caries susceptibility is attributable to a decreased number of surfaces available to the attack of the carious process.

Nutritional and metabolic requirements likewise are not constant throughout life. Requirements for normal growth, development and well-being are very high during the first months of rapid growth in infancy, high throughout the early years of growth, increasing to a maximum during adolescence and maturation, then decreasing in adult life to a lower level which is relatively constant through the adult life of any individual, except at times of increased requirement such as reproduction and disease.

Since the trends of dental caries incidence and of nutritional requirement parallel each other to some extent throughout life, and since evidence exists that the enamel is not an entirely static structure, there appears to be sufficient foundation to permit the formulation and experimental testing of the hypothesis: the initiation, progress and control of carious lesions may have a definite relation to and may at least partially be determined by the metabolic, nutritional status of the body. An attempt has been made to present, discuss and criticize the following experimental data in such a manner as to test the validity of this hypothesis.

General Nutrition and Dental Caries Incidence

Boyd and Drain (1928) observed arrest of decay in a group of 28 diabetic children in Iowa who had been under careful dietary

supervision for 6 months or more. Twenty-three had had definitely progressive carious lesions prior to the establishment of dietary control. The standard dietary formulae advocated by Boyd (1926) and Boyd and Nelson (1926) for the control of diabetes mellitus were used. The protein-carbohydrate-fat ratio of the diets was 7:9:21 and the fatty acid-dextrose ratio 1.5:1. The main constituents of these diets were milk, cream, butter, eggs, meats, vegetables and fruits, supplemented with adequate cod liver oil. The ash of the diet was definitely basic. Boyd and Drain believed that these diabetic diets were adequate for excellent growth and well-being and that the arrest of tooth decay had been promoted by correcting nutritional deficiencies. The occurrence of salivary calculus was observed to be almost universal on the teeth of the children with arrested caries. The degree of oral hygiene varied greatly, yet unquestionable evidence of arrested caries was found even in the most poorly kept mouths.

In order to test the dental caries-arresting value of these dietary formulae in subjects where insulin therapy and diabetes were not factors, Boyd, Drain and Nelson (1929) and Drain and Boyd (1930) prescribed typical diabetes-controlling diets for 3 groups of non-diabetic children. The diagnostic criterion of arrested tooth decay was "the dentin, which on previous examination had been soft, easily probed and easily dislodged, would become hard and impervious even to the sharpest explorers. Open cavities showed no sign of activity after they had first been described." Four orthopedic patients who had extensive carious lesions were placed under careful supervision and given a diet similar to those given the diabetic patients. In every case tooth decay was arrested, and dentin changed from soft to stony hardness with no evidence of advance of the destructive process. Observation was continued for 2 of these children who were served the same type of diet as previously, but who were permitted to refuse such food as they did not want. Active carious lesions were observed in both children within a few months. These authors specified that the diets of 5 healthy pre-school children living at home include daily: 1 quart of milk, 1 teaspoon of cod liver oil, 1 ounce of butter, 1 orange, 2 or more servings of succulent vegetables and fruits. Candy was allowed after meals only. Tooth decay was believed to be arrested in all 5 cases in less than 10 weeks on this normal, adequate diet. In another experiment, special diets were designed for 4 children with celiac disease, with special emphasis on avoidance of starch and fat by supplying energy with simple sugars and proteins. The diets of these children contained the same amounts of cod liver oil, milk, orange and tomato juice, vegetables and fruits as the diabetic formulae. Two of these children were 25 and 31 months old. No carious lesions were present at the beginning of the observation period and none developed during dietary control. The other 2 children were older and had extensive carious lesions when dietary control was begun. Caries activity in both cases was believed to be arrested in less than 10 weeks. These 4 cases are especially interesting, but the smallness of the group and the short observation period must be remembered. It is noteworthy that these children received as high as 60 per cent of their calories in the form of dextrose *per se* for periods of months. The diets for the celiac patients required practically no chewing and yet these diets were described as being as effective in the control of tooth decay as those which included foods demanding thorough mastication. The ability to determine an arrest in caries activity in an observation period of 10 weeks is widely questioned by some dental investigators.

Boyd and Drain (1932) summarized the dietary control of dental caries by the statement: "Arrest of caries is dependent on sclerotic changes in the dentin and enamel, which occur normally in the healthy tooth in response to physiologic or pathologic

irritation. Sclerosis has been demonstrated histologically in sound teeth of children who have received a suitable diet continuously, and at the bases of inactive carious cavities in the teeth of children whose diet has been made complete. The degree of sclerosis seems dependent on the adequacy of the diet."

A group of 53 orphanage children of an average age of 7.3 years was studied for a period of 8 months by Drain and Boyd (1935) to determine the ability of a low cost, adequate diet to control and arrest dental caries activity. No fancy foods or knickknacks were given. The regular institutional diet was fortified with ample milk, butter and eggs. Arrest of, or stationary, caries activity was observed in 42 of the 53 children by the end of the observation period. Nineteen of the children showed a definite improvement and 23, including 16 with no caries at the beginning, had no increased caries activity. Five of the 11 children whose condition became worse during the dietary observations were used for intensive metabolic studies. It was found that these 5 children were unable to utilize a sufficient amount of the nutritional essentials—calcium, phosphorus and nitrogen—as offered by the institutional diet. When a diet was given which contained each of these dietary essentials in more easily assimilable forms, the retention by these children improved, at which time the carious process became less active or completely inactive.

A summary of the value of dietary control in the arrest of tooth decay was presented by Boyd (1940). One hundred and seventy-five diabetics, ranging in age from 2 to 20 years, had been observed for an average period of 46 months. One hundred and twenty-seven of these were observed for a minimum of 2 years, 53 for 5 years or more, 18 for more than 8 years and 8 for more than 10 years. Thirty had teeth which were free from carious lesions and fillings at the first observation and remained so throughout the respective periods of observation. One hundred and forty-five had active carious lesions when dietary control was begun, or soon developed active lesions. The progress of tooth decay was arrested in 128 within 3 to 6 months, and in 13 partial arrest was attained. Four failed to cooperate, and no arrest was observed. Re-activation of the carious process was frequently traced to increasing laxity in self-adherence to the prescribed diets. It is most interesting to note that arrest of tooth decay frequently was observed in areas which were not self-cleansing. Anderson (1942) has reported that active carious lesions became inactive when the lesion was opened operatively in such a manner that self-cleansing became possible. However, Boyd's observation that arrest did occur in areas where self-cleansing could not occur eliminated the possibility that it was the cleansing nature of the diet which was responsible for the reduced activity of tooth decay.

Boyd (1943d) presented detailed records on the incidence of tooth decay in 111 children with diabetes mellitus who had been under dietary supervision for an average of 67.5 months, with a range of 36 to 144 months. In 34 per cent of the children, dental caries activity was checked completely throughout the entire observation period, and in an additional 55 per cent there was no progression for at least 18 months. In several cases, enamel defects that normally would have developed into dentinal caries were observed to remain unchanged for long periods without treatment. These data were presented to establish the pronounced lowering of dental caries activity in comparison with an average population of children. However, these children at the beginning of the observation period had as high a dental caries experience as other children of the same age. Eighteen subjects who did not have any arrest of tooth decay had not followed the prescribed diets, mainly because of unfavorable social or economic conditions. Omission of cod liver oil was a frequent deviation. With its subsequent use further progression was not observed. It was the impression of

the observers that cod liver oil contained one of the dietary factors involved but not the only one. Even children who did not use cod liver oil but who followed the prescribed diet in other respects had a significantly reduced dental caries increment.

Boyd (1942) graphically presented the dental histories of 55 teen-age diabetic children, 26 boys and 29 girls, who had been under observation and dietary control for not less than 41 months each and who had shed all carious deciduous teeth prior to the initial examination used in this study. The low incidence of dental caries in most of the subjects and the long intervals during which no progress in the carious lesions was observed was quite evident from the graphs. It was observed that when further tooth decay occurred, the increment occurred during periods which could be correlated with violations of the prescribed regimen. Of the 55 children, Boyd (1943a) reported that 20 children had no advance of caries for 3 years or longer; an additional 9 had none for periods exceeding 2 years. The average rate of tooth decay was less than one-fifth of what Boyd predicted for the general child population in Iowa from which these children came. Prior to diet control, the dental caries experience was similar to that of the average child population. Three children failed to show arrest of the carious process, having been unable to follow the prescribed diet due to socio-economic reasons. Their dental caries increment equalled or surpassed the predicted rate. When these 3 known dietary non-conformists were omitted from consideration, Boyd (1943b) observed that the average annual increment rate for the 52 diabetic children was less than 0.42 carious lesions as compared with Klein and Palmer's prediction of 2.0 carious lesions. It was observed also that there was a significant relation between the age at onset of dietary control and the annual dental caries increment. Boyd stated: "Those children whose dietary control was established prior to the age of six years were entirely free from caries of the permanent teeth when shedding had been completed. Furthermore, the incidence of decay after shedding was only 27 per cent as great in the 17 children who started the dietary regimen prior to the age of nine years as it was in the remaining thirty-five ostensibly conforming to the dietary regimen and whose control dated from a more advanced period of childhood."

When the same 52 children were considered in respect to pit and fissure caries, and proximal caries, Boyd (1943c) observed that 75 per cent of the occlusal surfaces and 98 per cent of the proximal areas were free from any clinically significant, destructive lesion.

Boyd (1944) reported that, during his studies on the relation of diet to dental caries, one sharp change had been made in the diabetic dietary formulae. This change consisted of the replacement of the original high fat diets by diets which offered only half as much fat and twice as much carbohydrate. No other change was made, so that this diet offered a caloric equivalence for full activity and adequate quantities of protein, minerals and vitamins. The protein:carbohydrate:fat ratio of this diet was 7:15:11 instead of the ratio of 7:9:21 of the high fat diet. The fatty acid:dextrose ratio was about 1:1.5 instead of 1.5:1. The dental caries experience previously reported by Boyd (1943d), of 59 girls and 52 boys who were observed recurrently for not less than 3 years each during the routine management of diabetes, was tabulated in respect to the fat content of the diet they had received. The children which had received only the high fat diet had practically the same duration of freedom from tooth decay and annual increment of DMF tooth surfaces as those children who had received the lower fat diet only. Forty-six of the children had received the high fat diet for a period, followed by the lower fat diet. During the period on the high fat diet the caries experience was slightly lower than in the latter period on the lower fat diet. Boyd attributed

the slightly higher incidence on the lower fat diet to the lesser amount of supervision and the shorter period of hospitalization necessitated by war shortages, rather than to the reduction in fat content and increase in carbohydrate content of the diet.

Ziskin, Siegel and Loughlin (1944) studied the dental caries experience of a group of 94 diabetic children under dietary control as part of an investigation on the relation of diabetes to certain oral and systemic problems. The children were from 4 to 19 years of age and consisted of 81 white and 13 Negro children. The diet recommendations were believed to be adequate for growth and maintenance, as well as satisfactory for the control of the diabetic process. The average dental caries experience of the 81 white children was compared with that of the Hagerstown, Maryland, children surveyed by Klein, Palmer and Knutson (1938). No significant reduction was observed in the dental caries incidence among the diabetic children. These investigators believed that these data reduced the strength of the hypothesis proposed by Boyd and co-workers that dental caries in diabetic children may be controlled by nutritional means via the general metabolism.

Howe, White and Rabine (1933) of the Forsyth Dental Infirmary in Boston presented their observations of 132 outpatients to whom dietary recommendations had been made over a period of 1.6 years. The following daily diet recommendations were made to the parents: 1 quart of milk, 1 raw vegetable, with special emphasis on cabbage and tomato, at least 2 cooked vegetables, 2 servings of fruit with special emphasis on oranges, 1 egg, meat or fish 5 times a week, and butter on vegetables and bread. Cod liver oil was not given routinely but was prescribed by the pediatrician when indicated. Candy was allowed only at the end of meals. The average number of new carious lesions found in 104 "cooperative" subjects was 0.73 instead of the average number of 3.51 cavities a year prior to dietary supervision. The average number of new carious lesions found in 28 "noncooperative" children was 3.83 instead of the previous average number of 3.39 a year.

Howe, White and Elliott (1942) compared dental observations of 189 children who had been under dietary supervision with those of 225 children of the same ages who had had no special supervision. The same dietary recommendations were made to this group as those described by Howe, White and Rabine (1933). The children under dietary supervision in each group showed a decrease of from 27 to 83 per cent in incidence of new carious lesions when compared to the unsupervised children in the same age group.

McBeath (1932) observed the effect on caries increment of supplementing the diet in three orphanges in New York State and in a state institution for mentally deficient children with milk, eggs, meat, vegetables, oranges, butter and three teaspoonfuls of cod liver oil daily. A definite reduction was observed in the dental caries activity of the children whose diet was supplemented. It was felt that there was no conclusive evidence for the super-importance of any one dietary factor.

McBeath (1933) continued the above studies by reversing the control and experimental groups for a second year of observations. The same protective result was observed in the group receiving the dietary supplements. A definite increase in caries activity was observed in the control group despite their previous period of dietary protection, which demonstrated the short-lived control of caries activity by dietary means in growing children.

Livermore (1942) reported the results of a two-year experiment with four groups of Indian children in Canada. The groups received: (a) uncontrolled diet, (b) controlled diet (vegetables, fruits, eggs, orange juice, meat, whole grain cereals, low carbohydrate desserts, and pastries never oftener than twice a week), (c) uncontrolled diet plus "one vitamin and one mineral capsule per

day" and (d) controlled diet plus "one vitamin and one mineral capsule per day." No statement was made of the composition of the vitamin or mineral capsules. The number of cavities was recorded every four months and all new cavities filled at each examination. The incidence of new carious lesions was lower with the controlled diet than with the uncontrolled diet. The addition of a vitamin and a mineral capsule daily reduced the incidence of new carious lesions with the uncontrolled diet below that observed with the unsupplemented controlled diet. However, the group with the supplemented controlled diet had the lowest dental caries increment.

Sprawson (1932a) stated that in a British institution housing 750 boys, the incidence of dental caries dropped from extremely common to almost complete absence when 1 pint of raw milk was given daily in addition to the institutional diet. Forty children of an average age of 4 years who had received raw milk since before 12 months of age had no carious lesions. At the time of admission to the home, they had rickets and enteritis. Fifty-eight other children in poor physical condition had been admitted to the home at a later age but still under 6 years. However, after several years in the home on the same dietary regimen as the other boys, it was stated that no tooth which had erupted since the beginning of milk administration had a carious lesion.

Sprawson (1938) described the results of an experiment to study the relative effect of supplementing an institutional diet with either 1 pint of raw milk or 1 pint of pasteurized milk per day for over 4 years. When a supplement of 1 pint of raw milk was given daily to 28 children from an average age of 4 years and 8 months to an average age of 8 years and 10 months, carious lesions were observed in only the 2 maxillary first permanent molars of one boy. When a supplement of 1 pint of pasteurized milk was given daily to 39 children from an average age of 4 years and 4 months to an average age of 9 years and 1 month, 25 carious permanent teeth were observed in 12 children. No control group was described, so that the incidence of carious lesions among a comparable group receiving the institutional diet only was not available for comparison. Sprawson recommended that 1 pint of raw milk be given daily to all children, beginning at as early an age as possible. He also stated that the value of milk was partially lost in the pasteurization process.

Roberts, Englebrecht, Blair, Williams and Scott (1938) divided 90 children who showed extremely progressive carious lesions into 3 groups. The first group received only the institutional diet, the second group received, in addition, a pint of reconstituted plain evaporated milk for each child daily, while the third group received a pint of reconstituted irradiated evaporated milk (70 units of vitamin D). The percentage increase in newly carious teeth was 28.7 for the children in the control group, 26.2 for those in the plain milk group and 21.5 for the irradiated milk group. The corresponding percentage increase in number of cavities was 54.6, 46.0 and 50.7, respectively. These differences were not significant but possibly indicated that there might be a slight inhibition of the rate of dental decay by the addition of milk to a diet.

Bunting, Delves and Hard (1929–30) observed the incidence of new carious lesions during 1 year in the children of 2 Michigan orphanages where a "well-fortified" diet was prescribed, with no sugar except where absolutely necessary. In 1 orphanage, 66 per cent of the 159 children had no active carious lesions, 9 per cent had 1 to 3 small cavities per child, and 25 per cent had minor defects which represented small or questionable carious lesions. Of the 107 children who had no active dental caries during the experimental year, 61 had had active carious lesions at the beginning whose development was arrested. A similar low caries increment was observed in the second orphanage. In 104 school children who were not under

dietary supervision but who were given hexylresorcinol as a mouth wash daily, 65 per cent had active and extensive carious lesions at the end of 9 months. Bunting *et al.* believed that a well fortified, low sugar diet was responsible for the lower dental caries incidence among these orphanage children. No data were presented to indicate the dental caries incidence of the same children before or after the experimental year.

Bunting, Hadley, Jay and Hard (1930) and Bunting, Jay and Hard (1931) reported the results of an experiment with a large number of children in which the effects of an adequate diet, hexylresorcinol mouth wash, and an adequate diet and hexylresorcinol mouth wash simultaneously were compared in three groups of children. Seventy-five to 80 per cent of those children who received an adequate diet, with or without the hexylresorcinol mouth wash, did not develop any active carious lesions. Only 18 per cent of the children receiving the uncontrolled diet plus the hexylresorcinol mouth wash had no active tooth decay.

Hubbell and Bunting (1932) observed an arrest of tooth decay in 58 per cent of 25 children whose home diet was supplemented by a quart of milk, 2 ounces of tomato juice and 8 drops of viosterol in oil daily. In a second group which served as a control, no new carious lesions were observed in 48 per cent of 30 children. The significance of this difference in such small groups was not tested but certainly would be questionable.

Bunting (1934a, b) and Koehne and Bunting (1934b) reported the dental caries incidence among 169 orphanage children who had been maintained on a diet which was stated to be inadequate in calories, calcium, phosphorus and vitamins C and D and contained no milk or butter. Seventy-five to 80 per cent of these children had had no active dental caries over periods of 1 to 4½ years, which represents a much lower dental caries incidence than in the average group of children of similar age. The authors believed that there were 3 possible factors responsible for the low incidence of tooth decay: low sugar, uniformity of diet, and hard fibrous fruit after meals. To test the effect of sugar in this deficient diet, 51 of these children were given approximately 3 pounds of candy a week for 5 months. At the end of this period 44 per cent showed evidence of active dental caries. Three months after discontinuance of the sugar feeding, however, there was believed to be no further extension of the carious lesions in any of these cases. Bunting stated that the 169 children from whom these 51 had been chosen for this experiment were the more resistant ones among 300 children. Later Jay, Hadley, Bunting and Koehne (1936) stated that despite all reasonable precautions, an effective bootlegging system soon became established to provide the control children with an amount of candy which could not be estimated. The value of these data must be questioned in view of the unknown amount of sugar in the control group. These studies are excellent examples of uncontrolled experimentation where the results were interpreted in terms of the effects of the added sucrose *per se* and with no consideration of possible changes which resulted indirectly from the addition of the sucrose.

Bunting (1934a, b) and Koehne, Bunting and Morrell (1934) studied the effect of sugar ingestion on the dental caries incidence in 23 metabolically normal girls, 6 to 13 years old, in the University Hospital at Ann Arbor over a period of 5 to 18 months. These girls received a basal diet which was believed to be adequate nutritionally, although it must be noted that they received no cod liver oil or sunlamp radiation even though hospitalized. Some received 100 gms. of sucrose as candy in addition to the basal diet. Of the 14 who received the basal diet only, 9 had arrested tooth decay while 5 had very few carious lesions. Of the 13 who received the basal diet and 100 gms. of sucrose, 9 had extensive active carious lesions; 4 had no active decay. Since these

workers believed that the subjects ate the usual portions of the hospital diet in addition to the candy, they stated that no reduction in the total calcium, phosphorus or nitrogen retention could be detected in the experimental subjects who received the sucrose supplement. As nearly as can be determined from the published data, the children with no dental caries activity had an average calcium retention of 5.3 mg. for each kilogram, and those with activity, 3.1 mg. The calcium retention of both groups was poor, presumably because of a lack of a vitamin D supplement. This experiment cannot be taken as an example of the effect of sugar when the diet is complete.

Waugh and Waugh (1940) made a superficial study of the effects of natural and refined sugars upon the dental caries experience in a few primitive Eskimos. The natural sugar sources studied were raisins, dried figs, dates, maple syrup or honey, while the refined sugar sources were figs preserved in 65 per cent cane sugar solution, sticky chewy chocolate bars, lollipops and sugar cubes. It was concluded from an average observation period of 5½ weeks that carious lesions were initiated or increased by refined sugars but not by the use of foods containing natural sugars. In view of the small number of subjects, and more especially of the extremely short observation period, these data certainly cannot be accepted as proof of a difference between natural and refined sugars.

Whyte (1943) determined the dental caries increment of 50 boys who were under constant institutional supervision at an isolated orphanage in Great Britain during the war. These boys were given 100 gms. of "macaroon bars" or "fudge tablets" each day for 62 days, followed by alternate 62-day periods of withholding and feeding the carbohydrate additions. Whyte observed a very small but consistently greater dental caries increment during the periods of carbohydrate feeding. The diet which these boys were fed was scarcely optimal in several respects: only 1 pint of milk a day, few fresh vegetables or fruits, and little meat. The effect of the candy bars on decreasing the ingestion of the basal diet is not clear. The significance of data based on the clinical evaluation of dental caries activity with such short experimental periods must be questioned.

Becks, Jensen and Millarr (1944) clinically observed an extensive arrest of caries when refined carbohydrates were largely eliminated from the diet by replacement with meat, eggs, milk, milk products and vegetables. These observations were made in California for a year or more on 1,250 individuals who had rampant caries and 265 who were completely free of caries. The authors drew attention to the fact that occasional individuals were observed who consumed large amounts of sugars without developing dental caries, while others who consumed negligible amounts of sugars developed rampant caries. It is of interest to note that most investigators place the emphasis in this study upon the reduction in refined carbohydrates rather than on the increase in fats, proteins and other essential nutrients which simultaneously accompanied the reduction in carbohydrates.

King (1946) has reported the results of a 2-year experiment where small groups of young children in Great Britain were given known amounts of boiled sweets or a chocolate biscuit each evening after cleansing the teeth, in addition to the usual 336 gm. wartime allotment per child per month. Either one or two sweets weighing 6.4 gm. each or one chocolate biscuit weighing 8.6 gm. were given daily throughout the 2-year experimental period. No increase in dental caries activity was observed in the control group of children during the 6-month period after which they were examined, nor in the two experimental groups during the 2-year period in which they were examined each 6 months. The basal diet of the institutions was stated to be high in vitamins A and D and fairly adequate in calcium and phosphorus. The

carbohydrate content of the diet was relatively high. King considered that these data were suggestive that small increases in the sugar content of the diet were not necessarily accompanied by an increase in the dental caries activity of young children. It is unfortunate that the control group was observed for such a short period.

Mack, Shevock and Tomasetti (1947a, b, c) studied the relative value of meat and of legumes in the feeding of growing children for a 14-month period in two orphanages in central Pennsylvania. Before the dietary plan was begun, dietitians assigned to the two orphanages evaluated the diet being served within each institution by weighing all raw foods prepared for a period of 2 weeks and measuring the portion served to each child at each meal and the amounts not eaten by each child. The Recommended Dietary Allowances of the Food and Nutrition Board of the National Research Council (1945) were used as the standard for energy and for all nutrients except phosphorus. The recommendations of Stiebeling and Phipard (1939) were used for evaluation of the phosphorus consumption at the two homes. The recommendation of Munsell (1940) for vitamin A was also considered, since much of the vitamin A, especially in institution I, was supplied from provitamin A sources. The caloric values of the diets in both institutions were lower than those recommended. The phosphorus consumption in institution I was slightly above the recommended level, whereas that in institution II was slightly below. The iron consumption met the recommendations for the younger age group but was slightly below the recommended level for the older children. Vitamin A intake in institution I was high enough by the National Research Council standard but fell short of the Munsell recommendation. The vitamin A intake at institution II was inadequate by both standards. Both institutional diets exceeded the thiamine recommendations. The diet of institution I slightly exceeded the riboflavin recommendation while that of institution II was high enough only for the younger age group. The younger age groups received sufficient niacin to satisfy the recommended allowances but the older age group did not. Ascorbic acid intake approached the recommendations on a calculation basis but, since most sources of this nutrient were excessively cooked fruits and vegetables in both institutions, losses probably were appreciable. No vitamin D supplement was given during the summer and only small amounts during the winter.

A master plan was designed for the experimental period at both institutions to coordinate the dietary programs as closely as possible except for the source of protein. The calculated provisions of the master plan exceeded the recommended allowances of the Food and Nutrition Board in all respects in order that the initially undernourished children would have an excellent source of all nutrients, with the only variable being the source of protein. In application, the amounts of the foods supplied under the master plan which were actually consumed by the children did not fully meet expectations. For example, the children in the younger age group in both institutions initially received less than the recommended caloric intake. When ample food was provided to supply the recommended energy value, several months elapsed before the children advanced their caloric intake to approach the recommended allowance. The older groups made considerable advance but did not reach the recommended caloric intake during tbe study. An average for the overall caloric intake indicated that the older children in institution I met 94 per cent of the caloric allowance and those in institution II met only 78 per cent through the entire period. Vitamin A intake exceeded recommendations of the Food and Nutrition Board but failed to reach the Munsell recommendation. In other respects the diets as consumed exceeded the recommended dietary allowances.

The protein allowances were supplied to

the children in institution II primarily through the use of meat, poultry or fish ten times weekly with the following distribution: meat and liver in the ratio of nine to one to the extent of 675 gm. for the younger and 900 gm. for the older age groups, poultry or fish to the extent of 75 and 100 gm. for the two respective groups of children. The diets in institution I provided main dishes of legumes supplemented by macaroni, peanuts and peanut butter at eight meals per week and meat twice per week. By supplying more green leafy vegetables in the diets of the latter institution, it was possible to plan menus for both institutions in which the caloric values as well as the nutrient content of both dietaries were similar.

Extensive medical observations, laboratory tests and dental examinations were made at the beginning, at the end of 8 months and at the end of the 14-month experimental period. In physical well-being, the children in both institutions showed marked improvement in most respects. The improvement in the children in institution I failed to surpass the improvement of the children in institution II in any respect, whereas those in institution II were superior to institution I in the following respects: epithelial cell condition; general condition of the skin, reversal atrophy of the filiform and fungiform papillae of the tongue; reduction of the enlargement of the cervical and isthmus of the thyroid glands; the skeletal mineralization process; weight status; hemoglobin; red blood cell count; blood plasma vitamin A; absence of fatigue; dark adaption; and in several other respects.

The dental caries evaluation was based on the number of decayed, missing and filled permanent teeth divided by the number of erupted permanent teeth (DMF/n). The average DMF/n values for the 61 children who remained in institution I for the entire period were 0.272 at the beginning and 0.230 at the end, and for the 41 children who were in institution II for the 14-month period were 0.224 at the beginning and 0.182 at the end. The decrease in the DMF/n value at the 2 institutions during the period was attributed to the eruption of permanent teeth which did not become carious and the lack of new carious lesions in permanent teeth which were not carious at the beginning of the experiment. A valuable addition to these data would be the actual average number of new carious lesions developed during the experimental period. From the data presented, it appears that there had been a low incidence of new lesions during the experimental period and that this was not related to the type of protein used.

In the course of making medical and nutrition observations and tests on three groups of orphanage children at the beginning of a series of longitudinal studies, Mack and Urbach (1947) at the Pennsylvania State College noted marked differences in the dietary regimens and in the physical status of the children, which prompted a more detailed examination of their comparative dietary records, and medical, dental and laboratory observations. The dietary intake of the children in institution I met or exceeded all dietary recommendations except vitamin A (77 per cent of the Munsell recommendations, 1940) and ascorbic acid (56 per cent of the Food and Nutrition Board's recommended dietary allowance, 1945). The diet for the children in institution II supplied 78 per cent of the caloric allowance recommended by the Food and Nutrition Board but met or closely approached all other recommendations except the Munsell allowance for vitamin A. The diet for the children in institution III provided 75 per cent of the caloric recommendations, failed to meet the amounts of calcium, iron and vitamin A recommended and was somewhat low in ascorbic acid from raw foods. In order to determine these dietary records, the weights of all foods were recorded, and the aliquot individual portions received by each of the children of the four age groups (5 to 6, 7 to 9, 10 to 12 and 13

to 15) were weighed or measured by dietitians associated with the study during the late summer for a 1-week period at institutions I and II, and for 2 weeks for the children in each part of institution III. The longer period for the last group was dictated by the less uniform type of menu received. Food purchase records and menus for a period of 1 year prior to this investigation were studied by the assisting dietitians in order to ascertain whether or not the records secured by weight and measure during the test periods were representative. It was concluded that the records were satisfactory except that the diets of all three institutions were probably lower in carotene and B vitamins from vegetable sources during the winter. The diets of institutions I and III probably were lower in ascorbic acid during other parts of the year because of the low citrus fruit purchases and the heavy reliance upon institutional canned and frozen foods during the non-growing season. The children in institution II consumed larger quantities of ascorbic acid throughout the year than was the case with the other two groups.

The children in institutions I, II and III received, respectively, 6.3, 5.7 and 4.2 quarts of milk per week. The egg consumption in institutions I and II averaged 5.5 per week with about half this amount for institution III. In legume, leafy, green and yellow vegetable consumption, the first two institutions were similar, with the children of institution III receiving about two-thirds as much on an age and sex basis. The diet of institution I provided more potatoes, sugar, cereals, bread, fats and proteins than the diet of either of the others. The caloric value of this ration was about 116 per cent of that in the recommended dietary allowance. The diet in institution II supplied more sugars and fats and about the same amount of cereals and bread as the diet in institution III.

An interesting observation was made on the sugar content of these diets. The baseline used for comparison of the sugar intakes was that value recommended in a moderate-cost dietary by the United States Department of Agriculture. The consumption of sugar in each institution was calculated with correction for the different ages and sexes of the children. The sugar intake in institution I was higher than that of the average population and 214 per cent of the value recommended by the USDA. The sugar intake of children in institution II was 153 per cent and that in institution III was 60 per cent of the same recommendation. The total carbohydrate consumption was lower in institution II than in I and lower in institution III than in II.

The 263 boys in institution I ranged in age from 7 to 15 years. The 100 boys and 78 girls in institution II and the 83 boys and 61 girls in the two parts of institution III were 5 to 15 years of age. The children in institution I were superior to those of the other two groups in physical status as shown by: skeletal maturity, height and weight for their sex and age, hemoglobin, red cell count, hematocrit, blood serum albumin levels, urinary thiamine excretion, heart and reflex functions and the absence of the appearance of fatigue. The children in institution II were superior to those children in institution I. The children in institution III were poorer in physical status in almost every respect tested than the children in institutions I and II.

The children in all three groups had routine dental care; this was provided in institution I through an attached dental department and in the other two by dentists in private practice. The dental examinations made in this study consisted of clinical charting and full mouth radiographs. The dental caries experience of the children was expressed on the basis of the total number of the decayed, missing and filled permanent teeth divided by the number of permanent teeth which have erupted (DMF/n). The average DMF/n values for permanent teeth for the children in the three institutions were 0.178, 0.242 and 0.341, respectively.

The percentage of older children was higher in institution I than in institutions II and III, which would tend to increase the dental caries experience in the former group. However, in spite of the age difference, the children with the higher average age group had the least dental decay.

An interesting similarity was observed between the dental caries incidence (DMF/n) of the children in institution III and that of 1900 Pennsylvania children surveyed by Ackerman (1943). The latter survey was made in children of the same general age groups and showed a DMF/n of 0.349 in contrast to the value of 0.341 for the children of institution III.

In 1945 the Italian Medical Nutrition Mission was sent from the United States to assess the effects of malnutrition on the Italian people. As a part of this survey, one team of workers studied the dental caries experience and the incidence of gingivitis in samples of the populace in four cities (Naples, Varese, Cagliari and Catanzaro). In all, 3,905 persons were examined. Those studied in Naples and Varese were from a low economic level and were unclean, underfed and suffering from various undiagnosed chronic ailments. Those persons examined in Cagliari and Catanzaro were better fed and represented a higher economic level.

The rates of tooth decay in these groups have been reported by Schour and Massler (1947). All examinations were made by means of mouth mirror and explorer in good natural light. The caries experience as obtained by counting the number of DMF teeth per person in the four Italian cities increased from 1.05 in the 11- to 15-year-old group to 10.80 in the 51- to 60-year-old group. These figures are low when compared with similar age groups in the United States, where the average number of DMF teeth per person for the 11- to 15-year-old and 51- to 60-year-old groups was 4.66 and 23.20, respectively. Likewise, there were considerably more individuals with no carious teeth in the Italian cities than in similar age groups in the United States. Attention should be drawn to the fact that these were accumulated lifetime manifestations of dental caries and not the increment in carious lesions during war years. The reasons for the lower prevalence of caries in Italy as compared with the United States were not clear. The people studied were relatively malnourished and oral hygiene was not commonly practiced.

Massler and Schour (1947) made an intensive dental study of 162 children in Naples, Italy, aged 10 to 18 years, who had suffered a definite retardation in growth during the war years. Thirty-five per cent of these children had no evidence of tooth decay. The average number of decayed, missing and filled permanent teeth per child was 1.67 of the 65 per cent with dental decay; 83.8 per cent showed only the initial stages of caries; 69 per cent of the first permanent molars were caries-free. This was an appreciably lower incidence and extent of tooth decay than has been reported for children of the same age range in Hagerstown, Maryland, by Knutson, Klein and Palmer (1938) where 91.5 per cent of the children had some degree of tooth decay, with an average number of 5.5 DMF permanent teeth per person. Since no data for similar age groups were presented for the period before the war or in the early years of the war, it is unknown whether the above dental caries attack rate for Neapolitan children was the same, greater than or less than the rate for children of the same racial and environmental backgrounds in earlier periods. These authors suggested that the low refined sugar intake in Italy and the high refined sugar consumption in the United States might contribute to the difference in caries attack rate and that the frequent vitamin B deficiency among the Italian children needed to be considered as a possible contributory cause.

The problem confronted in this difference is of fundamental importance in assessing the extent to which heredity, environment

and dietary habits are responsible for tooth decay. Clapp (1928) reported a low incidence of tooth decay in young adult Italians born in Italy and living in the United States who had maintained Italian dietary habits after moving to the United States. In contrast, Day and Sedwick (1935) observed an incidence of carious lesions in 500 children of Italian descent, whose diet was presumably Americanized, which was entirely comparable to the caries incidence of American children in general. These data indicate that the dietary habits are of greater significance than the hereditary influence, although the latter may be of considerable significance when the dietary habits are borderline in caries-producing properties. Some information of the fluorine content of drinking waters from the various districts studied would be valuable.

Toverud (1949) has reported the results of a series of surveys to determine the effect of general health supervision on the frequency of dental caries in groups of Norwegian children. Two communities were included in these studies: the first a rural community, Skedsmo, where the survey began in 1936; and the second, one-fourth of the city of Oslo, where observations were begun in 1939. Health supervision began in the prenatal period and was also undertaken with children who were as much as 7 years of age. An important phase of the program was the instruction of the mothers in the right choice of foodstuffs and in methods for the suitable preparation of food for themselves and their offspring. The diets recommended were such as would supply nutritional requirements during the various periods of growth and reproduction. A special effort was made with respect to calcium, iron, protein, vitamins A and D and the B complex. Where it was deemed necessary, extra vitamin C was recommended. Vitamin K was given to all pregnant women during the last 3 weeks before the expected date of delivery. Thiamine excretion was checked in pregnant women and synthetic thiamine was given where indicated. Whole wheat cereals were offered to the children, beginning in the sixth month of life. Vegetables in both raw and cooked forms were emphasized. The recommended diets in every case were low in sugar, sweets, cookies and pastries.

The overall health supervision program for pregnant women appeared to be beneficial, as demonstrated by the comparison of the stillbirth rate and the infant mortality rate observed among outpatients at the health station in Oslo with the same rates in the city of Oslo as a whole. For the years 1939 to 1946 in all the city of Oslo, 29,191 babies were born, of which 688 (23.2 per 1,000 births) were stillborn; of the 28,503 live infants, 768 (26.9 per 1,000 live births) died during the first year. In contrast, the infants of women instructed at the health station in Oslo totaled 1,553 in the years 1939 to 1947, of which 22 (14.2 per 1,000) were stillborn; of the 1,531 live births 18 (11.8 per 1,000) died during the first year of life. These data appear to be the more significant because such a difference could be demonstrated despite wartime regimentation and restriction.

The dental examinations of the children were grouped by years of age into 5 categories: 2½ to 3, 3 to 4, 4 to 5, 5 to 6 and 6 to 7 years. The dental caries incidences of the individual age groups were compared from year to year. In other words, the investigator compared the dental caries experience of a given age group of children with that of children of the same age in any preceding or subsequent year for which data were available. In the community of Skedsmo, 78.3 per cent of 23 children, 2½ to 3 years of age, in the years 1936 to 1937 had tooth decay; 27.1 per cent of their teeth were carious. However, in 1940 only 44.2 per cent of 43 children of the same age had dental caries and only 13.1 per cent of their teeth were carious. In children of this age group, the elapsed period of 4 years of health education was sufficient to cover the

entire period of tooth development, i.e., throughout pregnancy and postnatal life. Of 88 children 3 to 4 years of age, in the years 1936 and 1937, 92.0 per cent had some degree of tooth decay; 45.5 per cent of their teeth were carious. In 1940, 83.8 per cent of 37 children had tooth decay and 36.1 per cent of their teeth were diseased. In the 3 still older groups of children, there were no significant decreases in the dental caries experiences. Obviously, the older children did not come under the general health supervision program until after the teeth were largely or completely developed. Any alteration in the diet after this time would be effective in producing variations in the maturation, maintenance and oral environment of the teeth and not in influencing the major phases of development and calcification.

For the children in Oslo, the data were presented in somewhat different style; instead of comparison of age groups on a yearly basis, the data were only classified in accordance with whether the children registered with the health station before 1 year of age or after 1 year of age. In each of the 4 age groups between 2½ to 6 years, there was significantly less tooth decay among the children admitted for health supervision at the earlier age. For the 6- and 7-year olds, there was a slight but statistically insignificant lower degree of tooth decay among these children admitted at less than 1 year of age. Unfortunately, no data were available for children in the same district in Oslo prior to the beginning of the health station nor for those in the same district who were not under health supervision.

Just as this survey was reaching a stage where valuable data were becoming available, the war and German occupation drastically altered the Norwegian dietary. The full significance of these studies, in so far as permanent teeth are concerned, needed a much more prolonged continuation of the observation period. These projects had been under way barely long enough to give a picture of what health supervision might do to reduce the susceptibility to tooth decay in the deciduous teeth, and not nearly long enough to give any suggestion of the effect upon the permanent teeth, when war began with drastically altered dietary patterns.

Toverud suggested that the reduction in tooth decay could have been due to all or any combination of three possibilities: "(1) A supervision of the general health of the mother and child. (2) Regulation of the diet for the mother and child so as to meet the requirements for growth and maintenance. (3) Regulation of the diet and habit of living so as to reduce the local caries factors as much as possible." In the discussion of the pros and cons for each of the three premises, he emphasized his viewpoint on the value of excluding sugar and sweets from the diet in the following statements: "A more natural explanation of the cause of the decreased difference is given through the third factor" and "Besides acting as a local factor, uncontrolled indulgence of sugar and sugar products may also alter—reduce—a good resistance laid down during the pre-eruptive period."

Résumé: The investigations of Boyd and his group, Howe, White and Rabine (1933), Howe, White and Elliott (1942) and McBeath (1932, 1933) definitely indicated that the dental caries increment in the growing child could be reduced by dietary measures. The data of Mack, Shevock and Tomasetti (1947a, b, c) indicated reduction in DMF/n values when the diets of children in two homes were made adequate nutritionally. The standard formula advocated for the control of diabetes mellitus by Boyd (1926) and Boyd and Nelson (1926) was nutritionally complete in all known essentials. It contained little carbohydrate and a high amount of fat. Later Boyd (1944) reported use of a diet for the control of diabetes mellitus which was as adequate nutritionally but which contained one-half as much fat and twice as much carbohydrate. A compa-

rable reduction in the dental caries activity was observed when either diet was given. Boyd believed that this was an indication that the reduction in caries activity was due to the nutritional adequacy of these diets and not to an alteration of the oral environment. The fat content of the second diet still was much higher than that of the diet consumed in the United States.

The effect of supplementing the ordinary diet in an institution by milk, butter and eggs was observed by Drain and Boyd (1935). The production of an adequate diet by these supplements without the alteration of the basic institutional diet resulted in a substantial decrease in the dental caries increment. McBeath (1932, 1933) observed similar effects when the diets of four institutions for children were supplemented with milk, eggs, meat, vegetables, oranges, butter and three teaspoonfuls of cod liver oil daily. Boyd, Drain and Nelson (1929), Drain and Boyd (1930), Howe, White and Rabine (1933), Howe, White and Elliott (1942) and Livermore (1942) recommended that the daily diet of certain children who were out-patients contain adequate amounts of milk, cheese, meat, vegetables, fruits and butter. Those children who were cooperative had a substantial decrease in the caries incidence during the period of dietary control and observation.

Boyd, Drain and Nelson (1929) and Drain and Boyd (1930) prescribed a diet which contained 60 per cent of its caloric value as glucose for four patients with celiac disease and observed a reduction in dental caries activity. This was the only known record in which the caries activity was studied with an adequate diet having a high caloric content of soluble sugars without any starches. The number of cases described was very limited and the observation time was short. One of the most worthwhile studies that could be made at this time would be a more extensive, thorough investigation of the dental caries activity in the subjects receiving a nutritionally adequate diet of this type. An investigation on the effect of supplying part of the caloric intake in this diet as starch or cereals would be of great value to determine if the rate of clearance of the soluble sugars from the mouth would be retarded by the presence of the insoluble starch or cereals.

The statements of Sprawson (1932a, 1938) on the superior value of raw milk to pasteurized milk in the reduction of the dental caries incidence did not appear to be based on well controlled experimental evidence. The small reductions in the percentage increase of newly carious teeth and of new cavities reported by Roberts *et al.* (1938) when children were given one pint of reconstituted plain evaporated or one pint of reconstituted irradiated milk daily were not significant but possibly indicated a slight inhibition of tooth decay.

Bunting, Delves and Hard (1929–30), Bunting, Hadley, Jay and Hard (1930), and Bunting, Jay and Hard (1931) interpreted their data to indicate the value of a well fortified, low sugar diet in the reduction of dental caries susceptibility. The regular use of hexylresorcinol as a mouth wash resulted in no apparent reduction in the dental caries experience.

Bunting (1934a, b), Koehne and Bunting (1934b) and Koehne, Bunting and Morrell (1934) observed an increase in the susceptibility to dental caries when large amounts of sucrose were added to the diet as candy or table sugar. In the case of 51 orphanage children who received approximately 3 pounds of candy per week, several questions must be raised regarding the mechanism whereby the increase in dental caries activity occurred. The immediate explanation that might be proposed would be that the sucrose had changed the oral environment. However, this need not be the complete or the correct explanation. This addition of candy would approximate 190 grams per child per day and would be equivalent to an intake of 760 calories, if we assume an energy value for the candy of 4 calories per gram. How-

ever, there would be no accompanying increase in any known essential nutrient. This source of energy would be slightly greater than one-third of the recommended dietary allowance (Food and Nutrition Board, 1945) for a child 7 to 9 years of age. The effect of this great increase in energy consumption on the appetite and the quantity of the institutional diet eaten must be considered. The diet was admittedly inadequate in calcium, phosphorus and vitamins C and D and contained no milk or butter. Thus if the regular diet consumption decreased, as would be expected, because of the caloric increase, the overall nutritional adequacy would be reduced further. Whether the caloric change would affect retention of any essential nutrient would be open to investigation. It could not be concluded that the candy additions had increased the dental caries activity solely through an oral environmental effect until these questions were satisfied. A very valuable experiment could have been performed at this same orphanage if both a group receiving the institutional diet only and one receiving the institutional diet plus the candy supplements had been given milk, meat, butter, eggs, fruits and vegetables in sufficient quantity to make the diets nutritionally adequate. The comparison of the dental caries activity of these groups with those receiving the institutional diet alone and the institutional diet plus candy would have indicated whether the candy addition had altered the oral environment or the nutritional status. In addition, such an experiment would have indicated whether or not the inclusion of sugar in an adequate diet had had a greater influence on the dental caries activity than the inclusion of the same amount in an inadequate diet. Such an experiment is under way as a continuation of the studies of Mack and Urbach (1947). In institution I of the survey, the amount of sugar, which was already high, has been increased appreciably in an effort to test whether there is any effect on the low caries susceptibility of the children in this institution.

An attempt to answer some of these questions was made by Bunting (1934a, b) and Koehne, Bunting and Morrell (1934) in a preliminary study on 23 metabolically normal girls in the University Hospital at Ann Arbor. In this case a more adequate diet was used, the sucrose addition was only 100 grams a day and the same portions of food were assumed to have been consumed before and during the sucrose additions. In the group of children who received the sucrose supplement, there appeared to be an increase in the caries activity as compared with the group of children who received only the basic diet. The adequacy of at least vitamin D in the diet would be questioned, as neither cod liver oil nor irradiation was given. Since some of these children were hospitalized for as long as 18 months, it would seem advisable that they receive some vitamin D supplement during a period of growth as rapid as occurs in the 6- to 13-year range.

Drain and Boyd (1935) in the study of the ability of a low-cost, adequate diet to control the dental caries activity, observed 11 children out of 53 whose caries activity increased during the experimental period. When 5 of these children were used for metabolic studies, it was found that they were unable to utilize sufficient calcium, phosphorus or nitrogen from the institutional diet. However, when food was supplied more abundantly, retention improved and the dental caries activity was reduced.

These experimental data indicate very clearly that dietary control of dental caries activity is possible to a considerable degree. Part of the dietary control observed appears to have been due to an improvement of the nutritional adequacy of the diet. However, the exact extent to which the reduction in dental caries activity is due to systemic or oral environmental change cannot be evaluated at present. Nor is it known to what extent systemic changes may influence the

oral environment. In all experiments to study the effect of oral environmental changes, if the data are to be interpreted entirely as influenced by changes in oral environment, the nutritional adequacy of the diet must be guaranteed with ample allowances for individual variation in metabolic efficiency. Vice versa, in all experiments to study the effect of nutritional deficiencies, the oral environment must be maintained as constant as individual variation will permit. Many investigators have paid little attention to these self-evident principles. An evaluation of the experimental and survey data on the particular nutritional variations to be discussed in the following sections has been attempted with the above considerations in view. Since optimum nutrition should be the goal for the control groups of children, special attention is paid in these evaluations to the control rations. The reviewer feels that optimum nutrition of the individual is of at least as great importance as reducing the susceptibility to dental caries. Wherever the diets of the control children are obviously inadequate, or where the experimental treatment has rendered the diet more inadequate nutritionally in other ways than through the variant under investigation, this inadequacy is recorded and is of significant importance regardless of the low dental caries susceptibility which may have been achieved through the use of these rations.

Tooth Structure and Dental Caries Incidence

May Mellanby (1918) indicated her belief in an association of tooth structure and position with dental caries in the statement: "If the enamel on all parts of the crowns of teeth is abundant and sound, and if the teeth are adequately spaced, then such teeth are less likely to be attacked by caries and other diseases."

After a detailed examination of ground sections of 302 deciduous teeth from English children, Mellanby (1923a) observed that 84.5 per cent were hypoplastic and 83.8 per cent were carious. The superficial examination of these teeth had indicated gross hypoplasia in only 3 per cent. The microscopic enamel defects were reported as: "Enamel is more pigmented than is the case in well formed teeth, the pigment being sometimes chiefly confined to the striae of Retzius; at other times it is spread more diffusely. The groups of fibrous bundles are more numerous and the cross striations of the prisms are more distinct." The defects of the dentin were "interglobular spaces in varying numbers and sizes." These changes, as observed in ground sections, have become known in the literature as Mellanby hypoplasia in contrast to gross hypoplasia. Mellanby observed that the extent of disease varies greatly, but in general for any given age the worst formed teeth were the most carious. Of the 255 teeth which were hypoplastic, as determined by microscopic observation, 242 were carious; of the 47 teeth which were perfectly formed, 36 were free from disease. Thus 278 of the 302 teeth (92.2 per cent) were in harmony with an association between tooth structure and caries.

A study of the secondary dentin in these 302 human deciduous teeth was reported by Mellanby (1923b). There was a wide variation in the quality of secondary dentin, but this appeared to have no relation to the structure of the enamel and primary dentin. The data were interpreted by Mellanby to indicate that there was a higher dental caries incidence in the teeth with poorly formed secondary dentin than in those where it was well formed. Among the deciduous teeth examined, those which had resisted caries despite defective structure of the enamel and dentin, in general, showed well formed secondary dentin. However, those teeth which were carious but had normal enamel and dentin usually had badly formed secondary dentin. Mellanby believed that these facts indicated the importance of the production of normal secondary dentin as a second means of reducing the dental caries

incidence through normal structure. The question should be raised as to whether the poorly formed secondary dentin in these carious teeth is partially a result of the carious processes. Is it possible that secondary dentin formed less perfectly in advance of a rapidly progressing carious lesion?

Mellanby (1927a, b, 1930) further differentiated between "gross hypoplasia" and the "hypoplasia" revealed by microscopic examination of ground sections. In a survey of 349 deciduous teeth, she was able to correlate microscopic hypoplasia to the extent of 85 per cent with the roughness of the enamel as determined by a blunt probe passed over the buccal and lingual surfaces. Moderate and severe microscopic hypoplasia was observed in 85.6 per cent of 1,036 deciduous teeth and in 92.1 per cent of 266 permanent teeth. The secondary dentin of the deciduous and permanent teeth which were exceptions to the correlation between dental caries susceptibility and structure was minutely examined. Mellanby indicated that 72 per cent of the 111 deciduous teeth which were exceptions could be explained either on the basis of (1) well calcified secondary dentin in those defectively formed teeth which did not decay or (2) poorly formed secondary dentin in those well formed teeth which did decay.

Mellanby (1934) presented a summary of her observations on the association between structure and the incidence of dental caries. In 1,500 sectioned deciduous teeth, 78 per cent of those with well calcified enamel and dentin were free from caries while only 6 per cent of the very hypoplastic teeth were caries-free. Extensive dental caries was observed in only 7.5 per cent of the former teeth in contrast to 74 per cent of the latter. A similar association was observed in 275 sectioned permanent teeth.

Weaver (1935) criticized the interpretation of the data on the association between tooth structure and the dental caries susceptibility presented by Mellanby (1934). One of his criticisms centered about Mellanby's two hypotheses, that the susceptibility to dental caries was associated partially with: (1) the structure of the enamel and dentin and (2) the production and structure of secondary dentin as a defensive response to the stimulus of surface injury or irritation. Weaver believed that if the influence postulated by the second hypothesis were strong enough to explain the exceptions to the first hypothesis, it might well be capable of dispensing with the first hypothesis. Thus if the second hypothesis were applied to all the teeth in the survey, the first hypothesis might prove to be unnecessary. Weaver also criticized the method of aggregating data for mathematical convenience. The four grades of structure were reduced to two; a similar condensation was made in the case of dental caries. Therefore, those teeth which were free from detectable carious lesions and those which were slightly carious were combined to form one group for purposes of contrast with the group which consisted of the moderately and highly carious teeth. The teeth which Mellanby (1927b) grouped with the non-carious ones were those which had no definite carious lesion but in which occurred obvious enamel changes which she described as being "seen in dull 'chalky' patches or in very early fissure caries." "On histological examination of some of them there was definite evidence of caries." In Tables I and IV of Mellanby's (1927b) paper, the association between dental caries incidence and tooth structure was determined by consideration of the normal teeth versus those with dental caries of any extent. This presentation indicated as definite an association as when the comparison was made by grouping normal and slightly carious teeth versus moderately and severely carious teeth (Mellanby 1934).

Mellanby (1930), however, failed to find dental caries in dogs maintained on supposedly cariogenic diets and treatments: (a) soft and sticky foods, (b) variation of cereals and vitamins A and D, (c) the addition of large amounts of fermentable carbo-

hydrate, (d) daily feeding of *Streptococcus mutans* and *Lactobacillus acidophilus* and (e) suppression of the salivary flow by means of atropine. Dental caries did not develop even in those teeth which were badly formed due to the deficiency of vitamin D. When artificial breaks were made in the enamel, some carious activity was observed in two dogs. However, artificial dental caries was produced *in vitro* by incubation of dog teeth with a mixture of saliva and bread. Bacteria could be isolated from the dentinal tubules of these artificial lesions.

Edward Mellanby (1931) studied the association between the minute surface texture and the incidence of tooth decay in 1,500 teeth of English children. A high correlation was observed for all types of teeth.

The Committee for the Investigation of Dental Disease in the Children of Great Britain (1925) reported an incidence of 3.39 per cent grossly hypoplastic permanent teeth among the 4,000 children studied. The percentage of normal permanent teeth which were carious was 13.1, while the percentage of grossly hypoplastic permanent teeth which were carious was 26.9. The incidence of gross hypoplasia in deciduous teeth was 1.50. The percentage of normal deciduous teeth which were carious was 43.3, while the percentage of grossly hypoplastic deciduous teeth which were carious was 62.9. A most striking fact was that in the great majority (88.9 per cent of permanent and 88.3 per cent of deciduous teeth) it was the grossly hypoplastic area which was attacked by the carious lesion.

Day and Sedwick (1934a, b) observed no difference in the dental caries experience for the grossly hypoplastic teeth of the 318 children whom they studied for 18 months during a vitamin D experiment.

McCall and Krasnow (1938) made the statement that hypoplastic enamel of the permanent teeth was no more subject to tooth decay than normal enamel unless the structural defects were severe. They stated that in deciduous teeth the hypoplastic enamel was more susceptible to dental caries than normal enamel but the one governing factor seemed to be the environmental influence.

Davies (1939) investigated the association between surface structure and dental caries incidence in children attending public elementary schools in Great Britain. His findings were based on the gross study of one first permanent molar in each of 770 children. The gradings were repeated after 12 months and found to be the same in all but about 5 per cent of the cases. His data paralleled Mellanby's hypothesis that the liability of a tooth to caries was influenced by its structure, amongst other factors.

Helen Mellanby (1940) studied the association between tooth structures and dental caries susceptibility in the children of Finnish Lapps. This survey was made on 70 modern children between the ages of 2 and 18 years and on several children's skulls from 200 to 500 years old. A higher degree of hypoplasia and of dental caries was observed in the teeth of the modern children than in those of the skulls. A high degree of association was observed between the surface structure and the susceptibility to dental caries.

In a survey of the incidence of new carious surfaces, Lathrop (1941a) observed that hypoplastic teeth were equally distributed among the various nationalities represented in an American child-caring institution. However, the incidence of tooth decay in the group with hypoplastic teeth was as great as the incidence in the native American group and the incidence in both these groups was higher than in any other nationality group.

A study by Allen (1941) of the nutrition and dental caries incidence of 120 London elementary school children indicated that a definite association existed between the gross structure of the teeth and the amount of caries present: the better the structure of the teeth, the greater the resistance to dental disease.

Bibby (1943b) studied the relationship between microscopic hypoplasia (Mellanby), as determined by enamel surface texture, and dental caries susceptibility in 200 children in Boston, aged 6 to 16 years. A definite correlation between the minute surface structure of the teeth and the liability to tooth decay was observed.

Staz (1943, 1944) observed that 39 out of the 73 Johannesburg Indian children examined had hypoplasia, ranging from a generalized condition to only one affected tooth. He concluded from his data that the hypoplastic teeth were less susceptible to dental caries than normal teeth.

Résumé: The full significance of tooth structure in relation to the susceptibility to tooth decay cannot be evaluated on the basis of our present knowledge. In human material there appears to be a definite indication that, where the oral and systemic environment of the teeth are favorable to the processes of dental caries activity, those teeth which are most poorly formed and calcified have a higher susceptibility to tooth decay than normal teeth. However, if the oral and systemic environments are not favorable for tooth decay, there is no evidence that a tooth will become carious merely because it is imperfect structurally. Mellanby's (1934) inability to produce dental caries in the teeth of dogs even though the teeth had been poorly calcified indicates that in the dog there are other factors more important than the structure of the tooth itself. Such factors must be considered as possible reasons why human teeth decay even when normally formed, or do not decay despite imperfect structure.

Rickets in Relation to Tooth Structure and to Dental Caries Incidence

Edward Mellanby (1918a, b) observed that rickets in the dog was the nutritional disease associated with a deficiency of a fat-soluble constituent, now known as vitamin D. He observed that this vitamin had a specific controlling effect over many of the functions of growth in the body and in particular over the development of bone.

May Mellanby (1918) observed that a deficiency of this fat-soluble vitamin in young puppies also had a profound effect on the developing enamel of the permanent teeth, on the rate of eruption and on the position of the teeth in the jaw. When 8-week-old puppies were maintained on a rachitogenic ration for 15 weeks, the enamel of the permanent teeth which was formed during that period was soft and brown, particularly at the necks of the teeth. The eruption of the permanent teeth was delayed and the arrangement of the permanent teeth in the jaw was very irregular. When litter mates received 10 gm. of cod liver oil per day as a supplement to the rachitogenic ration, the structure of the permanent teeth, their position and rate of eruption were normal.

A deficiency of calcium in the dog in the presence of ample vitamin D (Mellanby 1923c) resulted in some defects of the enamel and the dentin, but these defects were not as severe as those produced by a deficiency of the fat-soluble vitamin. A deficiency of both calcium and vitamin D resulted in very badly formed enamel and dentin. If the cereal portion of the ration consisted of oatmeal or wheat germ, the tooth structures which developed during a period of deficiency were more defective than when white flour or rice was fed. Mellanby postulated a substance in the former cereals which inhibited calcification. Cod liver oil, however, overcame the effect of the oatmeal or the wheat germ.

The profound effect of the diet of the female dog during pregnancy and lactation upon the development of the deciduous and permanent teeth was described by Mellanby (1928). When the diet of the female dog was poor in vitamin D, the deciduous teeth of the puppies had defects in structure and their eruption was delayed. When the diet of the pregnant dog was rich in vitamin D, the offspring had normal deciduous teeth.

If the puppies from a dog which had received ample vitamin D during pregnancy were placed on a rachitogenic diet at weaning, the deficiency would produce milder changes in the developing permanent teeth than it would have in puppies born to vitamin D-deficient dogs.

Mellanby (1929) summarized her studies on the relation of vitamin D to jaw development and tooth structure in a well illustrated report in which the extensiveness of the damage to the enamel and dentin formed during rickets was stressed. Examples were presented to illustrate the rapid response toward normal calcification which occurred when a diet poor in vitamin D was supplemented with a source of vitamin D. However, when the source of vitamin D was removed from the diet of an animal, the response toward poor calcification was delayed for a period, the length of which appeared to be dependent on the body stores of vitamin D.

The microscopic examination of the deciduous teeth of about 100 normal puppies, worn down by natural attrition, indicated that there was rarely any secondary dentin present (Mellanby 1923b), either because of a lack of formation or of the advanced stage of resorption. However, when the early production of secondary dentin was stimulated by rubbing the teeth with a file, Mellanby (1930) observed that the structure of the secondary dentin was dependent on the calcifying power of the ration.

Mellanby (1930) reported further vitamin D studies on other species of laboratory animals, the rabbit and the rat, in which the importance of vitamin D during calcification of the teeth was again demonstrated.

Weinmann and Schour (1945b) observed the pathological changes which occurred in the incisor of the white rat on a rachitogenic diet low in phosphorus, deficient in vitamin D, but high in calcium. No hypoplasia was observed in the enamel formed during the deficiency period. Cystic degeneration occurred in scattered areas of the enamel epithelium in the incisal half of the incisor. The rate of dentin formation was retarded and there was an increase in the width of predentin. The newly formed dentin had a definitely interglobular calcification.

Sheldon, Bibby and Bales (1945) reported on the relationship between microscopic enamel defects and infantile debilities for a group of 95 teeth from 34 patients with detailed medical histories. In 24 patients, i.e., about 70 per cent of the cases, there was believed to be a positive correlation between the time of formation of a band of definitely defective enamel and the existence of some systemic disability. The nutritional and clinical records revealed 14 cases of vitamin D deficiency, 4 cases of vitamin C deficiency, 4 of vitamin A and 3 of calcium and phosphorus. These occurred singly or in combination, and sometimes in association with other diseases such as chicken pox, measles, pneumonia, pertussis, intestinal and gastric disturbances and upper respiratory infection in the children, and toxemia of pregnancy in the mother. In 23 per cent of the patients there were definite defects without history of systemic conditions which might have produced enamel changes. In 2 of the patients (6 per cent) there were insufficient enamel changes to be detected, although their histories revealed a vitamin D deficiency in both, one of which was complicated by eczema of the scalp.

The preceding studies indicate very clearly the necessity for adequate vitamin D to produce the normal development of the hard dental structures. Since there is no known residual metabolic malfunction after the healing of rachitic lesions, the only way in which infantile rickets might be expected to influence the dental caries incidence would be through the formation of teeth less resistant structurally to the carious process. The rickets to which reference is made in the following presentation is entirely infantile rickets except the one case where osteomalacia is specified.

In several of the following reports, the

diagnosis of rickets was described in children of school age. Except for rare cases of so-called late rickets, rickets is not diagnosable clinically at this age with any certainty. The clinical diagnosis of rickets is difficult in infancy, when rickets most commonly occurs, especially when the infants are growing more rapidly than average, but diagnosis is almost impossible in the older child in whom the criteria are the residual signs of infantile rickets.

During a survey of the dental caries incidence in 6,800 Swedish children, Forberg (1901) observed that 19.7 per cent of the teeth of those children who had had rickets were carious, while 16.5 per cent of the teeth of those children who had never had rickets, scrofula, anemia and chlorosis, or digestive diseases were carious. A slight increase in the percentage of carious teeth was also noticed in the children who had had scrofula, anemia and chlorosis or digestive diseases.

Dick (1916) observed what he considered to be distinct evidence of rickets in 80 per cent of 1,000 school children attending London City Council Schools in the East End. However, the diagnosis of past rickets in children of school age is sufficiently unreliable to make such a statement appear highly questionable. It was difficult to diagnose the presence of hypoplasia in the deciduous teeth since dental caries was universal and masked the hypoplasia. In 586 children in whom permanent teeth could be evaluated for hypoplasia, "20 per cent of them showed hypoplasia, frequently combined with decay, and 38 per cent had decayed teeth." No data were given on the incidence of hypoplasia in the children who did not have any evidence of rickets in Dick's diagnosis.

Dick (1918) compared the incidence of enamel lesions in 281 children from schools where the nutrition was distinctly below the average with those in 122 children from "good class" schools where the general state of nutrition was good. He observed "defective enamel" in 40 per cent of the "poor class" children in contrast to 13 per cent in "good class" children. Defective enamel included typical gross hypoplasia, honeycombed teeth, horizontal bands of thickened enamel and transverse grooves on the enamel, chalky appearance of the enamel and brown lines of Retzius. No attempt was made to correlate these findings with any specific nutritional deficiency.

Wells (1921) stated concerning dental caries in human rickets that "there is a striking tendency to very early caries; even before the tooth is fully cut the enamel at the cutting edge is often completely destroyed." No data were presented in his review article to substantiate the above statement.

Wilkins (1927a) studied the relation between the dental caries incidence and rickets, using chest deformity as the diagnostic criterion of rickets, in 5- and 6-year-old children in Birmingham, England. He considered that his figures tended to show "that rickets predisposes to decay of the teeth" but "this conclusion should be regarded as tentative only."

Wilkins (1927b) observed that there was a much higher dental caries incidence and higher number of carious and treated teeth for children in New Zealand than in Birmingham for all age groups from 5 to 22. In his discussion of possible factors related to the production of this difference, he considered that the greater prosperity, better infant care, more dairy products and meat, no margarine, and much more sunshine in New Zealand than in Birmingham were factors favorable to the production and maintenance of better teeth in New Zealand. On the other hand, he considered the greater frequency of eating and the higher sugar consumption in New Zealand to be adverse factors. Furthermore, he stated that "the grossest manifestations of rickets are much less evident in the New Zealand than in the Birmingham school children." Thus he felt that the adverse factors of greater frequency of eating and higher sugar con-

sumption were of greater importance than the favorable factors discussed.

Wilson and Surie (1930) studied the incidence of rickets and dental caries in 100 Indian children, 5 to 17 years of age, who were suffering from rickets of varying degrees of severity. Their observations were:

9 children with gross rickets, 100 per cent with caries, 100 per cent with hypoplasia
24 children with rickets, 79 per cent with caries, 96 per cent with hypoplasia
53 children with mild rickets, 74 per cent with caries, 90 per cent with hypoplasia
14 children almost normal, 21 per cent with caries, 85 per cent with hypoplasia

They interpreted these data to indicate that "the incidence of rickets and caries in parallel degrees of severity are co-existent."

Greenebaum, Johnson, Mitchell, Selkirk and Stillwell (1930) studied the dental caries experience of 13 breast-fed Negro children in Cincinnati, all of whom had a known degree of rickets at 8 months of age. At 4 years to 4 years and 9 months, 8 of these children had no carious lesions while the remaining 5 had one to 8 carious deciduous teeth. Of the 5 children with carious lesions, 2 had had marked, and 3 mild, rachitis. Eight of the 12 children in the control group had carious lesions. Greenebaum *et al.* believed that rickets had little effect in causing subsequent dental caries of the deciduous teeth.

In a later study of 17 Negro children, Selkirk, Greenebaum and Mitchell (1932) stated that a relationship was observed between rickets in infancy and the condition of the deciduous and permanent teeth up to 7 years of age in that the children who had had marked rickets had a slightly greater incidence of carious teeth per child than those who had had mild rickets in infancy. No comparison was made with the rate of incidence or number of carious lesions in children who had had no signs of rickets. The number of children was so small that no significance can be attached to the small differences reported. It was observed that the 12 children who had better nutrition at the time of the dental examination had a lower dental caries incidence than the 5 children who were in a poorer state of nutrition.

Greenebaum and Selkirk (1932) compared the dental caries incidence in 5- and 6-year-old Negro children who had had definite evidence of rickets at 8 months with a second group which was comparable except that rickets had been prevented by irradiation. All of the 10 children in the rachitic group had some dental caries activity, while 5 of the 12 children in the irradiated group had none. The incidence of carious lesions averaged 3.1 in the irradiated group and 2.1 in the rachitic group. Again, the dental caries activity was higher in those children showing malnutrition than in those whose nutrition was satisfactory at the time of the examination.

Mackay and Rose (1931) compared the incidence of dental caries and hypoplasia in 46 children with a history of rickets and 40 children with no history or diagnostic evidence of rickets at the time of the dental examination. All were London children of similar economic status who had been seen in private practice by Mackay. Gross hypoplasia was observed in 10 of the 46 children who had had rickets but in only 1 of the 40 children in the control group. However, there was only a slightly greater incidence of dental caries in the rachitic group than in the control group.

Hess and Abramson (1931) examined 2 groups of children aged 5 to 9 years in New York City, who had been observed throughout infancy. It was found that there was a higher rate of dental caries in the deciduous teeth of the 71 children who had had rickets than in the 24 children who had been protected. In all cases, the rachitic manifestations had been mild. The situation was not clear in regard to the permanent teeth, since only a very limited amount of data could be obtained at this age. A slightly higher dental caries incidence was observed

in the first molars of the non-rachitic group. Case histories were given of children who were free from dental caries despite previous severe rickets, and of children with dental caries although rickets-free.

Hess, Abramson and Lewis (1934) compared the dental caries incidence in 6- to 9-year-old children divided into a rachitic group and a group which had been protected against rickets. The deciduous teeth of the former group had developed carious lesions to a greater extent than in the latter group. The permanent teeth, however, showed a dental caries experience of about the same extent. The authors believed that this indicated that dental caries of the permanent teeth was not due to infantile rickets but to a nutritional disturbance occurring in childhood, shortly before or at the time at which the carious lesions appeared.

Kirsch and Rosenbaum (1932) studied the dental caries incidence in German children classified as (a) rickets-free, (b) questionable rickets and (c) rachitic. They observed a slightly higher dental caries experience in the deciduous teeth and a considerably higher experience in the permanent teeth of the children in the last two groups.

Eliot, Souther, Anderson and Arnim (1933, 1934) classified 451 New Haven children, aged 5 to 12 years, into 3 groups: 215 who had shown little or no sign of rickets at any time, 108 who had had mild rickets and 128 who had had moderate or severe rickets. Hypoplastic defects were found in 34 per cent of the last group but in only 4 per cent of the rickets-free class. Conversely, in practically every case in which severe hypoplasia was present, a history of moderate or severe rickets had been established. Dental caries was observed in 63 per cent of the children with a history of rickets but in only 43 per cent of the children with no signs of rickets. Dental caries was found more frequently in hypoplastic incisors and molars than in normal ones. Eliot *et al.* did not believe that the greater incidence of dental caries in hypoplastic teeth necessarily indicated a causal relationship between hypoplasia of the enamel and dental caries.

Shelling and Anderson (1936) observed an average incidence of 5 carious lesions in 126 children in Baltimore who exhibited X-ray evidence of rickets in infancy and early childhood. In a group of 150 children who received ample vitamin D, as viosterol, to protect them completely from rickets, the average incidence was 7.5 carious lesions. Enamel hypoplasia and malocclusion were more common in the rachitic group. They noted that 119 (93 per cent) of the rachitic group were Negroes while only 42 (28 per cent) of the protected group were Negroes. The influence of the greater resistance of the Negro children to dental caries formation upon the average dental caries experience of these 2 groups must not be overlooked. Repeatedly throughout the dental literature, statements are found that Negro children have a higher resistance to dental caries than white children in the same age groups. Unfortunately there appear to be no reliable data to substantiate these opinions at the present time, except the surveys of Hyde (1944), Mills (1937) and Rhoades *et al.* (1945). If such a difference in susceptibility does exist, it seems to the reviewer that the unequal racial distribution of these 2 groups might be an uncontrolled constitutional factor of sufficient size to render the comparison of dental caries experience valueless in this case.

Moore, Brodie, Thornton, Lesem and Cordua (1937) compared the sign of rickets and the dental caries experience of 550 five-year-old children from Portland, Oregon, with 383 from San Diego, California. It was observed that 90 per cent of these children exhibited 3 or more signs of rickets even though maternal statements indicated that some 80 per cent of the children in each city had received medicinal antirachitic treatment. Among the children of sunny San Diego, the percentage of children with signs

of rickets was nearly as great as in cloudy Portland. However, the dental caries incidence among the San Diego children was lower than in those of Portland, the numerical ratio being as 22 to 57. A negative correlation was observed between dental caries and rickets. An analysis of the mineral content of the municipal water supplies showed a much higher content of "Calcium, magnesium, sodium and other minerals" in San Diego than in Portland.

Taylor and Day (1939a, b) observed a low incidence of dental caries and of hypoplasia in 50 children with rickets at the Canadian Mission Hospital at Palampur, India. Of the 10 of these children in whom rickets was radiologically confirmed, 7 had no grossly observable hypoplasia, 2 had a slight opacity of the enamel of the permanent teeth and 5 showed a pitting of the deciduous teeth. Of these same children, 10 had carious lesions of "defective fissures" and 1 had carious lesions in the deciduous teeth. The diet of these children consisted mainly of carbohydrates with relatively little protein, no meats or fruits, and little milk or sugar. However, in 800 school children of the Indian "middle class" who were living on a diet similar to that of European standards, with soft, refined carbohydrates and sugar, the dental caries incidence was more than 6 carious lesions per child. Of 26 school children in an orphanage in Lahore, 8 were caries-free and the average incidence of carious lesions in the groups was 2.27. The iron content of the diet of these children was very low, since treatment with ferric ammonium citrate led to an increase in hemoglobin from 63 to 92 per cent within 1 month. The diet of all these children was very deficient in vitamin D and minerals and there was widespread evidence of osteomalacia and rickets among the population. However, Taylor and Day interpreted their data to indicate that a deficiency in vitamin D and minerals does not lead to high dental caries incidence or hypoplasia.

Taylor and Day (1940) and Day (1944a, b) reported that the incidence of carious lesions in 22 osteomalacious women in India was 1.54 per woman. Eight (36 per cent) of these women were caries-free. Hypoplasia was observed in only 7 of the women. Seven rachitic children borne by 5 of these osteomalacious women were observed. Only 1 carious lesion was observed in each of 2 of these children. Hypoplasia was observed in only 2 of the children and neither had any carious lesions.

In a further study of the relation of rickets to hypoplasia and dental caries, 200 boys in India were classified by Day (1944a, b) according to (1) no clinical signs of rickets, (2) slight clinical rickets and (3) moderate to severe clinical rickets. Day observed no relation between the incidence of rickets and the average number of carious lesions present or the percentage occurrence of hypoplasia.

As a result of an examination of 449 children in Palestine between the ages of 7 and 18 years, Rosenbaum and Mansbach (1944) concluded that there was no relationship between residual signs of rickets and the incidence of carious lesions in the permanent teeth. It is to be noted that the diagnosis of rickets had been made years after the active rachitic process had healed.

Sarnat and Schour (1941, 1942) in Chicago examined the medical histories of 60 random, non-luetic patients with enamel hypoplasia. Only 10 of these patients had a history of rickets and several of these 10 had had aplasia prior to the rachitic manifestations. It was concluded that rickets was not the only cause of enamel hypoplasia.

Résumé: Mellanby (1929) has shown the definite relation of vitamin D to the structure of the developing enamel and dentin in the permanent teeth of the growing puppy. When rickets was produced in the pregnant dog, the deciduous teeth of the offspring had changes in the enamel and dentin which were characteristic of vitamin D deficiency (Mellanby, 1928). Mellanby

(1930) also observed hypoplastic changes in the developing tooth structures of the vitamin D-deficient rabbit and white rat. However, Weinmann and Schour (1945b) observed retarded and imperfect calcification of the dentin but normal enamel formation in white rats on a rachitogenic diet which was low in phosphorus, deficient in vitamin D, but high in calcium. No explanation could be given for the differing observations of Weinmann and Schour, and Mellanby. The difference in rations must be considered as a possible factor.

In general, the data for children indicate that those who have had rickets during infancy have a higher incidence of hypoplasia and a slightly higher dental caries experience than those children in whom there have been no manifestations of rickets. However, the present diet in all cases appears to be of greater importance in the control of dental caries. Shelling and Anderson (1936) have presented evidence which indicates that there is no difference in the caries incidence of children who have not had rickets and those who have. However, inspection of their data showed an unequal distribution of Negro and white children in the two groups. The constitutional difference thus introduced might necessitate a re-interpretation of their data. Taylor and Day (1939a, b, 1940), Day (1944a, b) and Rosenbaum and Mansbach (1944) have observed no relation between rickets and hypoplasia or dental caries in Indian children. In each of these studies, the diagnosis of rickets was made from the residual signs years after the active rachitic process. The fact that no increase in hypoplasia was observed in these supposedly rachitic children might be explained by a difference between the diets of the Indian children and those of the western nations or by Sarnat and Schour's (1941, 1942) observation that rickets is not the only cause of hypoplasia. The low incidence of dental caries among the Indian children might be due to some factor or factors which are sufficiently powerful to overcome any susceptibility introduced by the rachitic processes. Along this line, Selkirk, Greenebaum and Mitchell (1932), Greenebaum and Selkirk (1932), and Hess, Abramson and Lewis (1934) have presented data which indicated that the diet at the time of observation is a more important factor than the increased susceptibility produced by rickets in a previous period. In view of the tremendous lack of knowledge of the relationship of diet during tooth development and maturation to susceptibility to tooth decay, it would appear to be much too premature to make any blanket statement to the effect that the present diet was of greater significance than the diet during development and maturation.

Vitamin D Supplements and Dental Caries Incidence

An investigation on the effect of vitamin D supplements upon the initiation and spread of carious lesions was the natural sequel to studies on the relation of vitamin D to tooth structure and on the relation of tooth structure to dental caries. Such an investigation would have to be conducted during the development of the permanent teeth and also subsequent to their eruption.

Mellanby and co-workers used children who had bone tuberculosis as the subjects of their first experiments on the effect of the addition of vitamin D to the diet. These children were confined in a hospital on the outskirts of Sheffield, England, where the normal hospital diet was described by Mellanby (1934) as "generous and by all ordinary standards would be considered very good." The total hardness of the drinking water, expressed as calcium carbonate, was 3.6 parts per 100,000. Throughout the period of investigation, the children were in bed and had little or no opportunity to obtain any food other than that supplied by the hospital diet.

In 1924, Mellanby, Pattison and Proud reported their observations of 3 groups of these subjects over a period of 8 months.

One group of 13 children received the regular hospital diet (diet C). A second group of 9 children received diet A which was characterized by "abundant calcifying vitamin and calcium, small amount of carbohydrate, not including oatmeal." A third group of 10 children received diet B which had "less calcifying vitamin and calcium, much carbohydrates, including oatmeal." The dental caries incidence and tooth structure at the beginning of this investigation were approximately comparable in the 3 groups. The data obtained as a result of 8 months of observations showed that "although there was an increase of caries in each group, the tendency for the disease to spread was less when the diet contained extra milk and fat-soluble vitamins A and D (present in such foods as eggs, cod liver oil, and to some extent in milk and butter) and that it was greater when the milk and fat-soluble content was cut down and the cereal moiety was increased."

Mellanby and Pattison (1926) reported a second experiment with the Sheffield children where the fat-soluble vitamins and the cereal content were varied and where the other factors, including total carbohydrate, were kept as constant as possible. The 3 groups of children were given: diet A_2, which included 21 gm. of cod liver oil daily, diet B_2 to which was added 21 gm. of olive oil daily, and diet C_2 which was supplemented with 10.5 gm. each of cod liver oil and olive oil. Very little egg was given in diet A_2, more in diet C_2 and a liberal supply in diet B_2. Less whole milk was given in diet B_2 than in A_2 and C_2, but the loss of salts was largely compensated for by the addition of skim milk. The bread was adjusted to the energy value of the other part of the diet, and in diet B_2 some was replaced by oatmeal. The children on the poor calcifying diet (B_2) had 3 times as much spread of dental caries as those on the best calcifying diet (A_2) and twice as much as on the intermediate calcifying diet (C_2).

The results of a third experiment in which an attempt was made to vary only the vitamin intake of the diet were presented by Mellanby and Pattison (1928). To test the supposition that vitamin A might be an important factor in limiting and inhibiting the initiation and spread of the carious process, vitamin A and vitamin D were added separately to the diet of different groups of the Sheffield hospitalized children. The authors reported that no final statement could be drawn from their incomplete vitamin A trials as to whether or not vitamin A played any role in prevention of dental caries. In 21 children whose average age was 5.5 years, the addition to the diet of vitamin D as irradiated ergosterol checked the initiation of new carious regions and diminished the spread of old ones. The data on the few children simultaneously maintained on the control diet with no additional vitamin A or irradiated ergosterol were not presented. The authors reported that the spread of dental caries in the control group was similar to that in the children of the same age in group C_2 of the second Sheffield investigation (Mellanby and Pattison, 1926).

A fourth study was made at Sheffield. In this case, Mellanby and Pattison (1932) gave a cereal-free ration, rich in vitamin D, to 22 children of an average age of 5.4 years over a period of 26 weeks. There were only 0.05 new carious teeth per child and 0.32 teeth per child which showed an increased activity in old sites of decay during this period. In addition there was hardening in an average of 4.7 of the 9.4 carious teeth present at the beginning of the dietary regimen. Again no data were presented for the control group, but the caries activity of this experimental group was compared with the observations of children under 6 years of age in the 3 previous experiments.

A more extensive investigation of the relation of vitamin D, both on the teeth during the development and after eruption, to dental caries initiation and spread was made under Mrs. Mellanby's supervision in three Birmingham children's institutions.

In 1931 the Committee on Dental Disease made an interim report on the results of the first 2 years of these studies. Each of the 3 institutions had its own water supply and the calcium content of the water differed in the 3 institutions. The total hardness of the water, expressed as calcium carbonate, was 16.0, 7.2 and 24.2 parts per 100,000, respectively. The ordinary routine of life was considered to be approximately the same in each of the homes. The standard dietary of the 3 institutions was considered to be generally similar, since the homes were administered by the same Guardians who distributed diet sheets and the food supplies. It was not possible to ensure that cooking procedures were identical. The diet judged by all ordinary standards was believed by the committee to be thoroughly satisfactory. The vitamin D content was found to be between 380 and 450 units per child per day.

In the first home, 28 or 42 gm. of treacle (golden syrup), depending on age, was added to the diet of each child daily. At the second home 14 or 21 gm. of olive oil was fed daily, and in the third home the same amount of cod liver oil was administered. The vitamin D content of the cod liver oil varied from 15 to 150 units per cc. Six months after the beginning of this investigation, another group of children was started in the second home. Each child in this new group was given daily a similar volume of olive oil in which was dissolved 625 units of vitamin D, as irradiated ergosterol, per cc. Caries increase was measured by the increase in the percentage of carious teeth and in terms of the average caries figure, ACF, which was the number of carious teeth divided by the number of teeth present.

The interim report presented evidence which indicated that in groups of children living in institutions, the addition to the standard dietary of fat-soluble vitamins over a period of 2 years significantly retarded the initiation of new carious regions and the progress of old lesions in the permanent teeth. There was no significant difference in the rate of increase of carious lesions between the children receiving the olive oil supplement and those receiving treacle. In the 2 groups of children living in the same institution, the addition of irradiated ergosterol in olive oil to the diet for a year and a half significantly retarded the progress of the carious process in the permanent teeth.

The final report of the Committee for the Investigation of Dental Disease (1936) summarized the results obtained over the 3-year observation period. The data presented indicated that:

(1) In the original permanent teeth, the incidence and the spread of dental caries were lower in the cod liver oil group than in the treacle and olive oil groups.

(2) In the newly erupted permanent teeth, the incidence and extent of tooth decay were significantly less in the cod liver oil group than in the treacle and olive oil groups. However, no difference was observed for the newly erupted first permanent molars.

(3) In the deciduous teeth, there were fewer carious lesions in the cod liver oil group than in the treacle group, but the incidence in the cod liver oil group did not differ from that in the olive oil group.

(4) In the original and newly erupted permanent teeth, there was significantly less dental caries, both in incidence and spread, in the irradiated ergosterol group than in the olive oil control group.

(5) For the deciduous teeth neither the incidence of new carious lesions nor the spread of old lesions differed appreciably between the irradiated ergosterol and control groups.

When the Birmingham experiments were begun by the Committee for the Investigation of Dental Disease (1936), it was hoped that it would be possible to test the effect of added vitamin D on the developmental structure of deciduous and permanent teeth. All the children in this section of the investigation were less than 5 years of age at the beginning. They were divided into 4 groups: 2 controls, olive oil and treacle, and 2 vita-

min groups, cod liver oil and irradiated ergosterol. Due to administrative difficulties, only 2 or 3 children in the olive oil group remained at the end of the experiment. Therefore, the treacle group was the only control. There were 31, 31 and 35 children in the treacle, vitamin D and cod liver oil groups, respectively. The number of first permanent molars which became fully erupted during the investigation was too small to be of statistical value, but there were indications that the additional vitamin D or cod liver oil during the developmental period had tended to improve the structure. The increase in both incidence and extent of dental caries was significantly less in the vitamin D group than in the treacle group. The group of children which received cod liver oil also showed a lower incidence and spread of dental caries than those in the treacle group, but it was only in the incidence that the difference could be considered statistically significant.

Weaver (1935) believed that the data presented for the Birmingham experiment could have an interpretation different from that of Mellanby (1934). Weaver pointed out that the teeth which were "dead" at the initial inspection of the Birmingham experiment were omitted from consideration, instead of observing them to determine if the progress of caries was altered by vitamin D supplements in the "dead" as well as in the vital teeth. In addition, Mellanby (1934) had made the statement that "comparatively little effect on the deciduous teeth could be expected in the older children as the blood supply to many of the teeth would be lowered owing to absorption of the roots, etc." The results of the experiment fulfilled this expectation. However, Weaver inquired whether the lower retardation in the deciduous teeth could have been due to the originally higher dental caries incidence in the deciduous teeth, or to the shorter average observation period of the deciduous teeth prior to their natural loss, or to both of these factors. Thus he questioned Mellanby's interpretation that the comparatively little effect of the vitamin D supplements on the deciduous teeth was the result of the poorer nutritive supply afforded by the reduced blood circulation during root absorption.

McKeag (1930) reported an investigation on the increase of carious lesions in 55 boys in Great Britain over a year of observation. The boys were divided into 3 groups, equal as to age and condition of the teeth. The 3 groups received: the control diet, the control diet plus 2 ostelin tablets which contained 4 minims of a preparation of the active medicinal factor of cod liver oil and two grains of calcium glycerophosphate, and the control diet plus two ostelin tablets plus $\frac{1}{10}$ grain of desiccated parathyroid gland. In the groups in which the ostelin tablets and the ostelin tablets plus desiccated parathyroid gland were given, the data presented showed a decrease of 35 per cent and 65 per cent, respectively, in the average number of newly carious teeth per boy.

Backer Dirks (quoted by the Committee for the Investigation of Dental Disease, 1936) reported an experiment which consisted of 3 groups of children aged 12½ to 16 years. These groups received the same basal diet with additions of sugar syrup, irradiated olive oil and cod liver oil. The incidence of new carious lesions in the children of the cod liver oil group was lower than that in the treacle group; there was a slightly lower incidence of new carious lesions in the children who received the olive oil supplement.

Jameson and Cox (1933) gave 10,000 units of vitamin D, as irradiated ergosterol, per day to 5 "selected" children in New Zealand for a period of 15 months with 2 interruptions of 6 weeks each. They believed that there was less advance of decay than in the control children.

Agnew, Agnew and Tisdall (1933) studied the dental caries increment in 3 groups totalling 350 children. These subjects lived in 4 orphanages in Toronto, Canada, on a

diet which had previously been considered adequate. The 3 groups consisted of: those children who received the institutional diet only, those on the same diet fortified with vitamin D and those receiving the same diet fortified with phosphorus and vitamin D. When vitamin D was received, there was a reduced dental caries increment, an arrest of previously existing carious lesions and an improvement of the general condition of the gingival mucous membranes. No statement was made on the effect of the phosphorus supplement.

Anderson, Williams, Halderson, Summerfeldt and Agnew (1934) studied the dental caries increase in the children of 2 orphanages in Toronto, for 1 year. The children in each orphanage were divided into 2 groups, the controls who received the institutional diet plus a plain ginger cookie into which had been incorporated about 250 I.U. of viosterol. The dental examinations of the 162 children were thorough; casts were made of the mouth at the beginning of the experimental year and again at the end. The presence and size of the carious lesions were determined by full length intraoral radiographs, as well as by the mirror and probe examination. These investigators observed a substantial reduction in the dental caries increment of the children in the experimental group between 3 and 10 years of age. However, no significant reduction was observed in the experimental children who were between 11 and 16 years of age. The lack of reduction might be attributable to the use of a substandard dosage of vitamin D at this critical period of growth.

Carmosin (1935) made a superficial study of the effects of calcium, phosphorus and vitamin D supplements on the increase in dental caries in 30 children in an orphanage in Philadelphia. His study consisted of 3 equal groups which received the institutional diet, the same diet supplemented with an unspecified amount of dicalcium phosphate in milk and the same diet plus unstated amounts of dicalcium phosphate and cod liver oil. He concluded from his data, which were not presented, that a supplement of dicalcium phosphate and cod liver oil was superior to dicalcium phosphate in milk as a preventive agent of dental caries. He observed that in all cases of bad decay the pulp showed a fine response in "throwing out" secondary dentin. Dicalcium phosphate alone gave little effect.

McBeath (1934) observed the increase in "percentage carious surfaces" of 425 children, 8 to 14 years of age, in 4 children's institutions in New York. There were 2 control groups in each of the homes, 1 on the regular institutional diet and 1 on the same diet with the addition of 1 pint of plain unevaporated milk daily. The children of the experimental groups received daily 100, 150 or 300 units of vitamin D in a pint of reconstituted milk. The increase in percentage of carious surfaces during the 7-month experimental period was much greater on the control regimens than on the vitamin D-supplemented ones.

McBeath and Zucker (1938) summarized the earlier observations of McBeath (1932, 1933, 1934) with the statement that the vitamin D given in a concentrate of cod liver oil in milk, as irradiated ergosterol or by irradiation of the skin reduced the incidence of new carious lesions in children 6 to 14 years of age. They believed that irradiated ergosterol was the least effective.

McBeath and Verlin (1942) continued McBeath's early studies by observing the increase of carious lesions in 5 groups of 40 children each. The children were housed in 10 cottages, with the occupants of 2 cottages assigned to each experimental group. The diet was planned by a dietitian and the food distributed from a central storeroom. However, the food was cooked and served in each cottage. The mean age of the groups varied from 10.1 to 12.5 years. The 5 groups were maintained on the institutional diet only and on the institutional diet supplemented daily with 800 units of viosterol, 400 units of vitamin D as cod

liver oil, 3200 units of viosterol or 800 units as cod liver oil, respectively. The results indicated that 800 units of vitamin D as supplied in cod liver oil daily gave the greatest protection; 800 units of viosterol gave the same degree of protection as 400 units of vitamin D as cod liver oil, while 3200 units of viosterol daily gave only a slight improvement in the dental caries reduction over the supplement of 800 units of viosterol. In the evaluation of these results, consideration must be given to the fact that the food consumed by the various groups may have been prepared in radically different manners, with the result that the diets actually consumed by the children may have differed in factors other than vitamin D.

Schoenthal and Brodsky (1933) studied the effects of diet on the incidence and spread of dental caries in 319 children in New York City aged 4 to 16 years, over a period of 13 months. These children were patients of a dental clinic and were classified into 2 groups: those who received an adequate diet and those who had a poor diet. The addition of vitamin D as viosterol or by ultra-violet irradiation did not reduce the incidence of new carious lesions in the group which had been receiving a well-balanced diet. However, the dental caries increment of the children receiving the poor diets was reduced by the addition of vitamin D to their diets. Ten to 20 drops of the viosterol preparation were given to each child, the amount depending on the age of the subject. The dosages of irradiation were said to have been sufficiently long to produce healing of infantile rickets. Irradiation was not believed to be as effective as viosterol, but there were only 11 children in the irradiated group, which did not provide a fair comparison.

Brodsky, Schick and Vollmer (1941, 1942) determined the effect of massive doses of vitamin D on the dental caries increment in tuberculous children whose ages varied from 2 to 16 years. The 33 control children who received only the hospital diet had an increase of 1.18 new carious lesions per child during the year's observation. Each of the 33 children in the second group received a supplement of 305,000 U.S.P. units of vitamin D and 2,455,000 U.S.P. units of vitamin A in 30 cc. of a fish liver concentrate at the beginning of the period and had an increase of only 0.39 carious lesions per child. Each of the 35 children in the third group received 600,000 U.S.P. units of crystalline vitamin D in one cc. of oil and had an increase of only 0.17 carious lesions per child.

Boyd, Drain and Stearns (1937) in Iowa have studied the effect upon the dental caries increment of daily supplements of 155 and 600 I.U. of vitamin D to an otherwise adequate diet. In the group of children who received 155 I.U. for 5 months, there was still minimal but definite caries activity. When the supplement was increased to 600 I.U. for 9 weeks, there was a cessation of caries activity. However, the effect of 600 I.U. of vitamin D per day was stated to be no greater than that produced by the Iowa workers in previous experiments described in the chapter "General Nutrition and Dental Caries Incidence," where 350 I.U. of vitamin D were given routinely per day.

Day and Sedwick (1934a, b) studied the effect of the daily addition of a concentrate containing 6,000 U.S.P. units of vitamin A and 1,470 Steenbock units (40,000 U.S.P. units) of vitamin D to the diets of 147 children in a Rochester public school. The control group consisted of 171 children. Both groups were observed at the beginning and twice during the 15-month experimental period. The average age of the 2 groups was 13 years and 10 months, and 13 years and 11 months, respectively, at the end of the test. No statement was made of the economic status of the families from which these children came nor of the type and quality of the diet. Inspection of the teeth was made by mirror and probe supplemented with radiographs. The experimental group had an increase in carious teeth of 5.28 per cent, whereas the control group had an in-

crease of 5.77 per cent. The increases in number of carious lesions per child were 7.52 and 6.77, respectively, and the increase in the "average caries figure" of Mellanby was 0.332 and 0.335, respectively. Day and Sedwick's data indicated that no beneficial effect was produced by the addition of a vitamin A and D concentrate, as judged by the average increase of carious teeth, the average number of new cavities and the average increase in the "average caries figure." In addition these authors observed that there was no protection in the newly erupted teeth of the experimental group. It is to be noted that the dosage of vitamins in this study was massive. Such an amount of vitamin D given daily could well be moderately toxic and might easily decrease, rather than increase, calcium retention.

In Stockholm, Sweden, Jundell, Hanson and Sandberg (1938) determined the average monthly dental caries increase for an average period of 15.1 months in 118 children who were given periods of quartz lamp irradiation or cod liver oil or both. The average monthly increase for an average period of 13.2 months in 44 control children was determined for comparison. The children had tuberculosis of the bones, joints or glands. The average ages of the experimental and control groups were 12.0 and 13.0, respectively. The average monthly caries increment for the experimental group was 0.27 and for the control group was 0.30. Jundell *et al.* concluded that irradiation and cod liver oil had no effect on the development of dental caries. They stated that no calcium or phosphorus deficiency existed in the diet of the children.

Goll (1939) gave a teaspoonful of cod liver oil daily to 56 German kindergarten children from February through May. New carious lesions were observed in 39.3 per cent of the 56 children who received the cod liver oil supplement and in 40.3 per cent of the 67 control children. No statement was made of the type of diet that these children received.

Strean and Beaudet (1945) described a series of 3 experiments in which tablets containing calcium fluoride alone, or with vitamins C and D, were administered to children for periods of 6 to 8 months. The one type of tablet contained 3 mg. of calcium fluoride, while the other contained 3 mg. of calcium fluoride, 30 mg. of ascorbic acid and 400 I. U. of vitamin D as calciferol. In the first experiment, which was continued for 6 months, 171 children, 8 to 13 years of age, residing in an orphanage, were divided into 3 equal groups. The first group was given 1 tablet containing calcium fluoride per day. The second group was given 1 tablet containing calcium fluoride, vitamins C and D per day. The third group served as controls. All 3 groups were receiving an additional supplement of cod liver oil daily, independent of the experimental procedure.

In the second experiment, which was conducted for 8 months at the same orphanage, 60 children were divided into 2 groups, one of which received the calcium fluoride plus vitamin pills, and the other of which served as controls.

The third experiment was conducted on 281 children who were private patients of dental practitioners. Children were selected for this experiment where there were 2 or more in the family living at home, in order that there could be both experimental and control subjects living in the same environment. In all 281 children studied, of which 140 were controls, half of the experimental children received 1 type of tablet while the second half received the other type. This experiment also lasted for 8 months.

In all 3 experiments, the children receiving the tablets had a lower average incidence of tooth decay expressed in terms of new carious lesions diagnosed during the experimental period.

In the third experiment, the children receiving the tablets with vitamins C and D in addition to calcium fluoride had a lower incidence of tooth decay than those receiving only calcium fluoride.

In the first experiment this difference

was not noted. According to the authors this discrepancy may have been due to the cod liver oil ingested by experimental and control children throughout the first experiment. This also was suggested as the cause of the low rate of incidence of tooth decay among the control children of the first experiment. The data were interpreted by the authors to mean that vitamins C and D augmented the effectiveness of the calcium fluoride.

The major criticisms of these experiments are their short duration, the lack of factual information regarding the dietaries of the orphanage children in the first and second experiments, and the failure to present average ages for any of the groups. The differences in the second and third experiments are particularly striking and need further investigation under more carefully controlled circumstances.

The Council on Dental Therapeutics of the American Dental Association (1945) has refused advertising claims concerning the relation of vitamin D and dental caries through the following statement: "It has been rather clearly demonstrated that vitamin D is of value in the formation of the hard dental structures, including the teeth themselves.

"Evidence that this vitamin aids in the maintenance of the fully formed tooth or in the prevention or retardation of dental caries at any stage in its progress is not supported by observations that have passed beyond the controversial stage. This being the case, advertising asserting that the ingestion of milk or milk products, with or without vitamin D, will aid in the prevention or retardation of dental caries tends to jeopardize the reputation of these foods, since persons who use them liberally are not necessarily free from dental decay or other dental disease.

"If it should be shown beyond reasonable doubt that vitamin D or products containing it are useful in maintaining normal tooth structure or in the prevention of dental disease, the Council on Dental Therapeutics will wish to foster as wide knowledge of such findings as possible. For the present, advertising claims for vitamin D as a factor in the prevention of tooth decay are not acceptable to the Council on Dental Therapeutics."

On the other hand, the Council on Foods and Nutrition of the American Medical Association and later the Council on Pharmacy and Chemistry of the same organization (1946) adopted the following statement: "There is clinical evidence to justify the statement that vitamin D plays an important role in tooth formation. Likewise experimental evidence justifies the statement that vitamin D is a beneficial factor in preventing and arresting dental caries when the intake of calcium and phosphorus is liberal and the diet is adequate with respect to other nutrients. Claims should not state or imply that vitamin D is the only important factor in caries prevention and arrest."

Neither of these claims represents evidence for or against the use of vitamin D or products containing vitamin D in the control of dental caries; they are, rather, the considered opinions of the men composing the respective councils of the two associations. The preponderance of evidence discussed in this review indicates a beneficial effect in the prevention and retardation of dental caries when adequate amounts of vitamin D were given to children with an otherwise adequate diet.

Résumé: The experiments reported by Mellanby and co-workers indicated that the addition of vitamin D to their basal diets resulted in a decrease in the dental caries incidence. It was never claimed that vitamin D deficiency was the only cause of dental caries but rather that if a partial vitamin D deficiency existed, the addition of adequate vitamin D to that diet would result in a decrease in the dental caries increment.

In the majority of the experiments which followed Mellanby's work, the data obtained indicate that there is a definite decrease in the dental caries increment and the spread

of old carious lesions when an adequate supplement of vitamin D is given. A few exceptions to this general statement have been presented. Schoenthal and Brodsky (1933) observed no decrease in the dental caries incidence of a group of children who received a good diet plus viosterol or ultraviolet irradiation. However, the group which received a poor diet showed a definite decrease in dental caries experience when given vitamin D. Anderson, Williams, Halderson, Summerfeldt and Agnew (1934) observed a substantial decrease in the dental caries experience if the children who received the vitamin D supplement were between 3 and 10 years of age but no decrease if they were between 11 and 16 years. At present this difference in age response cannot be explained. Two of the other negative results (Day and Sedwick (1934a, b) and Jundell, Hanson and Sandberg, 1938) were obtained with groups of children which had an average age of 13 years and 11 months, and 13 years, respectively. These negative results may have been due to the age of the group observed or to the previous adequacy of the diets with respect to vitamin D. Negative results were reported by Goll (1939) in kindergarten children. No indication of the vitamin D or mineral contents of the basal diets was given. Therefore, the adequacy of the diet for vitamin D, calcium, phosphorus, etc., could not be determined from the content of the research papers. If the diet in any of these experiments was fully adequate in vitamin D, a decrease in dental caries incidence with the addition of further vitamin D would not be expected. Schoenthal and Brodsky's (1933) data clearly pointed to the failure of vitamin D supplements to reduce the dental caries increment below that observed on a diet which is already adequate in vitamin D and minerals. From these studies, the amount of vitamin D needed daily by the growing child to produce the maximum reduction in dental caries susceptibility when the basal diet is otherwise adequate would appear to be around 400 I.U., the present recommended dietary allowance of the Food and Nutrition Board of the National Research Council (1945). The only evidence which indicates a possibly greater requirement for vitamin D is that of McBeath and Verlin (1942). However, their data resulted from a rather loosely controlled experiment, insofar as the preparation of the food of the various groups is concerned. In future experiments, investigators could contribute much more valuable data if they determined the calcium, phosphorus and vitamin D content of their basal diets and, whenever possible, the calcium and phosphorus balance of their subjects. Variable calcium and phosphorus contents and calcium-phosphorus ratios in the various basal diets may have resulted in different vitamin D requirements and, therefore, different results.

The relative value of various sources of vitamin D cannot be evaluated at this time. The loosely controlled studies of McBeath and Zucker (1938) and McBeath and Verlin (1942) suggest that the vitamin D of cod liver oil might be more effective in the reduction of the increment of carious lesions. The small group of children irradiated by Schoenthal and Brodsky (1933) were believed to be less benefited than those in the viosterol group, even though the form of vitamin D produced by irradiation is much the same as that in cod liver oil. This irradiation group was much too small to permit a fair evaluation. Thus the evidence at the present time is inadequate to support any claim for the use of one form of vitamin D in preference to the other for the maintenance of the dental structures. However, there is ample evidence to indicate that an adequate intake of vitamin D by children is of considerable value in the maintenance of normal dental structures. In no case described in this section have the investigators claimed that normal vitamin D metabolism was the only factor required for the maintenance of normal teeth.

Sunshine, Latitude, Winter Temperature and Dental Caries Incidence

Friel and Shaw (1931) quoted Osborn (1926) who estimated that, if the exposure to the sunlight in England were represented by 1, then the exposure in Johannesburg would be about 10. They observed that "rickets is practically an unknown disease" in South Africa but they found dental caries in 93.3 per cent of 600 children, 6 to 16 years of age, in government schools at Johannesburg. The dental caries rate was 482 carious teeth per 100 children. In contrast, the percentage of children with dental caries ranged from 20.4 to 45.3 in 4 orphanages. The authors believed that the amount of exposure to the sunlight was the same for all these children and that therefore vitamin D could not be responsible for the difference in the dental caries incidence of the children in the government schools and those in orphanages.

Osborne (1932) has drawn attention to the fact that there was a high dental caries experience in Australia despite the great amount of sunshine and the abundance of good quality fruits and vegetables.

Bunting (1937) in Michigan made the statement that "children in the South who have plenty of vitamin D in the abundant sunshine have no rickets, but they have just as much dental disease as do those in the North where rickets is common." However, no data were presented to substantiate this statement.

Ferguson (1935) classified the dental caries experience of 4,602 white recruits of the United States Navy by states. His data indicated a lower dental caries experience in the recruits from the southern states, with the lowest, Arkansas, having an average of only 3.00 defective teeth per man in 150 men. The men from the northeastern states had the highest rate, with Connecticut having the highest average of 12.54 defective teeth per man in 150 recruits. Ferguson particularly noted that most of the caries-free subjects were from the rural districts. He believed that the principal factors which affected variation in tooth decay were "climatic and occupational conditions, which determine the chief articles of daily diet." Ferguson also stressed the need for adequate exposure to sunlight and open air.

In a dental examination of 1208 midshipmen in school at Columbia University, Dunning (1944) observed a significant difference in the incidence of dental caries with respect to the region from which the men came. A high incidence of caries was found generally in the men from the northern latitudes and a low incidence in those men from the southern latitudes, particularly from the Southwest.

Mills (1937) analyzed the dental caries data of Messner, Gafafer, Cady and Dean (1936) of the United States Public Health Service, who had collected and tabulated the observations of approximately 8000 practicing dentists in many communities in 26 states. He found that there was an increase in dental caries experience with an increase in latitude for the 12- to 14-year-old boys living in rural districts and in towns of less than 5,000 population. The increase was roughly 15 more carious teeth per 100 children for each added degree of latitude, which amounted to well over 200 per cent from the Gulf to the Canadian Border. In cities of 10,000 or more population, a similar general relationship of dental caries to latitude was observed although much greater individual variations were present. This regional relationship held in Negro children as well as white, even though the overall dental caries experience of the former was definitely less than the latter. Mills stated: "The immediate explanation that comes to mind is the decline in sunlight intensity toward the north, since the relation of ultraviolet light to bone and tooth development has already been so clearly demonstrated."

East (1939) analyzed the relation between the mean annual hours of sunshine and the average number of cavities per year observed in 94,337 boys, 12 to 14 years of age,

from rural and semi-rural population groups below 5,000 (Messner, Gafafer, Cady and Dean, 1936). He observed the following relation: The average number of cavities per 100 boys was 290 in areas with 3000 plus annual hours of sunshine, 323 in areas with 2600 to 2999 hours, 374 in areas with 2200 to 2599 hours and 486 in areas with less than 2200 hours. The areas of more than 3,000 hours of sunshine per year included the southeastern states. The areas of low sunshine were located in a belt along the Appalachian Mountains from West Virginia to Maine, in Northern Michigan and in the Pacific Northwest. These areas closely paralleled the regions of low and high dental caries experience reported by Ferguson (1935).

Kaiser and East (1940) and East and Kaiser (1940) made a similar study on 296,605 rural boys and 285,103 rural girls whose dental caries experience was recorded by Messner, Gafafer, Cady and Dean (1936). The dental caries rate for boys and girls in the 9 to 11 age group decreased 3.9 and 4.7 per cent, respectively, for each 100 hours additional sunshine. The decreases for the 6 to 8 age group were 7.4 and 6.3, respectively, and for the 12 to 14 age group were 5.2 and 5.5, respectively. The increase in dental caries rate per added degree of latitude varied from 1.1 to 4.1 per cent. When the overlapping influences of sunshine and latitude were separated out, it was found that the relation of winter temperature to dental caries experience was slight in all boys and also in girls of the 9 to 11 age group. However, in girls 6 to 8 and 12 to 14 years old there was a lower dental caries rate with a higher winter temperature.

In a similar study on 528,842 children who lived in 156 cities with a population of 10,000 or greater, East (1941c) observed a 3.7 per cent decrease in the dental caries rate for each 100 hours increase in sunlight as compared with the combined value of 5.5 observed in rural children. East reported that the larger the city the greater the dental caries experience and expressed the view that this was due to the additional smoke and shade which would decrease the opportunity for exposure. When the winter temperature was considered, it was found that the higher the winter temperature for a city, the lower the rate of decay. These facts all seemed to point to the possibility that the more favorable the opportunities for exposure of the children to sunlight, the less decay would be experienced. As mentioned in detail in the introduction, East (1941c) had made a statistical analysis of these data which indicated that the "errors" or "bias" introduced by some 8000 different examiners were distributed as represented by the bell-shaped probability curve. He concluded that the data of Messner *et al.* were valid for purposes of comparing the caries prevalence among various age and population groups but would not necessarily be reliable as measures of the actual number of carious teeth in each of these population groups.

East (1941b) observed that the dental caries rate of the 9- to 11-year-old boys of 17 cities each in New Jersey and Tennessee were 2.89 and 1.79 carious teeth per boy, respectively, although the mean annual hours of sunshine were believed to be the same, 2,000 to 2,400 hours. In a study of the diets of the children of the two states, East observed that the flour and sugar consumption per capita in New Jersey was less than in Tennessee, while the milk, fruit and vegetable consumption was greater in New Jersey. However, the mean winter temperature of Tennessee was 10°F higher than that of New Jersey. Therefore, East proposed the explanation that the lower dental caries rate in Tennessee was due to "greater opportunity for exposure to solar ultraviolet energy."

East (1942) mentioned the observation of Hauck, Steenbock and Parsons (1933) and Morgareidge and Finn (1940) that fluorides were somewhat antirachitic in

doses toxic to animals. On this basis he postulated that, since low dental caries experience was observed in regions of the United States where both fluoride and sunshine factors were favorable, there might be a synergistic relationship between fluorine and vitamin D.

Although there was a very low dental caries experience in India, Shourie (1941) has drawn attention to the fact that "less caries was observed in Delhi in latitude 28° than in Madras at latitude 12°. It is well known that rickets is more common in North than in South India." This exception to the general finding of an increase in dental caries experience with an increase in latitude was based solely on one city in each latitude. No statement was made of other differences in the environment of the inhabitants which might over-balance the effect of latitude and hours of sunlight.

Blackerby (1943) observed that differences in dental caries experience existed between comparable population groups of closely related geographic areas. The dental caries experience among white children 6 to 15 years old was higher in East Tennessee than in Middle and West Tennessee, while the incidence in Middle Tennessee was slightly greater than in West Tennessee. Blackerby observed that there was an inverse relationship between the dental caries experience of these 3 divisions and the relative amounts of sunlight. He also observed an inverse relationship between the dental caries incidence and the concentration of carbonate, chloride, iron and fluoride in the public water supplies.

Résumé: On the basis of the statistical analyses made by Mills (1937), East (1939), Kaiser and East (1940), East and Kaiser (1940) and East (1941b, c), a high correlation was observed between an increase in the latitude or a decrease in the hours of sunlight per year and an increase in the dental caries experience. It must be remembered that these statistical analyses were made on the data compiled by Messner, Gafafer, Cady and Dean (1936) from the reports of dentists, physicians and school nurses in the individual communities. The number of communities considered in the latitudes and in the sunlight studies and the impressive differences would indicate that the correlation between latitude or hours of sunlight and the dental caries experience was real and not a result of the variation between the evaluation of the individual examiners. The most evident explanation of this correlation, if it is one of cause and effect, would be the increased exposure to sunlight made possible by the overall increased hours of sunlight, the increased winter temperature and the greater exposure to sunlight in country districts.

Vitamin A and Dental Caries Incidence

The effect of vitamin A deficiency on the formative tissues of the incisor of the white rat was described by Wolbach and Howe (1925b, 1933). Atrophic changes in the ameloblasts were observed, followed by atrophy of the remainder of the enamel organ, which finally consisted of the remnants of the epithelial papillae and squamous cells. The odontoblasts on the labial (enamel-covered) side retained their normal structure and function for relatively long periods of vitamin A deficiency. However, the odontoblasts on the lingual (cementum-covered) side became atrophic and depolarized simultaneously with the ameloblasts' changes. The dentin near the formative end on the lingual side became very thin and atypical, lacked the normal tubular arrangement and contained vascular inclusions. As a result of the active deposition of dentin on the labial surface of the incisor and the greatly reduced rate of dentin formation on the lingual surface, the dentin on the labial surface appeared extraordinarily thick when compared to the remainder of the tooth.

Schour, Hoffman and Smith (1941) confirmed and enlarged on Wolbach and Howe's observations. The primary effect of vitamin A deficiency was a failure of the odontogenic

epithelium to undergo normal histo-differentiation, particularly on the lingual surface. Thus the normal organizing influence of the odontogenic epithelium to cause pulpal cells to differentiate into odontoblasts was lacking. The earliest response which could be recognized was a morphological and functional alteration of the lingual odontoblasts rather than a morphological change in the epithelium. Along with the failure of histodifferentiation, there was a continuation of the proliferation of the odontogenic epithelium which resulted in an invasion of the pulp by epithelial cords arising from the lingual odontogenic epthelium.

Boyle (1933) observed similar changes in the developing tissues of the incisor of a prematurely born, vitamin A-deficient infant.

Bloch (1930, 1931) studied a group of 64 blind Danish children and young individuals who had had xerophthalmia during infancy (3 months to 2 years of age). At the time of the examination all but 19 were believed to be normal except for the blindness produced by the xerophthalmia. These 19, however, had pronounced signs of previous rickets and were eliminated from the survey because of the possible involvement of rickets in tooth structure and dental caries incidence. Of the remaining 45 children, 6 had perfectly normal teeth in every respect. In the 39 children with dental caries, the first molars were most affected. Bloch stated that as to form, position and consistency of the first molars, no conspicuous abnormality was present. He believed that vitamin A deficiency was unlikely to alter the calcification of the first molars, as they are partially calcified prior to xerophthalmia. The incisors were normal in 24 of the 45 children, but there was a slight hypoplasia of the enamel and dentin in 21. Bloch concluded that a vitamin A deficiency in infancy did not increase the disposition to caries.

Shourie (1942) observed that 39.8 per cent of the children in Madras, India, did not have dental caries. He investigated the relation between deficiency diseases and dental caries, using Bitot's spots and phrynoderma as an index of vitamin A deficiency, and angular stomatitis as the index of riboflavin deficiency. In children with deficiency symptoms, 32.8 per cent showed caries in deciduous teeth and 5.3 per cent showed caries in permanent teeth. In children without deficiency symptoms the comparable percentages were 25.8 and 6.1. These data indicated that there was no association between the deficiency signs observed and the dental caries incidence.

Sarnat and Schour (1941, 1942) analyzed the dietary history of 60 children in Chicago with enamel hypoplasia. They concluded that there was no indication that any of these children had ever been vitamin A-deficient during the period when the hypoplasia occurred.

Day (1944a, b) made a survey of the relation of nutritional deficiencies and dental caries in Northern India. He observed as high an incidence of phrynoderma as rickets. In 15 cases of phrynoderma found among 200 boys there was no relation between vitamin A deficiency and dental caries.

Mellanby and Pattison (1928) tested the supposition that vitamin A might be an important factor in reducing the incidence of carious lesions in hospitalized children at Sheffield, England. Their single trial resulted in differences so small that the authors felt that no inference could be drawn as to the importance of vitamin A. The actual data were not presented.

Résumé: No evidence except the one case described by Boyle (1933) has been found in the literature to show that vitamin A deficiency in man resulted in a malformation of the teeth. There has been no direct evidence that the addition of vitamin A to a diet will reduce the dental caries incidence. In those experiments in which cod liver oil supplements were found to decrease the dental caries susceptibility, there may have been occasions when not only a borderline

vitamin D deficiency but also a vitamin A deficiency was relieved. If such cases occurred, the reduction in dental caries incidence might have been due partially to the vitamin A of the cod liver oil.

Wolbach and Howe (1925b) observed a metaplasia of the epithelium in the salivary glands of the vitamin A-deficient rat. The removal of the salivary glands of the rat has been shown to increase the susceptibility to dental caries (Cheyne (1939b) and Weisberger, Nelson and Boyle, 1940). Also an association between the rate of salivary secretion and dental caries in adolescent children has been described by Trimble, Etherington and Losch (1938) and Cushman, Etherington and Thompson (1940). In view of these data, it would be interesting to know if the salivary flow decreases markedly in vitamin A deficiency in man and if this produces any marked change in dental caries incidence in vitamin A-deficient individuals.

The Vitamin B Complex and Dental Caries Incidence

There have been numerous studies on the relation of vitamin B-complex deficiencies to the maintenance of dental structures in experimental animals, but few studies have been made on the relation of vitamin B-complex deficiencies in man to the incidence of tooth decay.

In the examination of monkeys which had been made deficient in folic acid, Waisman, Rasmussen, Elvehjem and Clark (1943) found that there was a pronounced gingivitis with occasional necrosis of the gums, often involving the mucous lining of the cheek. In some cases, extensive, gangrenous, perforating areas developed in the cheeks from infectious foci in the mucosa of the oral cavity. As yet no investigations have been conducted to determine if folic acid deficiency may increase the susceptibility of the monkey to dental caries.

The deficiency of nicotinic acid in the dog has been recognized by the production of a condition similar to the oral condition in human pellagra, with highly inflamed gums and swollen, dark red tongue. Becks and Morgan (1942) studied the effect of nicotinic acid deficiency on all the oral structures of the dog and found that no dental or osseous changes accompanied the gingivitis. However, the deficiency of the filtrate factor (pantothenic acid and other unidentified members of the vitamin B complex) resulted in malformation and resorption of the peridontal structure which resulted in widespread osteoporosis. Sandwith (1905), Roberts (1915) and Gorkill (1934) stated that there was a low incidence of tooth decay in human subjects who had pellagra.

Goll (1940) tested his hypothesis that the deficiency of thiamine might be the cause of dental caries by feeding a type of cake prepared from thiamine-rich foodstuffs to a small group of German children. In this way their thiamine intake was increased by 0.8 mg. per day. However, no reducduction was observed in the 9-month period during which this supplement was fed.

Shourie (1942) investigated the relation between the signs of vitamin A and riboflavin deficiencies and the dental caries incidence. The data indicated that there was no relation between these deficiences and tooth decay.

Kniesner, Mann and Spies (1942) studied the dental caries incidence in 41 patients who were hospitalized in Alabama for treatment of vitamin B-complex deficiencies. They believed that the dental caries experience was as low as or lower than in normal individuals of the same age groups.

Mann, Dreizen, Spies and Hunt (1947) have compared the lifetime dental caries experience of 124 subjects with evidence of deficiency diseases at the time of the dental examination to that of 99 subjects who were well nourished at that time. Included in the malnourished group were "34 patients with evidence of pellagra, 84 with riboflavin deficiency, 29 with the initial nervous syndrome, 15 with nutritional macrocytic

anemia, 27 with clinical evidence of thiamin deficiency, 11 with vitamin A deficiency and 17 with clinical scurvy. Sixty-two of these patients showed clinical evidence of multiple deficiency diseases operating simultaneously which Spies, Bean and Ashe (1939) have shown to be the rule rather than the exception in cases of prolonged malnutrition." These subjects ranged in age from 6 to 75 years, with 72 between the ages of 11 and 40. Clinical diagnosis was supplemented by blood vitamin level determinations and dietary assays. The control group of 99 well nourished subjects was selected at random from a company in Birmingham, Alabama, the clinic personnel and freshman medical students. The age range was from 8 to 54 years, with 87 between the ages of 11 and 40. The large majority of both groups were natives of north central Alabama, which has around 60 per cent sunshine per year where 100 per cent represents continual sunshine from sunrise to sunset for all 365 days per year.

The dental examinations were made with the aid of mouth mirror, explorer and roentgenograms. The average lifetime dental caries experience as expressed by the average DMF tooth surfaces was 4.54 for the malnourished subjects and 14.94 for the well nourished subjects. Several tests were made to determine how the oral environment varied between these 2 groups of subjects. By the Fosdick, Hansen and Epple (1937) test of enamel decalcification only 25 of 124 malnourished subjects were positive, with none of the readings exceeding 2 plus. By comparison, the well nourished group showed an activity rating distribution similar to that of the general population, with between 25 and 30 per cent of the group in each of the 2 and 3 plus ratings and an additional 15 per cent in each of the 1 and 4 plus ratings. When the occurrence of *Lactobacillus acidophilus* was determined, 27 of the experimental subjects had a zero count and an additional 76 had counts ranging from 1 to 5,000 per cc. of saliva. Among the well nourished subjects only 3 had a zero count and 4 had a count above 10,000 bacteria per cc. of saliva, with the remaining 92 distributed among the intermediate readings. The average buffer capacity of the malnourished patients was about 50 per cent greater than that of the well nourished patients.

No statement was made in these studies of how long the deficiencies had existed in these patients. Indeed the probability is that there would be no way in which this could be even roughly estimated. Since measurements of decayed, missing and filled tooth surfaces represent the lifetime dental caries experience and not the dental caries activity at the time of examination, the lower dental caries experience of the malnourished patients would imply that these individuals had had a lower dental caries experience throughout most of their existence. Whether the nutritional deficiencies had existed throughout a comparable portion of their lives cannot be determined from these data. It is evident that under the dietary regimens of these malnourished individuals, there is no rampant dental caries activity in periods of single or multiple deficiencies of ascorbic acid, vitamin A and the members of the vitamin B complex studied. Whether the correlation between these deficiency states and the low lifetime dental caries experience is one of cause and effect or of incidental nature cannot be determined from our present knowledge. Since these malnourished patients were attending a nutrition clinic at the time of the dental examination, curative procedures probably were initiated, thus preventing further study of whether the increment of new carious lesions was low during a period of observation in the deficiency states. The main question arising from the above data would be the comparability of the malnourished and well nourished subjects. The authors stated: "The income of the patients in the experimental group was such that they could afford little else but

corn bread, syrup and fat pork, the dietary staples in this section of rural Alabama." By contrast, one would expect that the class of subjects used as the controls would have much higher incomes and therefore a greater choice and selection of foods. This different food distribution, with possible increases in refined foods and decreases in natural products, may have resulted in diets more adequate with respect to the nutrients which were low or deficient in the diets of the experimental subjects but less satisfactory with respect to other nutrients.

Dreizen, Mann, Spies and Skinner (1947) described a clinical study where the dental caries increment was determined for a group of malnourished children and for a group of well nourished children of comparable age. The DMF tooth surfaces and increment of new carious lesions in a year were determined for 72 children with clinical evidence of nutritional deficiency diseases. Each child had been examined periodically in the nutrition clinic at Birmingham, Alabama, for several years prior to the time this investigation was begun. The diets of the mothers of these children usually were inadequate during pregnancy and lactation. Few of the children received orange juice or vitamin D supplements during childhood. As the children grew older, they usually had poor appetites, ate irregularly and many preferred high carbohydrate foods. These 72 children were divided into 2 groups: group 1 was composed of 47 children which were malnourished throughout the observation year, while group 2 was composed of 25 malnourished children who received a quart of milk daily for 6 days a week during an 18-month period which included the observation year. A third group consisted of 25 well nourished children who were free from evidence or history of nutritional deficiency disease. Of the children in group 1, 44 had clinical evidence of riboflavin deficiency, 2 of nicotinic acid deficiency, 4 of thiamine deficiency, 4 of ascorbic acid deficiency and 9 of vitamin A deficiency. In group 2, 20 of the 25 children had signs and symptoms of riboflavin deficiency, 2 of thiamine deficiency, 12 of ascorbic acid deficiency and 5 of vitamin A deficiency. Thirty of the children in groups 1 and 2 showed evidence of multiple deficiency disease. No evidence of rickets was seen in any of these children. The average carbohydrate intake per child per day was 248, 253 and 296 grams, respectively, for groups 1 to 3. These values constituted 54, 55 and 49 per cent, respectively, of the total caloric intake. The average refined carbohydrate intake per day per child was 52, 48 and 67 grams, respectively. These values constituted 12, 11 and 11 per cent, respectively, of the total caloric intake. The average value for the DMF surfaces for the children in group 1 was 4.52, for group 2 was 2.80 and for group 3 was 7.34. The average increase in DMF tooth surfaces per child during the observation year was 0.95, 0.96, and 2.38 DMF surfaces. In other words, the incidence of new carious lesions in the group of malnourished children and in the group of malnourished children which received a quart of milk 6 times a week throughout the observation period was only 44 per cent of that observed in the well nourished group.

The data presented in the above study appear to answer some of the questions raised about the report of Mann, Dreizen, Spies and Hunt (1947) especially with regard to the actual development of new carious lesions during an observation period in these deficiency states. Some additional data are presented on the comparative total and refined carbohydrate intakes of the three groups of children. The groups of malnourished children had somewhat less total and refined carbohydrate intakes per day on a gram basis and about the same amounts on a percentage basis as the well nourished children. An unexplained point in the data presented was the fact that the two groups of malnourished children had almost identical carbohydrate intakes despite the fact that the record group received

a supplement of 1 quart of milk per day, which would contribute about 44 gms. of carbohydrate and 624 calories. On the basis of present knowledge, it is not possible to explain the lower dental caries increments among the malnourished children in this study, especially since so little is known of the various foodstuffs which made up the bulk of the diets and which may have been of greater importance than the deficiencies recorded in the malnourished children.

Résumé: The prevalence of vitamin B-complex deficiencies merits more study concerning their relation to dental caries susceptibility. From animal experimentation, it has been shown that certain deficiencies lead to disturbances in the normal maintenance of the supporting structures. The effect of vitamin B-complex deficiencies of the borderline type over prolonged periods possibly might be responsible for some cases of tooth mortality due to pyorrhea and alveolar atrophy.

Vitamin C and Dental Caries Incidence

The scorbutic lesions of the human mouth are remarkably constant (Dalldorf, 1939). The lesions of the gingiva occur only when teeth are present and are most severe around broken teeth. The gingival lesions begin on the papillae, first as hyperemia with dilated thin-walled vessels which have a tendency to hemorrhage. Disintegration of the epithelium follows, and infection with ulceration, granulations and gangrene may result. The gums become inflamed and spongy and bleed easily. In cases of severe deficiency they become sufficiently large to obstruct mastication. These changes are accompanied by a rarefaction of the alveolar bone resulting in a loosening of the teeth and frequently the loss of one or more.

Jackson and Moore (1916) observed pulp changes in the teeth of scorbutic guinea pigs and designated them as the processes of "necrosis." Zilva and Wells (1919) investigated these changes further and described them as "fibrosis" or "fibroid degeneration."

Hojer and Westin (1925) made a histopathological study of the jaws and teeth in scorbutic guinea pigs. There was a gradual change and disappearance of the odontoblastic layer, accompanied by an amorphous calcification of the predentin and the absence of Tomes' canals in this region. There was commonly a dilation of the vessels of the pulp with occasional hemorrhage.

Westin (1925, 1931) stated that the pathology in the pulp and odontoblastic layer of the teeth in scorbutic human beings was nearly identical with the pathologic changes observed in the incisor of the scorbutic guinea pig. In the teeth of adults with scurvy, the dentin is found to be resorbed and porotic, particularly around Tomes' canals. The small amount of replacement dentin present was of the osteodentin type. The pulp was found to be atrophic and hyperemic. Degeneration of the odontoblasts, the formation of cysts and foci of denticle-like regions of calcification were observed. However, Westin did not find any evidence from any of his subjects to indicate that there was an increased susceptibility to dental caries in the scorbutic state.

Boyle (1934) examined the teeth of two scorbutic infants but observed no changes similar to those in the developing dental tissues of the scorbutic guinea pig.

Howe (1920) did not observe tooth decay in guinea pigs which had been fed diets containing "very excessive amounts of sugars and starches" for periods of up to one year, even though the teeth were covered with adhesive masses of the sugars and starches. However, when young guinea pigs were fed a scorbutic diet consisting of skim milk and rolled oats, lesions developed in the molars and the incisors. Howe believed that this softening and carious process was unmistakable and could be produced with regularity. The feeding of sugars in the deficient diets seemed to produce no additional deleterious effect on the teeth.

Howe (1924a, b) reported on his dietary

experimentation with about 69 Rhesus monkeys and 6 Java macaques. The dietary regimen was mainly scorbutic, with its intensity varied by the addition of orange juice. Four of the Java macaques were fed the scorbutic diet. "In two, the mandible was extensively decalcified. Caries was found in the first permanent molars and at the gingival margin of the central and lateral incisors." Of 26 other animals receiving a scorbutic diet with some variations, 8 had caries, 4 of these "had received orange juice only when they showed pronounced symptoms of scurvy, and then only for a period sufficient to insure their living. Three more that had caries had 10 cc. of orange juice daily. One had 30 cc. daily and yet had caries." Speaking of some other experiments on scurvy and calcium deficiency, Howe stated: "Of thirteen animals that have had caries, five have been on a pronounced scorbutic diet, five had received 10 cc. of orange juice daily, and three have received from 26 to 30 cc. daily." Howe observed that an increase of from 30 to 60 cc. of orange juice was followed by improvement in the animals. Of an experiment on the effect of local carbohydrate fermentation, when sugar, candy and white flour were fed to six monkeys, Howe stated that in one case carious lesions were found but in the other five monkeys none were observed. Howe stated that "dental caries appears to result as a consequence of disordered calcium metabolism and not from some specific agency."

Hanke (1929) made a critical dental examination of 114 patients in Chicago between the ages of 6 and 60 years. This examination later (1930) was increased to include 191 cases. On the basis of this examination he distributed the patients into 4 classes—free from dental disorders, uncomplicated caries, caries and inflamed gums and inflamed gums without caries. Each class was then broken down in respect to an estimation of their diet according to the deficiency of vitamins C and D, individually and together. Of the 17 individuals who had no dental disorders, 11 were believed to have no dietary deficiency. Of the 61 individuals who had dental caries but no gingival lesions, 22 were believed to be deficient in both vitamins C and D, and 39 deficient in vitamin C. Thirty-eight of the 65 with dental caries and gingival irritation were believed to be deficient in vitamin C and D, while the other 27 were believed to be deficient in vitamin C. Of the 48 individuals who had gingival irritation or pyorrhea or both but no dental caries, 19 were believed to be deficient in vitamins C and D, while 28 were suspected of being deficient in vitamin C. Hanke believed, therefore, that vitamin C deficiency was associated intimately with the development of gingival irritation and dental caries and that vitamin D deficiency might be partially responsible.

Hanke (1932) reported the results of a 3-year investigation in 40 private patients. During this period a very liberal diet was advocated in which was included daily: 8 ounces of orange juice and the juice of 1 lemon, 8 to 32 ounces of milk, 1 to 2 eggs, lettuce, fruit, vegetables and meat. A great improvement in the gingival conditions was observed under this type of dietary regimen.

Hanke (1933) presented the data obtained in a 3-year study at an orphanage in Mooseheart, Illinois, on the relation of a citrus fruit juice supplement to the diet of a large group of children. Three hundred and twenty-three children between 10 and 17 years of age were observed to determine their rate of dental caries development during a year under the ordinary dietary regimen of the home. This diet contained a fairly liberal amount of fruits (other than citrus), vegetables, milk, butter and meat. The diet was believed to be adequate except in vitamin C. In the succeeding year, each child received a pint of orange juice and the juice of one lemon daily in addition to the ordinary diet, which appeared to be quite similar to that of the previous year insofar as volume and

distribution of food purchases were concerned. In addition, 99 other children who received only the unsupplemented institutional diet during the experimental period were observed as a further control group to test the dental caries-producing property of the unsupplemented institutional diet during the experimental period. In the 323 children during the control year, there was a high incidence of dental caries and gingival abnormalities and a slightly subnormal growth rate. The 99 control children who received only the institutional diet during the actual experimental period had a similarly high incidence of tooth decay and gingival lesions. The 323 children who received a pint of orange juice and the juice of one lemon in addition to the regular diet were stated to have had a reduced rate of dental caries activity and of gingival lesions and an increased rate of growth.

For 18 months following the experimental period, the 264 children remaining of the original 323 experimental subjects received the regular diet supplemented with approximately 3 ounces of orange juice per day. By the same tests as were applied previously, the institutional diet during the recheck period was believed to be comparable to the diet during the control and experimental periods. An increase in the dental caries activity and in the extent of gingivitis was observed, even though 3 ounces of orange juice were consumed daily.

The number of children with active carious lesions during the control period was found to be 256, or 79.2 per cent of the group, while the number of children with active carious lesions during the experimental period was 112, or 34.6 per cent of the group. Of the 211 children who had no active carious lesions during the experimental period, 161, or 49.8 per cent of the entire group, had had active carious lesions at the end of the control period but during the experimental period the caries activity was arrested. It would appear to be evident from these data that the increase in the number of children with no active carious lesions during the experimental period was due to the arrest of caries activity in lesions which had been diagnosed as active at the end of the previous control period. Indeed the entire validity of these data would appear to be dependent on the ability of the dental examiners to evaluate the activity of each carious lesion at each examination.

Hess and Abramson (1931) stated: "Some years ago we saw a number of children who had suffered from scurvy during infancy but whose teeth subsequently did not show more than the average degree of caries." No data were given on the dental caries increment of any children during a period of scurvy.

McBeath (1932) added various vitamin C-containing foods—bananas, oranges and tomato juice—to the regular diet of a small number of children in a New York orphanage. He was able to observe no beneficial effect of these foods on the dental caries activity of the children who received them. He concluded that vitamin C had no effect on the dental caries increment of the children observed. The diet to which these foods were added was not described. The age of the children, the state of their teeth and the duration of the experiment were not presented.

Grandison, Stott and Cruickshank (1942) gave a daily supplement of 200 mg. of synthetic vitamin C to each of 20 children for a period of 2 years. These English children, aged 4 to 14 years, were nearly all of tuberculous parentage. They were given a detailed dental examination every 3 months but received no dental treatment unless necessary. The control children were of normal parentage and each child was "paired" by sex, age and dental picture with a corresponding experimental subject. They were likewise inspected quarterly but "complete dental treatment was given at each three-monthly inspection." The children receiving vitamin C showed 1.5 times more new caries in teeth erupted and sound at the start of the experiment, and 1.8 times more new cavities in the occlusal surfaces

of teeth erupting during the experiment, than the control children. The authors stated: "It would be unfair to attribute the greater percentage of decay in the experimental group to the ingestion of vitamin C. It is more likely to arise from the disadvantages of lack of treatment, although the persistence of difference for the occlusal fissure cavities of erupting teeth makes this explanation less forcible. The fact that the subjects are nearly all of tuberculous, and the controls of normal, parentage may also play a part. The clinical fact remains that whatever the explanation, there is no evidence of a positive influence of these massive doses of vitamin C in any way modifying the usual course of caries development. There was no evidence of arrested decay in any carious tooth."

Sandburg and Dagulf (1939) observed no correlation between the ascorbic acid of the blood in the spring and in the fall with the dental caries experience of 190 subjects, 7 to 20 years of age.

Burrill (1942) determined the blood plasma vitamin C concentration of 1,291 patients in Illinois whose dental caries susceptibility had been clinically estimated. The vitamin C blood plasma range for the group without dental caries was 0.04 to 1.84 mg. for each 100 cc.; for the group with moderate dental caries it was 0.08 to 2.16 mg. for each 100 cc. There was no significant difference in vitamin C concentration among the three groups.

Lüthi (1941) of Bern, Switzerland, observed a slight difference in the vitamin C retention of children during saturation studies. It was found that the children with extensive dental caries had a slightly greater deficit than those with little or no dental caries. However, the average deficit of the children with extensive dental caries was not considered by Lüthi to be great enough to indicate actual hypovitaminosis.

Dierks (1943) concluded that sufficient vitamin C was a prerequisite for the prevention of "secondary caries," on the basis of observations made on the children in three German institutions. One hundred mgs. of crystalline ascorbic acid were given to each child daily for one year. No cases of "secondary caries" developed, as was shown by oral inspection and radiographic examination. The incidence of "secondary caries" was not described for any control group of children. The possible effect of vitamin C in the prevention of "primary caries" was discussed. However, on the basis of the limited data and lack of a control group, it was felt that no conclusion could be drawn. Under the term "secondary caries" Dierks considered all lesions, regardless of cause, which occurred in the hard tooth substances around the edge of where "primary caries" had been treated.

Résumé: The effects of ascorbic acid deficiency on the developing dental tissues of the guinea pig have been described by numerous workers, and similar changes in the pulp and the odontoblasts of the teeth of scorbutic human beings have been described by Westin (1925, 1931) without showing apparent relationship to caries susceptibility. However, Hanke (1933) reported a striking reduction in dental caries susceptibility by use of citrus juices. Present knowledge would indicate that the effect of citrus juice may not be entirely due to the ascorbic acid content. Various other components of the juices, such as the ash, the acid base ratio of the ash, the content of other essential nutrients, the increased water consumption, the detergent properties, etc., must be considered. If citrus juices have nutritional value in addition to their ascorbic acid content or are related to the susceptibility to tooth decay, as might be the case if Hanke's results are valid, then studies of these relations and the search for the active moiety should be conducted. As this problem stands at the present time, there appears to be an encouraging positive relationship clouded by questions of the ability to diagnose arrest of activity in carious lesions.

The experiment of Grandison, Stott and Cruickshank (1942) cannot be taken as evidence for or against the control of dental

caries by vitamin C ingestion because of the differences in composition and treatment of the control and experimental groups.

The limited data of Dierks (1943) indicating a relation of vitamin C therapy to "secondary caries" would suggest the need for repetition with larger groups of children plus a suitable control group. In addition, a thorough investigation of the incidence of "primary caries" in the same groups would be important.

No satisfactory evidence has been reported which shows that scurvy in any degree produced an increased susceptibility to tooth decay in man. In addition, no evidence has been reported which shows that the addition of ascorbic acid to a partially deficient diet would decrease the susceptibility to tooth decay. The importance of vitamin C in the maintenance of the gingiva and supporting structures of the teeth has been demonstrated adequately in experimental animals and in man.

Vitamin K and Dental Caries Incidence

Little is known of the relation of vitamin K to the development and maintenance of the teeth. Granados and Dam (1945) have reported preliminary observations on the relation of vitamin K deficiency, as produced by a diet containing 1 per cent sulfadiazine, to the growth, enamel pigmentation and rates of eruption and attrition of the incisor teeth of the white rat. A definite vitamin K deficiency was produced, since the prothrombin time of the rats which received no vitamin K was between 110 and 363 seconds, in comparison with 33 seconds in those rats which received a supplement of 1 mg. per cent of synthetic vitamin K. The incisors of all rats receiving diets which contained sulfadiazine showed a striking reduction in the rates of growth, eruption and attrition but no decrease in the rate of enamel pigmentation. The vitamin K supplement had no effect on these abnormalities.

The effects of prolonged administration of synthetic vitamin K have been studied by Hatton, Dodds, Hodge and Fosdick (1945). In the first experiment, low levels of synthetic vitamin K (2-methyl-1,4-naphthoquinone or the sodium bisulfite addition product) from 0.005 to 0.125 per cent of the diet, were fed for three generations. On the basis of the food consumption records, it was calculated that the rats in the low and high vitamin K groups each ingested 1 mg. and 25 mg., respectively, per day. No differences in the incidence of carious lesions were observed in any of the three generations regardless of the amount of vitamin K ingested. In the second experiment, the Hoppert-Webber-Caniff caries-producing ration containing 60 per cent of coarse corn meal was supplemented with 0.1, 0.3 and 0.8 per cent synthetic vitamin K. The diets containing 0.3 and 0.8 per cent produced measurable reductions in the rate of growth. Again there was no reduction in the incidence of carious lesions.

These studies on rats were preliminary to studies on the effect of vitamin K upon the incidence and progress of dental caries in man (Burrill, Calandra, Tilden and Fosdick, 1945). For this experiment, 119 subjects were selected from the Northwestern University dental students, each of whom had a greater than average susceptibility to dental caries but had at least 12 unfilled proximal surfaces in positions where decay could occur. These subjects were divided into 2 groups, as nearly alike as possible in susceptibility to dental caries, and were instructed to chew 1 pellet of gum for 10 or more minutes after each meal. No attempt was made to control the composition nor the quantity of food consumed by the experimental subjects. The gum given to the first group of 55 subjects was a regular commercial product. That given to the second group was identical except that 0.75 mg. of sodium bisulfite addition product of 2-methyl-1,4-naphthoquinone had been incorporated into the sugar coating of each pellet. The flavor of the gum was not altered

by this addition and neither the subjects nor the examiners knew which gum had been assigned to the subject. A control group of subjects, selected from the same dental student classes, were examined as minutely as the other 2 groups but were given no gum. Due to lack of cooperation, 10 of the subjects in the plain gum group and 9 in the vitamin K gum group had to be dropped from the experiment. A reduction in the incidence of new carious lesions was observed in both experiment groups. At the end of the 18-month period, on the basis of radiographic examination the authors stated that the incidence of new carious lesions in the subjects who had chewed vitamin K gum was 69 per cent lower than that of the control group. The incidence of new carious lesions in those subjects who had chewed plain gum was stated to be 60 per cent lower than that of the control subjects. On the basis of clinical and radiographic examination combined, the increment of new carious lesions in the vitamin K gum subjects was calculated to be 54 per cent lower, while the increment of new lesions was found to be 21 per cent lower than that of the control subjects who chewed plain gum. There was stated to be no apparent relation between the chewing of either gum and the inhibition of the carious process in lesions which were present at the beginning of the experiment.

The data presented by Burrill *et al.* merit further consideration. For example, by multiplying the average number of new cavities per person by the number of persons in their respective experimental groups, the total number of lesions observed by these workers can be calculated. Thus the total number of new carious lesions observed at the end of 18 months by radiograph in the 55 members of the vitamin K gum group was 9 (0.17×55), while during the same period 10 (0.22×45) new lesions were observed in the 45 members of the plain gum group. The number of new cavities observed by clinical and radiographic examination combined was 35 (0.64×55) for the former group in comparison with 50 (1.10×45) new lesions in the latter group. In view of the difficulties encountered in making an accurate diagnosis of the initiation and progress of carious lesions, it is believed that these differences are too small to justify any conclusion to the effect that vitamin K as administered in this experiment had produced a reduction in the incidence of tooth decay. No statistical evaluation of the data had been made by these investigators nor was such an evaluation possible from the data presented in the paper.

The effect of synthetic vitamin K in chewing gum on the incidence of carious lesions has been investigated by the Medical Department Professional Service Schools at Washington, D. C. (1946). Sixteen men each chewed gum containing 1 mg. of synthetic vitamin K for at least 10 minutes after consuming food or liquids. A similar group of men chewed plain gum at the same intervals. The men in each group were examined clinically and by radiographs at intervals of 3 to 4 months. Analysis of the data indicated that there was no difference between the number of new carious lesions in the experimental and the control groups.

Previously Fosdick, Fancher and Calandra (1942) reported that a definite reduction in the amount of acid produced in incubated saliva occurred when synthetic vitamin K was present. Armstrong and Knutson (1943) and Armstrong, Spink and Kahnke (1943) have presented evidence which indicated that the antibacterial action of synthetic vitamin K was not specific but was characteristic of a number of quinones, some of which did not have vitamin K activity for animals. It is clear from the investigations of Burrill *et al.* (1945) and the Medical Department Professional Schools (1940) that vitamin K was not being tested as a dietary or nutritional factor but rather as an enzyme inhibitor. Since vitamin K is probably a nutritional essential for man, the

discussions of the above data are merited in the review.

Résumé: At present little is known to have been published on the effect of vitamin K deficiency on the teeth and supporting structures either during development or after maturity. Two reports have been published on the relation of vitamin K in chewing gum to the incidence of new carious lesions. In one of these, the authors concluded that a reduction in tooth decay had been produced over the 18-month experimental period, but this conclusion was not clearly substantiated by the data; in the other no relation was observed. In both cases the subjects were young adults. An experiment with children as subjects, when the expected increment of carious lesions is highest, might give more definite results.

Seasonal Variation in Dental Caries Incidence

Price (1930) stated, without figures, that "a survey of my clinical records indicates approximately four times as much dental caries in the winter and spring as in the summer and fall." He believed that the seasonal variation was "because of the rise and fall, at different seasons, of the level of the activators in the foods, some of which are vitamins."

Erpf (1938) determined the "caries susceptibility index" of 143 dental students in San Francisco at different seasons of the year as follows:

Fall 1934	4.36
Winter 1934–35	4.76
Spring 1935	2.72
Summer 1935	2.28
Fall 1935	2.70
Winter 1935–36	2.87
Spring 1936	1.08
Summer 1936	0.58

It can be seen that there was a decrease in the incidence of detectable carious lesions during the spring and summer of these 2 years. No attempt was made to discuss the much lower "caries susceptibility indices" which were observed in the year 1935–36 in comparison with the previous year. Erpf stressed the need for adequate controls in all dental caries experiments, but especially in experiments of such short duration that any seasonal variation could be an appreciable factor.

McBeath and Zucker (1938) have presented data which also indicated a seasonal variation in the incidence of carious lesions in New York children. They observed in a group of 218 children an increase in carious surfaces of 2.07 per person during 100 days in the autumn-winter period in contrast to 3.09 for 100 days in the winter-spring period. In another group of 68 controls the increase in carious surfaces during the summer was only 0.64 per person. From their experimental groups, they concluded that 800 I.U. of vitamin D as cod liver oil were necessary to reduce the increase in carious surfaces in experimental children during the winter to the level observed during the summer months.

The staff members of the Ellen H. Richards Institute (1942) have recorded quarterly observations of 63 children for one year. They found that "the incidence of dental caries was least in June and greatest in November and January."

Lathrop (1941b, 1943) enumerated the seasonal occurrence of new carious surfaces in 82 school boys at Letchworth Village in New York State between the ages of 9 and 22 years, with the majority between 10 and 15 years as follows: October to January, 240; January to June, 89; June to November, 68; November to April, 132. These data were obtained with dental mirror and explorer, and bite-wing roentgenograms.

Résumé: The data indicate that there may be an association between the season of the year and the dental caries incidence. The recognition of new carious lesions is difficult but, insofar as they can be diagnosed as such, the greatest incidence appears to occur in the fall and winter and the lowest incidence in the spring and summer. A possible explanation of the seasonal

variation in caries activity would be the relative hours of sunlight in the various seasons. The greater availability of fresh fruits and vegetables during the summer, however, must also be considered as a possible factor. The association between seasons of the year and dental caries incidence merits further intensive investigation as a possible route through which etiology and control of dental caries might be evaluated. In such a study the collection of further data may be limited by present inability to diagnose accurately the activity and rate of progress of carious lesions.

Calcium, Phosphorus and Dental Caries Incidence

Toverud (1923, 1926) observed a low calcium and high magnesium retention in rats on a calcium-deficient diet. The blood calcium fell from 11 or 12 mg. to 5 mg. per 100 cc. serum, but no tetany was observed. Bones from these rats showed a great reduction in total ash, calcium and phosphorus, with a possible increase in magnesium. There was a slight tendency towards a reduction in the total ash, calcium and phosphorus content of the molars and a small increase in the magnesium content. There was a definite decrease in the total ash, calcium and phosphorus content of the incisors and a definite increase in the magnesium content.

Gaunt, Irving and Thomson (1939) observed that a calcium and phosphorus deficiency in the rat resulted in a progressive increase in the width of the predentin, from the normal of 10 to 20 microns to 90 to 100 microns. The newly formed dentin was imperfectly calcified and with chronic deficiency had vascular inclusions. However, Gaunt and Irving (1939) found no changes in the enamel organ or in enamel formation and calcification.

Albright, Aub and Bauer (1934) studied the calcium metabolism in 16 cases of proved hyperparathyroidism. In cases of marked disease, the loss of calcium from the bones was very great while the teeth remained intact. The absence of lamina dura in the tooth socket was a manifestation of this decalcification. The teeth remained well calcified but frequently were lost because of the decalcification of the alveolar bone.

Hansen (1938) conducted metabolic studies in three types of clinical disorders which were associated with a disturbance in calcium and phosphorus metabolism. In osteogenesis imperfecta, where there was failure of the mesenchymal tissues to develop normally, the teeth showed no particular gross or radiographic abnormality. In severe decalcification of the skeleton due to primary carcinoma of the liver, clinical examination showed no disturbance of the teeth. The bone, in cases of hypoparathyroidism, appeared to be normal, but there was a marked degree of hypoplasia with enamel defects and many carious teeth.

Siegel, Stowe and Ziskin (1942) made clinical and radiographic examinations of the jaws and teeth of children with such diverse chronic conditions as: renal rickets, refractory rickets, Cooley's anemia, hyperparathyroidism and generalized xanthomatosis. As far as could be determined, the development of the teeth was not impaired in any case. The dental caries experience in the deciduous and permanent teeth did not vary materially from the average in normal children.

Kronfeld (1940), Schour (1938) and Robinson (1943) have summarized the literature on calcium therapy in dentistry and concluded that there was no known benefit on the dental caries incidence.

Lennox (1929a, b) proposed the hypothesis that dental caries was one result of a phosphorus deficiency, and considered that the intense sunlight of South Africa eliminated vitamin D deficiency as a possible cause of the extensive dental caries there. He believed that the phosphorus-deficient soil, the inadequate phosphorus content of the foods grown on phosphorus-deficient soils, the poor choice of foods and the improper methods of cooking among civilized

races made it impossible to obtain an adequate intake of phosphorus. During inadequate phosphorus consumption Lennox felt that there would be a leaching of phosphates from the teeth in an attempt to make available the necessary phosphorus for metabolism and that one external manifestation of this leaching was dental caries. Later Lennox (1931a) postulated that the action of phosphorus in maintaining teeth was manifest through the saliva. As an illustration to support the phosphorus hypothesis, Lennox (1929a) stated that the African tribes which drank milk had a low dental caries experience even when the starch content of the diet was high. However, upon contact with civilization at the mines where they worked, Lennox stated that a rapid deterioration of the natives' teeth was observed. No specific dental caries experiences of such tribes were given and other variants in the diet were not evaluated.

Lennox (1929a, b, 1930a, b) deduced from food composition tables that the average diet of civilized races in South Africa was deficient in phosphorus but contained adequate calcium and magnesium. Illustrations were presented to indicate that the replacement of the refined foods with unrefined ones comparable to those used by the "raw" native would increase the phosphorus of the diet to an adequate concentration.

In a further attempt to eliminate the possibility of a calcium deficiency, Lennox (1931b) determined the dental caries incidence in the 119 school children at Tsumeb in the district of Grootfontein, where the drinking water contained 0.141 mg. of calcium, expressed as calcium oxide, per liter. If a child consumed an average of 2 liters of this water per day, he would obtain 0.282 gm. more calcium than a child living in a district where the drinking water was soft. The dental caries experience was not reported, but Lennox stated that it was not lower there than in parts of South Africa where the water was not as hard and that the teeth in this area probably decayed more rapidly than in other regions. Although no data were presented to substantiate the claim, Lennox stated that the routine administration of 1 gm. of sodium glycerophosphate daily to all patients arrested decay and also pyorrhea.

Friel and Shaw (1931) have pointed out that the farm animals in South Africa frequently suffer from phosphorus deficiency but that the farmers of the same regions were a collection of superbly formed men and women.

Hewat (1931) observed a low dental caries experience in 32 orphanage children in New Zealand and implied, without any supporting evidence, that this low rate was due to a higher dietary phosphorus than that of children residing at home.

Osborne (1932) drew attention to the excellent physical development of the Australians as an indication that they were not phosphorus deficient, despite a very definite phosphorus deficiency in the soil.

Torrens (1941) recommended the use of two nutritive mineral tablets per day. No data were presented to substantiate the statement that the regular use of these tablets led to a definite control of dental caries. However, Dale (1942) drew attention to the fact that each of these tablets contained 0.10 grains of sodium silicofluoride and that the control of dental caries described might have been due to the 0.8 mg. of fluorine consumed daily.

Malan and Ockerse (1941) studied the effect of an increase in the calcium and phosphorus intake of Pretoria school children in South Africa, ranging in age from 6 to 14 years, upon the dental caries increment over a 3-year period. The 85 control children received the typical diet of the district, which supplied a calculated average of 0.43 gm. of calcium and 0.79 gm. of phosphorus daily. The 97 experimental children received the same diet plus one tablet each per day which contained 0.5 gm. of calcium and 0.5 gm. of phosphorus. The total calcium and phosphorus intake of the

children in the experimental group is still somewhat lower than the Recommended Dietary Allowances (1945). During the school vacations, which totalled 100 days per year, no tablets were provided. No significant difference in the dental caries susceptibility of the deciduous and permanent teeth of the two groups was observed.

Harootian (1943) reported studies on the effect of feeding 5 grains of bone flour 3 times daily to 9 psychotic patients in the Worcester State Hospital, 22 to 46 years of age, whose life dental caries incidence was high. During the 9 months of observation, only 1 carious lesion developed. However, no similar data for a suitable control group were reported. Harootian postulated that the described effect was due mainly to the fluorine content but that a supporting effect of the calcium, phosphorus and unidentified constituents of the bone flour could not be excluded.

Taylor (1941, 1942) suggested that there was a relation between the fluorine content of the drinking water, a high calcium and phosphorus content of the locally grown produce and the dental caries incidence in Deaf Smith County, Texas. However, McClure (1944) compared the calcium and phosphorus concentrations in grain grown in that district with produce from other regions and did not find any appreciable difference.

Hubbell and Koehne (1934) observed the calcium, phosphorus and nitrogen retention of Michigan children whose caloric intake was increased 16 to 18 per cent by addition of sugar without alteration of other constituents of the diets. They observed a slight tendency to increase the nitrogen and phosphorus retention and to decrease the calcium retention. These children received no supplemental vitamin D and consequently the diet was not complete. The calcium retentions were relatively low in both the experimental and the control groups.

Boyd, Drain and Stearns (1933) studied the retention of calcium and phosphorus in relation to activity of dental caries in 98 Iowa children. For 28 children with no dental caries the daily calcium and phosphorus retentions for each kilogram were 17 and 15 mg., respectively. For 38 children with active dental caries the values were 10 and 9 mg., respectively. The differences found were statistically significant.

Drain and Boyd (1935) made metabolic studies of five children whose dental caries activity had increased during a supervised period in an orphanage in which the institution diet was protective against dental caries for 80 per cent of the children. When the five children were given special metabolic study with a more abundant diet than that supplied by the orphanage, the calcium and phosphorus retentions increased from relatively low values to higher ones as the study progressed. Concurrently with the increase in retention the dental caries became arrested.

In the preceding study by Drain and Boyd it was noted that the institution diet protected 80 per cent of the children against dental caries and left 20 per cent unprotected. An explanation offered for this distribution of dental caries is that children have individual variations in efficiency. With similar diets of borderline inadequacy certain children are protected while others are not. Instances in which the inefficiency was marked and measurable have been cited by Stearns (1942) and Jeans (1944). One of these offers excellent evidence of the relation of calcium retention to dental caries. A 14-year-old girl was brought for care because of rampant dental caries, 28 new cavities being present. An excellent diet, low in free sugar, was given under close control and supervision. Because no change was observed in the dental caries status and because calcium retention was poor, the amounts of vitamin D and of milk were increased. Calcium retention continued poor and the dental caries status the same. Poor utilization of the fat-soluble vitamins was suspected and confirmed. The

girl had very poor dark adaptation when tested. Administration of bile salts produced immediate improvement in dark adaptation and rapid improvement in calcium retention. The improvement in dental caries status paralleled that of calcium retention. In this instance the diet had remained essentially the same throughout the experimental observation period. The dental caries activity was arrested only when calcium retention improved.

Siegel, Waugh and Karshan (1940) found no correlation between the retention of calcium, phosphorus and nitrogen and dental caries in children 7 to 15 years of age in three Eskimo settlements. In one community the diet was very low in calcium and moderately high in phosphorus, while the other two had diets high in calcium and phosphorus. Two of the communities had potentially acid diets and the third had a potentially basic diet. The authors believed that there was no basis for the view that freedom from dental caries in primitive peoples is due to nutritional superiority of "natural" as compared with "civilized" diets.

In one of the groups of the Eskimo study, positive daily calcium balances of 51.6 and 58.2 mg. for each kilogram of body weight were found for children who were free from dental caries and for those who had dental caries, respectively. In another of the Eskimo groups negative calcium balances of 33.7 and 3.0 mg., respectively, were found. The values found for both these groups are definitely abnormal and could not continue without disaster to the children. While they may be true findings for the two-day balance periods, they cannot represent the continued regular day-to-day metabolic behavior of the subjects of the study. The per cent of calcium intake retained by the high retention group is approximately twice the maximum ordinarily found. The values reported are so far from normal expectation that the placing of reliance on the conclusion is difficult.

Résumé: No data have been presented that indicate any increase in the dental caries experience of children receiving diets partially deficient in calcium or phosphorus or both. Supplementation of a diet moderately low in calcium and phosphorus with additional amounts of these minerals by Malan and Ockerse (1941) did not result in reduction of the dental caries increment over a 3-year period. The data presented by Harootian (1943) and Taylor (1941, 1942) must be considered further experimentally before acceptance.

The few cases studied in which the metabolism of calcium or phosphorus or both had been disturbed by hyperparathyroidism, hypoparathyroidism, osteogenesis imperfecta, renal rickets, refractory rickets, Cooley's anemia, generalized xanthomatosis and primary carcinoma of the liver have not shown any increase in the dental caries experience. For some of these conditions alterations in the teeth would not be expected. For example, osteogenesis imperfecta represents an abnormality of the osteoblasts of bone, and no primary defect in mineral metabolism exists. Also in the removal of mineral from bone, as in hyperparathyroidism, presumably osteoclasts play an active part. The teeth have no cells corresponding to osteoclasts and any loss of mineral from the teeth must occur through a different mechanism. Critical and individual consideration is needed in correlating the status of the teeth with these various disturbances of mineral metabolism.

On the other hand, no one has shown the concurrent existence of dental caries activity and good retention of calcium and phosphorus. Boyd, Drain and Stearns (1933) Drain and Boyd (1935), Stearns (1942) and Jeans (1944) have presented strong evidence of a relationship between dental caries and retention of calcium and phosphorus. All the children studied had inactivity or arrest of the carious process when retention of calcium and phosphorus was good.

Drain and Boyd (1935) observed that

children have individual variations in efficiency of utilization of nutrients and that with similar diets of borderline inadequacy certain children are protected against dental caries while others are not.

Stearns (1942) reported instances in which utilization was poor even when the diet was fully adequate by common standards. Improvement of conditions required for absorption resulted in increased retention of calcium and phosphorus and in arrest of dental caries. These data indicate clearly that consideration of the individual's ability to utilize the minerals present in the diet is more important than the actual amount in the diet.

Calcium and Phosphorus Concentrations of the Blood and Dental Caries Incidence

Klein and McCollum (1931b) believed that a relation existed between the phosphorus concentration of the blood and the incidence of dental caries in the white rat. They stated that when blood phosphorus fell below the critical level of 10.5 ± 0.5 mg. of phosphorus per 100 grams of serum, dental caries occurred more frequently.

Karshan, Krasnow and Krejci (1931a, b) studied the calcium, phosphorus and protein concentrations in the blood of subjects in New York which were immune or susceptible to dental caries. There was no significant difference in the concentrations of these measured constituents.

In Stockholm, Sweden, Jundell and Magnusson (1933) found that the calcium content in the sera of 12 caries-free children averaged 10.55 ± 0.25 mg. per 100 cc. and of 20 children with extensive caries 9.92 ± 0.13. The corresponding inorganic phosphorus values were 5.01 ± 0.16 and 4.71 ± 0.10. These differences were found to be insignificant.

Dobbs (1932c) determined the blood calcium and phosphorus concentrations of 1000 children under 3 years of age in Rochester. He found that the average value of calcium was 10.38 mg. per 100 cc. but exceeded 11 mg. in 55.6 per cent of the cases. The average value of phosphorus was 4.2 mg. per 100 cc. but was below 3.2 mg. in 53.4 per cent of the cases. Dobbs believed that a low blood phosphorus and a high blood calcium predisposed to abnormally formed teeth and dental caries. No significance can be attached to these data since no attempt was made to classify the children according to their dental caries activity at the time of the chemical determinations. Hawkins (1931a, b, 1932) had reported previously that he believed low phosphorus and high calcium blood concentrations to be responsible for dental caries.

Schoenthal and Brodsky (1933) determined the calcium and phosphorus concentrations in the sera of 200 children in New York City at the beginning of and during a 13-month period of vitamin D therapy. They found no correlation between calcium and phosphorus of the serum and dental caries.

During 2 years of dietary investigation Hanke *et al.* (1933) followed the total calcium and acid-soluble phosphorus values of the blood of 323 children 11 to 17 years of age at Mooseheart, Illinois. The children received a control diet during the first year. During the second year a daily supplement of 1 pint of orange juice and the juice of 1 lemon was reported to have reduced substantially the dental caries increment among the children. However, no change in calcium and phosphorus concentration accompanied the reduced increase in new carious lesions.

Detailed studies of the chemical composition of the blood of 12 caries-free and 17 distinctly caries-susceptible children 9 to 16 years of age were made by Hubbell (1933). The values for blood calcium, inorganic acid-soluble phosphorus, carbon dioxide capacity and pH were the same for the 2 groups.

Kerley, Lorenze and Godfrey (1935) determined the blood calcium and phosphorus concentrations in 48 children in New York City who were susceptible to dental caries.

Since all the patients had a phosphorus content between 4 and 6 mg. per 100 cc. serum and 46 had a calcium content greater than 9 mg. per 100 cc. serum, the authors concluded that there was no correlation between the calcium and phosphorus levels of the blood and dental caries. However, a significant relation was found to exist between the adequacy of retention of calcium and phosphorus and the resistance to tooth decay.

Extensive metabolic studies of children with dental caries were made by Boyd, Drain and Stearns (1933). They concluded that the levels of calcium and phosphorus in the blood and the acid-base relationships were not of primary significance in the production of dental caries. However, a positive correlation was found to exist between the adequacy of calcium retention and the resistance to tooth decay. Little is known of the relation of nutrition to the calcium and phosphorus contents of saliva. However, the relations of calcium and phosphorus in the saliva to dental caries incidence have been studied intensively by numerous investigators. An adequate review of their data is presented in the section on Oral Environment and Dental Caries.

Résumé: No relation between the calcium or the phosphorus concentration of the blood and the dental caries incidence has yet been demonstrated. Probably the retention of calcium and phosphorus is of greater importance and a better diagnostic sign than blood concentrations, since the mobilization of calcium and phosphorus from the body reserves could be occurring for long periods prior to any significant decrease in the blood concentration.

Acid-Base Balance and Dental Caries Incidence

Simonton and Jones (1927) described what they considered to be a clinically unrecognized form of rampant tooth decay in the pre-school children in Honolulu, which they termed odontoclasia. Subnormal bone growth was observed during the early months and was believed to be related to the tooth decay.

Jones, Larsen and Pritchard (1930a, b) reported the incidence of odontoclasia in 1810 young, non-Caucasian children as 33.3 per cent of babies under 1 year of age, 58.2 per cent between 1 and 2 years of age, 72.4 per cent of 2-year-olds, and 78.4 per cent of 3- and 4-year-olds. The incidence was highest in Orientals, next in Hawaiians and both higher than in Caucasians. Odontoclasia was not associated with rickets, malnutrition or any other known disease. Most of the children had been breast-fed and had lived in a subtropical, sunny environment. Certain diets that contained no milk and had a high carbohydrate content were reported to have been compatible with sound enamel for hundreds of years. Candy and sweets were not believed to be a causative factor. Cod liver oil, egg, orange juice, tomato juice and milk in adequate amounts, alone or together, did not prevent the rapid disintegration of the teeth during periods of active bone growth. It was stated, however, that the children who received enough fruit and vegetables to produce an alkaline ash invariably were observed to have sound enamel or arrested decay. On the other hand, active decay was reported to be present always in the children whose diets contained excess acid mineral elements. The authors postulated that a diet which tended to increase the amount of base in the tissue fluids and oral secretions would proportionately decrease the liability of enamel to decay.

Jones, Larsen and Pritchard (1932) observed enamel defects which they described as closely simulating decay in unerupted teeth of breast-fed infants when the mother's diet contained inorganic acid elements in excess.

Jones, Larsen and Pritchard (1934) made a survey of the alkaline and acid ash po-

tentialities of typical Hawaiian diets. When 30–40 per cent of the total caloric intake was in the form of grain foods, they calculated the potential alkalinity to be 6 to 10 cc. of normal alkali daily. When roots and tubers (taro or poi and sweet potatoes) replaced the grains as the source of carbohydrates, the potential alkalinity of the diet was 36 to 45 cc. of normal alkali daily. The authors stated that a high incidence of dental caries was observed in the people on the former dietary regimen but a markedly lower incidence in those on the latter.

Ker (1936) presented a critical review of the studies of Jones and co-workers. He believed that there were many other factors to be checked before any credence could be given to the favorable effects of diet with a potentially alkaline ash in the prevention of dental caries.

From limited diet surveys in New York City, Kugelmass and King (1933) made the observation that children receiving a potentially alkaline diet were more likely to be free of caries than those receiving a potentially acid diet.

According to Price (1935), four out of five primitive racial stocks, whose native foods he evaluated, were living on diets which were potentially acid-forming. On changing to civilized habits, an increase in susceptibility to dental caries occurred, but Price believed that there was no increase in the potential acidity of the new diets.

Forshufvud (1937–38) reported that dental caries occurred in the molars of the white rat when the acid-base balance of the blood was disturbed by frequent, alternate injections of sodium bicarbonate and ammonium chloride. Thomas and Bodecker (1942), however, were unable to produce dental caries in the rat with a similar experimental procedure.

Résumé: At present there has been presented no conclusive evidence on the relation of the potential alkalinity or acidity of the dietary ash to dental caries susceptibility.

Hardness of Public Water Supplies and Dental Caries Incidence

Devot (1855) examined the distribution of dental rejections among French recruits for the years 1831 to 1849, inclusive. The rejection rates varied from 36 per 100,000 in Pury de Dome to 6,760 per 100,000 in Dordogne. There was a definite geographical distribution, with the lower rejection rates in the high regions of south central France and in the Brittany peninsula, while the higher rejection rates were in northern and eastern France. There was a tendency for the rejection rate to be higher at the mouths of the rivers except the Rhone. Devot concluded that there was a higher dental caries incidence in the low countries, a lower dental caries incidence in the warm countries and a relation between the dental caries experience and the composition of the drinking water. Subsequently Devot's data were interpreted by Magitot (Chandler, 1878) to indicate a racial explanation of the observed geographic distribution of dental rejections.

As the result of a survey on the relation of the hardness of the drinking water to the dental caries incidence, Röse (1894) reported that 98.2 to 99.2 per cent of the 12,161 German children living in six localities where the hardness of the water was between 1.7° and 2.8° had carious lesions in 27.2 to 36.9 per cent of their teeth. Among the 911 children from 3 areas where the hardness of water ranged from 11.7° to 19°, however, the percentages of children with caries were 91.8, 87.7 and 70.8 while 90.7 per cent of the 3,595 children from areas with water hardness between 1.1° and 5.6° had dental caries, with 25.1 per cent of their teeth affected. In the 2,708 children in 19 communities with water ranging from 5.9° to 44° of hardness, the percentage incidence of dental caries was 73.6 to 95.5, with a maximum of 27 per cent and a minimum of 10.2 per cent of the teeth carious. In addition, Röse felt that there was a tendency

towards a higher dental caries experience when white bread was consumed instead of rye bread.

Röse (1908) summarized all his studies on the relation of hardness of drinking water to dental caries incidence showing a consistently inverse relationship between water hardness and dental caries.

Röse observed also that there was an association of the color of teeth with the hardness of water and the freedom from carious lesions. Yellowish teeth were least susceptible, white intermediate and gray-blue most susceptible. Yellowish teeth were most common in districts where the drinking water was hardest, while gray-blue teeth were most common where the drinking water was the least hard.

Förberg (1901) also reported a relation in Swedish children between the frequency of dental caries and the hardness of water. His data indicated that there was a definite inverse relationship between the dental caries incidence and the hardness of the water supply at any age between 8 and 15 years.

In a survey of a small number of English children over 12 years of age, Cook (1914) correlated the temporary, permanent and total hardness of the drinking water with the percentage of children having 4 or more carious teeth. He observed a high inverse relationship with the temporary, permanent and total hardness of the water and concluded that "the harder the water, the better the teeth."

A survey was made under the supervision of the Medical Research Council of Great Britain (1925) to determine if there were an association between hard and soft water supplies and the dental caries experience in school children. The data indicated that the children in the country districts where the drinking water was hard had a lower dental caries incidence in their deciduous and permanent teeth than those children in country districts where the drinking water was soft. No appreciable difference was observed in the dental caries experience of the children living in towns with soft and those with hard drinking water.

Mills (1937) compared the dental caries data on 12- to 14-year-old children in United States cities of over 10,000 population (Messner, Gafafer, Cady and Dean, 1936) with the hardness of the water supplies in their cities. The hardness in p.p.m. and average number of carious teeth per 100 children were:

Below 50	496
50–99	397
100–199	476
200–299	355
300–399	382
400–499	342
500 plus	318

The detailed dental caries rate and hardness of water were given. There were numerous exceptions to the definite trend of a lower dental caries incidence with increasing hardness of water. This would be expected in any survey in view of the numerous other factors which might be present in the various cities to alter the caries incidence and in view of the method used by Messner *et al.* (1936) for the collection of the caries data.

Mills (1937) also observed a relation between the dental caries experience in the United States and the slope of the river drainage areas. The dental caries incidence tended to increase from the head waters toward the mouth. The same was true where a state had a more or less general slope. Mills stated: "There would therefore seem to be some factor in soil or water which tends to produce less caries in the upland regions or head waters of rivers and more caries down near the mouth. Whether this be greater leaching of the soil in the lower regions or greater hardness of the drinking water in the uplands is unknown." It is interesting to note that Devot (1855) observed a lower rejection rate due to dental causes in the higher departments of France than in the regions at the mouths of the rivers except the Rhone.

Chapin and Mills (1942) studied the dental caries experience in the 12- to 14-year-old children in the Panama Canal Zone, where the hardness of the drinking water was less than 50 p.p.m. The dental caries incidence which they observed was equalled "only in certain northern cities using river or lake water (Cincinnati; Milwaukee; Portland, Maine)." It was interesting to note the apparent but temporary immunity that American-born Americans residing in the Canal Zone had.

From the data of 109 cities in the United States in the survey on dental caries of Messner, Gafafer, Cady and Dean (1936) and the studies of the hardness of public water supplies of Collins, Lamar and Lohr (1934), East (1941a) demonstrated an inverse relationship between high hardness of the drinking water and high incidence of carious teeth in each of the age groups, 6 to 8, 9 to 11 and 12 to 14. No significant relationship was found, however, between either the mean annual hours of sunshine or the latitude and the hardness of water.

Ockerse (1944a, b) reported observations on the relationship of the fluorine content, the hardness and the pH values of drinking water to the caries incidence in 78,563 South African children from 109 cities and towns and 86 districts. He observed a highly significant relationship between the fluorine content of the water and the dental caries incidence and also between the hardness of drinking water and the dental caries incidence. There also appeared to be an association between a high pH of the drinking water and a reduced dental caries incidence. This is the only known extensive study in which the association of the fluorine content and of the hardness of the drinking water with the dental caries incidence have been considered simultaneously. The detailed data obtained in each town and district contained a few examples of relatively low dental caries experience in the regions with water supplies of low fluorine content and a high hardness, while a few regions also were found where there was a relatively high dental caries experience when the water supply contained from 0.8 to 1.1 p.p.m. of fluorine and low hardness. These apparently independent effects, if demonstrated to be real, need further elaboration, since they may influence the results of artificial fluorination of drinking water.

Dean (1938) published observations of a few districts in the United States which indicated that reduction in dental caries incidence occurred when the fluorine content was high even when the hardness of the water was low.

Blackerby (1943) observed a slight inverse association between the carbonate, chloride, fluoride and iron concentrations of Tennessee drinking water supplies and the dental caries experience in different regions of the state. In addition there was a relation between the hours of sunshine and the dental caries incidence. This is an example of the great necessity for careful evaluation of all data in such a complex field. The differences observed by Blackerby were all interesting trends, but in each case the differences in hours of sunshine and water concentrations were small. One or more of these factors might have contributed to the decrease in dental caries incidence observed. On the other hand, it might be that none of them was responsible. This statement is not a criticism of this work but is meant rather as a suggestion for the need of careful evaluation and consideration of every possible variant in a survey of this type.

Résumé: The data reviewed indicate that there is an association between an increasing hardness of the drinking water supplies and a decrease in the dental caries experience. This association has been studied in greatest detail by Ockerse (1944a, b) who considered the pH and the fluorine content of the drinking water in addition to the hardness. His data clearly indicate that there is a definite association between the hardness of the drinking water and the dental caries

experience, independent of the fluorine association. However, Ockerse's data do not rule out fluorine as a possible contributing cause for the reduction. In the other surveys no consideration is taken of the fluorine concentration of the water supplies. Future surveys should be designed to study, not only the association of hardness and of fluorine content, but also the association between the concentration of other factors in the water supply and the dental caries incidence. The fact that hard waters frequently contain fluorine and that fluorine-bearing waters frequently are hard makes investigations to study their separate effects difficult but none the less important.

Relation of Soil Fertility to Dental Caries Incidence

Taylor (1941, 1942) suggested that the low incidence of tooth decay among the native-born continuous residents of Deaf Smith County, Texas, could be due partially to the high calcium content of the soils and the high calcium and phosphorus content of certain food plants growing thereon. McClure (1944) later presented data which indicated that foods grown in this area did not have higher calcium or phosphorus values than comparable food from other parts of the United States.

Albrecht (1947) has made an extensive survey of weathering factors affecting soil fertility, its effect upon the composition of foods, and the possible relation of varying degrees of soil fertility to the dental health of the residents in these districts. The data presented by Schlack and Birren (1946) on susceptibility to dental caries of 69,584 naval inductees were segregated by Albrecht according to the districts of major degrees of soil fertility. These men represented 93 per cent of the original group from which the remainder had been eliminated for dental reasons. This selection may have reduced the regional differences which Albrecht deduced. The United States was divided from east to west into five districts on the basis of soil fertility: the east coast, the central eastern states, the midwestern states, the far western states and the west coast. The numbers of carious lesions per man for these districts were 17.55, 14.95, 12.08, 13.10 and 15.50, respectively. The east coast was further subdivided, with the men from New England states having an average number of carious lesions of 21.3, in contrast to 19.6 for the men of the middle Atlantic states and 13.4 for the men of the southern Atlantic states.

There are possibly other conditions which may have been superimposed upon the dental caries distribution described by Albrecht, based on Schlack and Birren's survey of naval inductees. One should be interested in considering further the association between increased annual hours of sunshine and reduced incidence of tooth decay described by Mills (1937) and by East (1939). In addition, one should consider the distribution of fluorine and hardness to drinking water supplies in relation to caries susceptibility. Either or both of these variants may have had a causal relation to the data on regional caries susceptibility summarized by Albrecht. Further examination is needed to determine, not only for dental caries but for numerous degenerative diseases, whether the examples of alleged lower food quality which have been related to soil fertility do have a causative relation to the differential incidence of these diseases in various parts of the country. In an age when there is so much interchange of food products among the various parts of any nation, one would expect that it would be difficult, in view of the present inadequate knowledge of the subject, to generalize very widely as to the relation of soil fertility to degenerative diseases. For example, in the heavily populated urban areas of the eastern seaboard, a larger percentage of the cereal grains, beef and lamb must come from the more fertile regions of the middle west. During winter months especially, appreciable quantities of the vegetables and fruits must come from the

south and west. If a causative relation between soil fertility and tooth decay should be demonstrated unequivocally, then it would seem that the foods brought into the eastern regions might have reduced the susceptibility to carious lesions, and possibly to degenerative diseases also, beyond what it might otherwise have been if all foods consumed had been locally produced.

Fluorine and Dental Caries Incidence

No attempt will be made in this section of the monograph to present and discuss the possible nutritional and oral environmental roles of fluorine in the reduction of the dental caries incidence, since the detailed direct and indirect evidences related to that subject are recorded in the section on Fluorine and Dental Caries. At this point attention will be drawn to the data which indicate that fluorine or some accompanying factor in the drinking water supply has played a definite nutritional role in the development and maturation of the teeth which have been developed.

From various surveys in the United States, Deatherage (1942, 1943a, b) has obtained evidence that a partial reduction in the dental caries increment continues at least until early adult life even when the fluorine-bearing waters were not consumed after the first 8 years of life and that little reduction in dental caries increment occurs when the exposure to fluorine-bearing waters began after the first 8 years of life. These data were collected from questionnaires given to selective service inductees and correlated with their total incidence of decayed, missing and filled teeth. Relatively large samples were represented in the various groups.

Strean and Beaudet (1945) conducted an experiment where calcium fluoride tablets were fed to children for periods of 6 to 8 months at a level of 3 mg. of calcium fluoride per day. It was reported that appreciably lower numbers of new carious lesions were observed in the children receiving the tablets than in the control children. The effect was believed to be augmented by the simultaneous ingestion of vitamins C and D. The short duration of the experiments raises questions as to the validity of the findings.

The dental caries experience of children of Japanese ancestry who were relocated during the war by the War Relocation Authority was studied by Klein (1945). One group of 120 children was housed in a camp in California where the drinking water supply contained not over 0.1 p.p.m. of fluorine. A second group of 180 children of similar age was located in a camp in Arizona where the drinking water contained 3.0 p.p.m. The dental caries experience of both groups was determined upon arrival in the relocation zones and again after 2 years of exposure to the respective water supplies. Klein concluded that among the children between the ages of 8 and 10 who were transferred to an area where the drinking water contained 3.0 p.p.m. of fluorine, the incidence of new carious lesions in previously noncarious erupted teeth was reduced approximately 60 per cent below that which was observed in the children who were located in an area where the drinking water contained only 0.1 p.p.m. of fluorine. Those children who were over 11 years of age at the time of the first exposure to fluorine-bearing waters had no significant reduction in their dental caries increments when compared to children of similar age relocated in an area with fluorine-low drinking water.

Klein (1946a) analyzed the above data with respect to the individual teeth. First and second molars and second bicuspids already erupted into the oral cavity were protected significantly against caries attack, provided they were exposed to the fluorine-bearing waters within a short time after eruption. The data on erupting second molars and second bicuspids were few in number but suggested that teeth which erupted during exposure to fluorine-bearing waters receive greater protection against the carious process than those which have

finished erupting. These data may indicate that the effect of the fluorine-bearing waters is almost entirely during development and maturation of the teeth.

Ammonia and Dental Caries Incidence

The occurrence and possible significance of certain aciduric bacteria, particularly *Lactobacillus acidophilus*, in the human oral flora have been discussed in the section of the monograph entitled Oral Environment and Dental Caries. Numerous studies have reported that these bacteria were scarce or absent in the oral flora of persons who have no active carious lesions.

Kesel, O'Donnell, Kirch and Wach (1946) observed that during an 8-day incubation period cultures of human saliva collected from individuals in Chicago who had no active carious lesions developed an ability to inhibit the growth of *L. acidophilus* and the conversion of glucose into acid because of the production of ammonia nitrogen from urea and amino acids. The inhibition of *L. acidophilus* was not believed to be due to the alkaline reaction produced, since the same degree of alkalinity produced by sodium acetate did not produce the amount of inhibition that ammonia did. Six amino acids were found to be present in the saliva analyzed, but no apparent correlation between the amounts of these substances and dental caries activity was found in 18 subjects of varying caries activity as measured by *L. acidophilus* counts. When dibasic ammonium phosphate was used clinically in a mouth wash or dentifrice for approximately 5 months by persons with active carious lesions, there was a marked reduction in the salivary *L. acidophilus* counts. The tests had not been in progress sufficiently long, however, to determine whether a reduction in the dental caries activity had been produced. The *L. acidophilus* counts are inadequate as diagnostic means for the evaluation of dental caries activity. If it should be determined after continued investigation that ammonia nitrogen production in the oral cavity is a prerequisite for the control of dental caries, and that there is a greater production of ammonia nitrogen in the oral cavity of individuals who are immune to caries attack, then it will become essential to know how the necessary substrates for the continued formation of ammonia in the saliva of caries-susceptible individuals can be provided by dietary or other means which can be applied to the mass control of tooth decay.

Stephan and Miller (1944) at the University of Chicago have studied the effect of using a saturated solution of urea as a dentifrice over a 2-year period. In their six subjects who had used this dentifrice and who had had a high caries increment during the period of observation prior to beginning the experimental period, a large decrease in the number of surfaces attacked by the carious process was observed.

Relation of Prenatal Care and Infant Feeding to Dental Caries Incidence

Toverud and Toverud (1930a, b, 1931) studied the calcium and phosphorus balance of 43 Norwegian women during pregnancy. Their diets contained 0.6 to 1.5 gm. of calcium per day and 0.7 to 1.5 gm. of phosphorus per day. However, a negative calcium and phosphorus balance was found frequently during the last part of pregnancy, even though the calcium and phosphorus intake appeared to be adequate. When a quart of milk was given daily in addition to the regular diet, the calcium and phosphorus balance improved greatly. Frequently the addition of cod liver oil improved the balance. Toverud and Toverud believed that poor calcification of the developing enamel and dentin predisposed to dental caries and that dietary precautions should be taken to insure a positive balance of calcium and phosphorus during pregnancy to provide optimum conditions for calcification.

Toverud and Toverud (1931) stated that eight children from Norwegian mothers who

consumed a minimum of milk (below one-half liter per day) during pregnancy showed twice as high an incidence of dental caries as did the three children whose mothers had consumed a high-milk, high-vegetable and fruit diet during pregnancy. The children were 3 to 14 years old at the time of this survey.

Losch and Morse (1936) observed a lowered rate of dental caries incidence in a selected group of Boston children of preschool and early school age. In relation to prenatal care, 94 per cent of the mothers whose children were free of carious lesions had had medical supervision during full-term pregnancy. Only 63 per cent of the mothers of highly caries-susceptible children had had such care. Regular medical supervision of care and feeding during infancy had been received by 88 per cent of the caries-free class, in contrast to 38 per cent of the highly caries-susceptible group. They postulated that dental caries in children was apparently influenced by illness during pregnancy and infancy but that no single factor determined dental caries immunity.

Berk (1943) at the Forsyth Dental Infirmary studied the relation of increasing numbers of pregnancies and the effect of diet during pregnancy upon the dental caries incidence. One hundred and ninety-eight 5-year-old children were selected, 106 of which were first-born and 92 fourth-born or later. The dental caries experience of the former group was 8.82 carious teeth per child and of the latter group was 10.42. These children were then divided into groups depending on the number of carious teeth per child: 5 or less carious teeth, 5 to 15, and 15 or more. The first and third groups contained 33 and 38 children, respectively. These 2 groups were compared in respect to first-born children, milk consumption during gestation and vitamin D supplements. There was a slightly higher percentage of first-born children in the low-caries group than in the high-caries group. Berk believed that the occurrence of multiple pregnancy was not itself an important factor in the incidence of dental caries. The mothers of the children in the low-caries group had a much higher milk consumption during gestation than the mothers of the high-caries group. Vitamin D supplements were received either throughout life or during the winter by a much higher percentage of the low-caries children than of the high-caries children. It was concluded that an adequate prenatal diet was an essential factor in the calcification of the teeth but that the present diet seemed to be the most significant factor in dental caries control.

Stuart (1945) has reported various findings, collected through examinations of newborn infants and infants during the neonatal period, which appear to have a relationship to the diets of their mothers during pregnancy. A poor diet was strongly correlated with retardation in the infants' osseous development. The difference between a good or excellent diet and a fair diet was not striking, although there was a somewhat greater percentage of retarded infants in the fair diet group. In the group of infants whose mothers had a very poor diet during pregnancy, few infants had an advanced and many had a retarded osseous development. From a standpoint of the protein intake during pregnancy, there was even a stronger relationship than to the quality of the entire diet. For example, in the group of infants whose mothers had had an excellent protein diet 57 per cent had an advanced osseous development, while 14 per cent were retarded; in the group of infants whose mothers had had a poor protein diet, none were advanced and 71 per cent were retarded. When the osseous development of the infants was compared to the calcium content of the maternal diet, there was a somewhat lower correlation than in the protein comparison.

The development of teeth as determined by the degree of calcification recorded in lateral radiographs of the head of living full-term infants at birth had much the

same relationship to the overall quality of the maternal diet as appeared in the comparison with the osseous development. In a comparison of the protein in the maternal diet to tooth development, it was found that 37 per cent of the infants from the mothers with excellent protein diets had advanced development, while 16 per cent were retarded; in contrast, none of the infants in the very poor protein group had advanced tooth development, while the teeth of 71 per cent were definitely retarded in development. Almost as strong a correlation was observed between the calcium content of the maternal diet and the stage of tooth development in their offspring.

In a survey of 6,800 Swedish children, Förberg (1901) concluded that there was an association between breast feeding and good teeth.

Frick (1901) classified the quality of the teeth of 200 English children by values from *1* to *5*. The value *1* was given to children of all ages with perfectly healthy jaws and dentitions with few carious lesions, while the value *5* was given to children under 5 years of age whose deciduous teeth had been attacked by severe carious lesions. Values *2*, *3* and *4* represented intermediate dental conditions. The average quality of the teeth of any group of patients was determined by dividing the sum of these values by the number of patients. For the entire 200, the average quality of teeth was 2.87. When the group was divided into those breast-fed and those artificially nourished, the average quality of the teeth was 1.58 for the former and 4.16 for the latter. Frick concluded that the increased predisposition to tooth decay which had been noted in the preceding 50 years had been due in part to a lowered resistance produced by the artificial feeding of infants.

Pedley (1916) reported the observations of Wheatley, the School Medical Officer for Salop, England. The number of carious teeth per child in 5,707 breast-fed children aged 5 to 6 was 6.7; among 3,375 bottle-fed children of the same age, incidence of carious teeth was 7.1. At the age of 12, the number of decayed teeth per child in 3,571 breast-fed children was 4.7, while in 1,900 artificially fed children the number of teeth decayed was 5. Wheatley concluded that the influences producing carious lesions were nearly as active in the breast-fed children as in the artificially fed children. He attributed the small difference to "the fact that in artificially fed children, the noxious habit of feeding on soft starch food is commenced earlier."

Durand (1916) made a study in Seattle, Washington, of the relation of breast feeding and artificial feeding to the dental caries incidence of 1,000 children between the ages of 2 and 7 years. His data indicated that there was a greater dental caries experience among the children fed sweetened condensed milk during infancy than among those which were breast-fed or received cow's milk. In 3 groups (61, 32 and 104 children) who had received sweetened condensed milk for 5 months or longer of the first year of life, 72, 53 and 74 per cent, respectively, of the children had carious teeth. In 2 groups (859 and 418 children) fed breast milk for similar periods during that time, dental caries developed in 43 per cent and 28 per cent, respectively. In 2 groups (232 and 102 children) fed cow's milk mixtures for similar periods, the incidence of caries reported was 42 and 29 per cent, respectively.

As the result of a survey of a small group of Norwegian children ranging from 3 to 14 years old, Toverud and Toverud (1931) stated that 70 per cent of the children with macroscopic hypoplasia of the enamel in the permanent teeth had received artificial feeding in infancy. The other 30 per cent with such defects had received breast milk for 2, 3 and 4 months, and in one case for the entire first year.

Mackay and Rose (1932) studied the relation of the duration of breast feeding and dental caries in 73 children in Great Britain.

When the duration was under 1 month, the number of carious teeth per child was 6.0. When the duration of breast feeding was between 1 and 4 months, between 4 and 8 months, between 8 and 13 months, and over 13 months, the number of carious teeth per head was 5.4, 4.7 and 7.6, respectively.

In New York, Kugelmass, King and Bodecker (1934) studied the nature of infant nutrition in respect to the duration of breast feeding, vitamin supplements and the potential reaction of the mineral ash of the diet. They concluded that dental caries was neither minimized nor prevented by breast feeding nor by vitamin supplements. The children who were maintained during infancy on potentially basic dietaries that contained early additions of semi-solid foods showed a consistently lower dental caries experience.

Day and Sedwick (1935) made a survey in Rochester of the relation of breast feeding to the dental caries incidence of 370 children at puberty. The average caries figure of the 282 children who had been breast-fed was 1.00 while the average caries figure of the 88 who had been bottle-fed was 1.06. If the 92 children who had been breast-fed for 7 to 10 months were considered, the average caries figure was 0.94, which Day and Sedwick believed might indicate a slight superiority for breast feeding. However, these data were not tested for statistical significance.

After a study of the incidence of carious teeth in 2,894 English children, Read and Knowles (1938) selected 12 caries-free children and 12 with extensive caries. The feeding of these 24 children in infancy was studied. In 11 of the caries-free children, but in only 4 of the caries-susceptible group, the diet during infancy was adequate. Read and Knowles stated that breast feeding without vitamin supplements could not be considered adequate.

Miller and Crombie (1938) selected 25 children in Great Britain who were caries-free and 25 who were caries-susceptible, with an average of 12 carious lesions each. These children were from families of the same economic status. Of the 25 caries-free children, 22 had been breast-fed, 12 had received cod liver oil and 14 had received orange juice. Of the 25 who were susceptible to dental caries, 15 had been breast-fed, 4 had received cod liver oil and 9 had received orange juice. There were 15 first-born and 8 fourth or later in the caries-susceptible group. These authors concluded that careful infant feeding, especially breast feeding, was an essential factor in increasing the resistance to dental caries.

Goll (1939) presented data from 232 German children on the relation of breast feeding to the dental caries incidence in deciduous teeth and concluded that after one month there was no favorable effect of breast feeding.

Sognnaes and White (1940) made a survey, at the Forsyth Dental Infirmary in Boston, of prenatal history and infant feeding in 32 children 4 to 13 years of age. They observed a direct association between good prenatal care and a lowered dental caries incidence in the teeth of the children, and a lower dental caries experience was observed in the children who were breast-fed for a period than in those always artificially fed.

Knowles (1948) reported the results of a survey on the effect of breast feeding on the dental caries experience of a group of 3- and 4-year-old English children housed in wartime day nurseries. The conclusion was formulated that day nursery children who were breast-fed for 3 months or more had a higher dental caries incidence than those who were artificially fed.

Résumé: The evidence indicates that there is a direct association between good prenatal care and a reduced dental caries experience in the deciduous teeth. Toverud and Toverud (1930a, b, 1931) have observed a definite negative calcium and phosphorus balance during pregnancy, which could be reduced by supplements of milk or cod liver oil or both to the diet.

Förberg (1901), Frick (1901), Pedley

(1916), Durand (1916), Mackay and Rose (1931), Read and Knowles (1938), Miller and Crombie (1938), Goll (1939), and Sognnaes and White (1940) have presented data which indicated that children who were breast-fed had a slightly lower predisposition to dental caries than those who had never received human milk. Goll (1939) believed that there was no favorable effect of breast feeding after the first month, while Mackay and Rose (1931) observed no beneficial effect after the first 8 months. Knowles (1948) concluded that children breast-fed 3 months or more had more tooth decay than those artificially fed. Durand (1916) indicated that cows' milk was as good as breast milk but that there was an increased predisposition to dental caries if sweetened condensed milk was used. Kugelmass, King and Bodecker (1934) believed that the potential alkalinity of the diet during infancy was of greater importance in the prevention of dental caries than the source of the milk.

On the basis of these data no final conclusion can be drawn as to the comparative value of human milk and cows' milk in infant feeding. The relation of rickets to tooth structure and to dental caries incidence has been discussed previously. Eliot (1925) has drawn attention to the fact that rickets occurred commonly in rapidly growing breast-fed infants. The amount of vitamin D in breast milk does not increase greatly even when large doses of vitamin D are given to the mother (Polskin, Kramer and Sobel, 1945). Before vitamin D was recognized and made available for infant feeding, the incidence of rickets was very high in both breast-fed and artificially fed infants. However, a consideration of the occurrence of rickets in the groups selected for the study of infant feeding has not been made. Variables other than vitamin D might also be involved which would influence the significance of the results. At present any difference reported between the dental caries incidence in breast-fed and artificially fed children should be questioned until groups of children are available who have had adequately supplemented diets during infancy.

Relation of Social and Economic Status to Dental Caries Incidence

On the basis of the discussions and conclusions in previous sections, there appear to be several factors which might help to produce a lower dental caries experience among the families in the higher income groups than in the lower income groups: availability of medical, dental and nutritional treatment and advice, financial ability to purchase an adequate well balanced diet if it is desired, less crowded living conditions, more outdoor exposure during vacations and possibly in other less obvious ways. On the other hand, a higher intake of more refined, luxury foods at the expense of and uncompensated for by more nutritious foods might tend to produce a higher dental caries experience among the higher income groups. The balance which such factors as the above would produce in the dental caries experience would be expected to vary from community to community in relation to the general diet of that district, the racial composition of the various income groups, the hardness and fluorine content of the drinking water, etc.

Dick (1918) made a comparison of the incidence of enamel lesions in 281 English children from "poor-class" schools, where the state of nutrition was considered to be distinctly below normal, with 122 children from "good-class" schools, where the state of nutrition was considered to be good. "Defective enamel" was observed in 40 per cent of the children in the "poor-class" schools in contrast with 13 per cent of the children from the "good-class" schools.

Reiser (1931) made a survey of the school children of Zurich to determine the effect of family income and of school dental clinic care upon the dental caries incidence. He observed that in the 1,569 children of the first grade and the 1,216 of the sixth grade

there was a higher dental caries experience among the children from the families in the lower income groups. In the 1,190 subjects of the third grade who had had 3 years of dental clinic care, however, no difference in dental caries incidence was observed. The children of the sixth grade had had dental care at school for only the preceding 3 years, which apparently had not been sufficient to have any effect on the higher dental caries incidence of the children from the lower income groups. Reiser attributed the lowered dental caries incidence in the third grade children of the lower income brackets to the dental care provided.

Miller and Crombie (1938) compared the dental caries incidence of 75 children of a poor district in Newcastle with 75 children from Ponteland, a self-contained village in the country, who received a good diet containing a pint of milk per day. Eight of the Ponteland children had perfect dentitions in comparison with 2 of the Newcastle children. Fifty-two of the Ponteland children had gross carious lesions, while 70 of the Newcastle children had gross lesions. None of the other possible variants, such as the composition of the drinking water, was discussed.

In a survey of 2,097 children in Chicago schools, Greenwald (1939) observed that the children of low social and economic status up to the second grade had a higher incidence of carious teeth, missing first molars and first molars needing extraction than the children in the higher income groups. With increasing age after the second grade, there was a tendency for the incidence of dental caries to approach the same level. Greenwald postulated that the difference in dental caries incidence might be attributed to prenatal conditions, possibly the inadequate diet of the mothers of the lower income levels, to the poorer care of the children's teeth or to a combination of both.

Schiotz (1939) observed about seven times as many children with good teeth, as far as dental caries was concerned, among the economically well situated people of Oslo, Norway, as among the poorer families.

Mellanby (Editorial 1940c) made a study of the dental caries incidence of the 5-year-old children in 33 public schools in London. The number of caries-free children decreased from 6.1 per cent in the schools of higher income districts to 2.8 per cent in the schools of the poor districts, while the average caries figure increased from 4.4 to 4.9.

Klein and Palmer (1942) and Klein (1942) presented data which showed no difference in dental caries incidence among well-to-do and relatively poor children. The children of families with high incomes received diets of better quality and quantity and were more advanced in skeletal age. Clear-cut differences in such environmental factors as diet, housing and dental care, however, did not produce an appreciable difference in the caries susceptibility of the two groups.

Wilkins (1941) compared the dental caries incidence of English children in the elementary schools of poor and fairly well-to-do sections with the incidence in children in private schools. His data indicated a slightly higher average number of defective teeth per child and a slightly lower number of perfect sets of teeth in the poorer children than in the fairly well-to-do children. The average number of defective teeth in the 106 children in private schools was 1.16 in comparison with 6.8 for the 1,220 elementary school children; 73.5 per cent of the former had perfect sets of teeth in contrast to only 6.1 per cent of the latter group.

Shourie (1942) presented data on the dental caries incidence and nutritional deficiency signs in children attending various schools in Madras. The children of well-to-do families showed no signs of deficiency disease, while many children of the poorer classes showed signs of one or more deficiencies. There was little or no difference in the percentage of children who were free from caries.

Hyde (1944) made a survey of the socio-economic aspects of dental caries in groups

of selectees at the Boston Armed Forces Induction Station. The percentages of men between the ages of 21 and 38 years rejected for military service were grouped into a six-category scale of desirability from A for the finest communities to F in slum regions. The greater rejection percentages noted in the communities of low socio-economic standing were considered to be due to less reparative dental care, poorer personal dental hygiene and poorer nutrition. To test these hypotheses, a further study in greater detail was undertaken with 3,899 men who were mainly between 18 and 20 years old. The average number of decayed, missing and filled (DMF) teeth was 17 per person for desirability level A and gradually decreased to about 14 per person for level F. The average number of decayed teeth ranged from 1.5 per person in the best community level to over 3.0 per person in the poorest communities. The average number of missing teeth did not vary significantly between groups. The average number of filled teeth decreased from 8 per person in the best economic levels to 4 per person for the poorest communities. Two alternatives were suggested to explain the slightly higher DMF in the economically most desirable communities. Filled teeth might include caries-free fissures that were filled and might not have become carious. In the second place, the communities of low socio-economic level contain higher percentages of foreign-born and of races considered to have better teeth than the American average. To test the latter alternative, only those communities were considered wherein one nationality predominated. The percentages of men from communities of a predominant nationality disqualified for military service because of dental deficiency were as follows: Irish 6.8, English-speaking foreign and old American 4.4, Italian 4.1, Portuguese 2.0 and Jewish (Russian) 1.9. An additional study of nationality effect was made in the more detailed examinations of 2400 white and 1212 Negro selectees in a period of 1943. The lowest average DMF teeth was observed in the Chinese, Negro and Russian Jewish groups and the highest average in the Canadian, Irish and English groups. The number of filled teeth also varied greatly with nationality. The Portuguese and the Negroes had the lowest number of filled teeth while the Italian, Yankee, Irish and English groups had the highest number of filled teeth. The average number of missing teeth was roughly proportional to the incidence of tooth decay. The average number of decayed teeth was least for the Chinese and Russian Jewish groups and highest for the Negro, Portuguese and Irish groups. These nationality differences could easily have contributed to the slightly lower number of DMF teeth observed in the selectees from less favorable home communities.

Résumé: There has been no general agreement as to the relation of social and economic status to the dental caries experience of children. It would appear under certain circumstances that there is a definite difference between the dental caries incidence in children from various classes. Undoubtedly there are many factors which might modify this difference: the availability of free dental care, the general caries susceptibility of the race studied, the price and availability of foodstuffs of all types, the composition of the water supply, the availability of sunshine, etc.

Relation of Physical Characteristics of the Diet to Dental Caries Incidence

Klatsky (1945) has made an anthropologic survey of the masticatory apparatus of modern man as compared with that of primitive races and prehistoric remains. He concluded that the masticatory apparatus of modern man is of greatly inferior development and his teeth and periodontal tissues are more susceptible to disease than those of ancient and primitive peoples. This was true despite the fact that the survey indicated that civilized man is far superior to

man of the past and to contemporary primitive races in general build, organic development, average stature, resistance to disease and length of life span. Klatsky postulated that the inferior development of the masticatory apparatus of modern man was not due to nutritional causes but more probably was a result of the lack of function and reduced amount of mastication due to high amounts of soft and liquid foods in the modern diet.

Neumann (1946) has recorded some philosophic observations on the distribution of dental caries in various nations of the world and has presented some suggestions as to the causes of tooth decay. This writer notes that the cause of tooth decay has been attributed by various investigators to widely different factors such as heredity, hormonal disturbances, racial susceptibility, deficiencies of certain elements in the local soil and water and consequently in the dietary, over-refined food, vitamin deficiencies and excessive sugar and starch consumption. Neumann discussed each of the above hypotheses in terms of the dental caries incidence of selected nations and concluded that the environment and food habits of these nations did not permit the acceptance of any one of these hypotheses. In a further examination of diets used in these countries, he postulated that the primary etiologic factor of dental caries was a deficiency of tough food requiring mastication. This deficiency was not believed to result in too little detergent action of the food during eating, but to cause a disuse atrophy provoking a decalcification of the dental structures. The term "disuse odontoporosis" was coined for this postulated condition with the suggestion that it might be comparable to the osteoporosis observed in bones after a period of disuse. Neumann suggested that the prevention of dental caries could be achieved ideally by the addition of soft sugar cane to the diet or more practically through the consumption of some tough breadcrusts daily. Obviously the above hypothesis of the cause and prevention of dental caries is based entirely on presumptive reasoning. This postulate seems especially extended in view of the fact that decalcification of formed teeth has never been demonstrated. Yet no one can deny that the use of cutlery and modern refining and cooking procedures have to a large extent relieved the teeth of civilized man from the tasks of biting and chewing for which they are admirably constructed.

Heredity and Dental Caries Incidence

In any consideration of the relation of heredity to dental caries there are certain factors, environmental and dietary, closely associated with the family unit, which are difficult, if not impossible under most conditions, to dissociate from the constitutional factor.

Black (1899) drew attention to certain of these: "When the family remains in one locality, the children living under the conditions similar to those of the parents in their childhood, the susceptibility to caries will be very similar in the great majority of cases. This will hold good even to the particular teeth and localities first attacked, the order of occurrence of cavities, and the particular age at which they occur." Thus the locality in which the family lived, the food tastes and cooking habits of the family must be evaluated in any study of heredity.

Mummery (1907–8) laid much emphasis on heredity as a factor in dental caries and saw little hope for improvement of the teeth of European children, since they were nearly all born of susceptible parents.

Bunting (1909) determined the caries experience in a group of 112 Negro children and 62 white children in Detroit, all of which were over 10 years of age. The Negro children had an average of 6.2 per cent of the erupted teeth which were carious, in comparison with 9.2 per cent for the white children.

Mills (1937) used data contained in the bulletin on dental caries incidence in the

United States by Messner *et al.* (1936) to compare the relative amounts of tooth decay in Negro and white children residing in comparable areas. He concluded that Negro children in all areas surveyed had a lower dental caries experience than white children of the same age. Moreover, the same relationships of dental caries incidence to latitude, to distance from the mouth of rivers and to the degree of hardness of the drinking water held for Negro as well as for white children.

Staz (1938) compared the dental caries experience of 300 each of primitive Bantus, urban Bantus and Europeans in the age range from 15 to 30 years. The incidence of tooth decay was much lower in the primitive Bantus than in the urban Bantus and much lower in the latter than in the Europeans examined.

Blackerby (1939) reported the results of a survey of the dental experiences of 1,117 Negro children of 32 schools and 11,674 white children of 328 schools, with geographic representation from East, Middle and West Tennessee in each group. The age range was from 6 to 17 years. Although 80.2 per cent of the white children were found to need dental care, only 67.5 per cent of the Negro children were found to need dental treatment. For each child with evidence of tooth decay, the white children had an average of 5.18 defective teeth in contrast to the value of 3.39 for the Negro children. The difference between white and Negro children became gradually less with increasing age, until in the 15- to 17-year age group the difference was considerably reduced.

Hyde (1944) reported in a survey of selectees at the Boston Induction Station that a smaller number of DMF teeth was observed among Negro selectees than among selectees of Canadian, Irish and English origin.

Bachrach and Young (1927) observed tooth eruption and dental caries in 301 pairs of twins, of whom 130 pairs were described as identical and 171 as fraternal. Identical twins exhibited a closer affinity in regard to tooth eruption than fraternal twins as a whole, and than fraternal twins of like sex. The proportion of pairs of teeth in unequal stages of eruption was 15.0 $\pm$ 0.5 per cent in identical twins and 21.7 $\pm$ 0.5 per cent among fraternal twins. This significant difference could not be attributed to sex differences in the fraternal twins since the proportion for unlike and like sexes was shown to be 24.3 per cent and 19.4 per cent, respectively, which were both significantly larger than the percentage for the identical twins. The total incidence of dental caries in the permanent teeth was practically the same for fraternal twins of like sex and for identical pairs. The incidence of hypoplasia in the permanent dentition did not differ between the two types of twins. However, the proportion of identical twins in which hypoplasia was present in both twins was significantly higher than in fraternal twins. Normal occlusion was significantly more frequent among identical twins than among fraternal ones of like sex.

Denney (1930) discussed several lines of families in regard to the number of teeth decayed. No designation of the teeth affected, of dietary habits or of change of dietary habits was given. Each of three pairs of identical twins was described as having the same type of mold and approximately the same number of carious lesions.

Goldberg (1930) reported a striking similarity of the teeth and their arrangement in identical twins. He found that the incidence of carious lesions on the smooth surfaces was very haphazard. From the comparison of decay in pits and fissures, however, he concluded that "heredity affects dental decay only inasmuch as it controls the shape of a tooth and its pits and fissures and its position in the dental arch, but has no influence over the chemical composition or microscopic structure that will encourage or prevent decay." No supporting evidence was given for the part of this statement

concerning the lack of relation between heredity and the chemical composition and microscopic structure of human teeth, nor are such data known to have been reported elsewhere.

Dahlberg and Dahlberg (1942) made a survey of the dental caries experience of 37 pairs of identical twins at an average age of 9.92 years and of 89 pairs of fraternal twins at an average age of 10.30 years. They stressed the fact that the environmental factors, especially with respect to diet, were necessarily much more similar for identical and fraternal twins than for other siblings. This similarity tended to lessen the value of twins for the study of the effect of heredity upon dental caries experience. Nevertheless, they concluded that their data indicated a strong influence of heredity in establishing a degree of resistance or susceptibility to the processes of tooth decay.

Montelius (1933) observed shovel-shaped incisors in 3,520 of 4,474 Chinese (79 per cent) and 5 cusps on the second lower molars of 2,505 (56 per cent) of this group. He found some individuals with 4 cusps on one side and 5 on the other. In many cases the fifth cusp was very small. He concluded that these were hereditary traits.

Bossert (1933) studied the incidence of carious lesions in relation to the steepness of the fissures between the cusps in 100 upper right first permanent molars of New York City children. He observed that "the steeper the sides of the valley, the greater the likelihood of caries in the pit." Bossert (1937) continued his study on the relation of the physical measurements of the occlusal surfaces to development of carious lesions in the upper right second deciduous molar. His subjects were 300 New York City children, aged 2 to 8 years, of whose teeth he made models. The same tendency of the narrower, deeper fissures to increased susceptibility to decay was described.

The dental classifications of 198 children and their parents were plotted by Schoenthal and Brodsky (1933) but failed to show any striking relationship.

The 264 children who had been observed during a 3-year period of dietary experimentation were classified by Hanke (1933) according to their degree of dental caries experience at the beginning of the experiment. His data indicated graphically what a pronounced effect some constitutional factor or factors played throughout the control, experimental and recheck periods of observation. In each period the increment of carious lesions was characteristic of the past experience of that group. In each group the increase in carious lesions was greatly reduced by the citrus fruit juice supplement. During the recheck period there was an increase in the dental caries increment of each group over the rate observed in the original control period.

McBeath (1933) reversed the control and experimental groups in one of his vitamin D studies. His data indicated that hereditary factors had not been responsible for the beneficial effects of vitamin D administration. During the second year, the original experimental group received no additional vitamin D. The dental caries increment was greater than the degree observed in the previous year of vitamin D therapy and was comparable to the dental caries increment of the original control group. A reduction in dental caries experience was observed again in the group with the vitamin D supplement. McBeath stated that these data indicated that in these two groups of children the nutritional variant had been of greater importance than the constitutional ones.

Day and Sedwick (1934a, b) observed a continued tendency for those children studied in Rochester who showed an originally low dental caries incidence to maintain a lower absolute increase in the average caries figure than those with a higher incidence at the beginning of the observation period.

Bunting (1934b) stated that "inherited tendencies or inherent individual characteristics were, in a small percentage of cases,

more important determinant factors in caries susceptibility than ordinary dietary conditions." No data were presented in support of this statement.

Johnson (1940) studied the effect of crossbreeding dogs of contrasted types on the development of skull form and dental occlusion. It was found that when dogs of contrasting facial form were crossbred, the F1 generation had skulls which showed a great similarity to the long-muzzled normal ancestral type. In the F2 generation, however, much greater diversity in facial form was produced, ranging from a type comparable to the dominant, long-muzzled form to a type approaching the recessive, short-muzzled form. The independence in genetic constitution of the maxillary structures from the mandible and the independence of the teeth from both was clearly evident. These data indicated the scope of variations of skull form and of tooth size and position which could occur in the dog under the influences of the germinal constitution of the individual. Experiments were performed also to modify the internal environment of the animal. When certain endocrine glands were removed during growth, there was a general arrest of growth but no appreciable change in the skull form. A low calcium diet given during the period before the adult dentition was established produced no differences in the form and proportions of the skull. Surgical removal of the osteogenetic cartilages and the body of the sphenoid at the base of the skull resulted in no marked change in the constitutional form of the skull. Therefore it appeared, under the conditions of these three experiments, that the constitution of the dog had a greater influence in the development of facial form than endocrine deficiency, calcium deficiency or partial surgical removal of active growth centers. In addition to the effect of crossbreeding dogs of contrasted types upon the development of skull form and dental occlusion, Johnson (1945) has observed that severe enamel hypoplasia could be produced at will by the same procedure.

Brekhus (1941b) summarized the status of heredity in relation to dental caries: "At the present time there is no justification for making any definite statements as to the part it may play."

Klein and Palmer (1940) observed that there was a greater similarity in the dental caries incidence of siblings than of unrelated children. These studies were based on observations of 301 school children of known dental caries susceptibility who were compared with 488 siblings. Klein and Palmer, however, drew attention to the fact that such a study did not mean that this similarity was due solely to hereditary factors, since it was not possible to eliminate the various conflicting factors associated with the family as a unit.

Klein and Shimizu (1945) have made detailed dental examinations of 3,490 married men and women residing at a War Relocation Center. Analysis of the data from this survey indicated that persons who have high levels of experience with dental disease, as indicated by large numbers of decayed, missing and filled teeth, tend to marry persons who, on the average, have more than the usual number of decayed, missing and filled teeth for their age and sex. A parallel situation was found to hold for persons who have good teeth.

When the children of 1,150 families of Japanese ancestry were given detailed dental examinations and their dental caries experience compared with that of their parents, Klein (1946b) observed that there was a remarkably consistent relation between the dental caries experience of the parents and that of their offspring. This relation was sufficiently strong to make it difficult to exclude the view that dental disease susceptibility involves strong familial vectors which very likely have a genetic basis, perhaps sex-linked. The relation observed can best be expressed by quotation: "For all ages

combined, sons of fathers with low DMF rates average 4.4 DMF teeth, sons of fathers with middle DMF rates average 5.5 DMF teeth, while sons of fathers with high DMF rates average 6.3 DMF teeth." "For all ages combined, sons of mothers with low DMF rates average 4.2 DMF teeth, those of mothers with middle DMF rates average 5.4 DMF teeth, while sons of mothers with high DMF rates average 6.6 DMF teeth." Very similar data were obtained with the relation of the daughters' carious teeth to those of the mothers and fathers. In the section on Experimental Dental Caries, investigations demonstrating definite relationships between heredity and dental caries in the white rat, hamster and cotton rat have been reviewed.

Résumé: At present there are no data that point to an absolute relation in human beings between heredity and dental caries, uninfluenced by other factors. In experimental animals such a relationship has been demonstrated. The results of Hanke (1933) do indicate possible constitutional factors predisposing to a definite dental caries susceptibility which can be altered by dietary measures but not completely overcome. There constitutional factors are not necessarily hereditary but feasibly could be due to environmental effects during the development of the teeth. In addition, even though all of the 264 subjects in Hanke's experiments ate food prepared and served in the same fashion, there can be no guarantee that they consumed equal amounts or the same distribution of these foods, or that their metabolic processes made equal use of the foods consumed. The greater similarity in the dental caries incidence of siblings than of unrelated children observed by Klein and Palmer (1940) and Klein (1946b) indicates a difference between families which might be due to heredity or to the various factors associated with the family group. The relation of heredity to dental caries incidence in man merits considerable investigation. It constitutes a problem of the greatest complexity because of the superimposed factors of diet during tooth development and after eruption. In any consideration of heredity and dental caries, these factors would have to be weighed before the full effect of heredity on tooth structure, position and susceptibility to tooth decay could be evaluated. Likewise, in the studies of nutrition and dental caries, adequate consideration of the possible effect of heredity on tooth structure and tooth decay must be evaluated whenever possible.

Calculus and Dental Caries Incidence

An inverse relationship between dental caries activity and calculus formation has been mentioned in dental literature frequently in the last few decades. In almost every case the expression of this hypothesis has been based on impressions from clinical observations but was not founded on data collected in controlled experiments. Boyd (1926) has stated that calculus was observed to be almost universal on the teeth of children in whom arrested decay was produced through the use of an adequate diet. The fundamental importance for investigations to determine whether or not there is an inverse relationship between calculus formation and dental caries is obvious. A great amount of work has been done already on the parasitic Actinomycetes and other filamentous microorganisms of the mouth which have been found to be intimately associated with the inorganic components of calculus (Rosebury, 1944). Little is known, however, of the metabolic differences which occur within the oral cavity when calculus formation begins after an inactive period or ceases after a period of active formation. If there is an inverse relationship between dental caries activity and calculus formation, the metabolic changes resulting in arrested dental caries activity may be responsible for the greater availability of calcium and phosphorus in the

oral cavity which permits the formation of calculus.

RELATION OF PREGNANCY AND LACTATION TO DENTAL CARIES INCIDENCE

The adage "a tooth for every child" has echoed through dental literature for generations. If this is to be proved or disproved, both the effect of the nine months' gestation period and the effect of lactation from parturition to weaning upon the incidence of tooth decay must be evaluated. Experimental evidence has indicated that calcium is added to the fetus chiefly during the last trimester and half of it in the last month. Therefore any depleting effect of the additional calcium and phosphorus requirement of the maternal host need not be expected to appear in the teeth at least until nearly parturition; then the even higher calcium and phosphorus requirement during lactation would be encountered. Few investigations of the effect of reproduction on the susceptibility to dental caries have considered the duration of pregnancy or lactation in their discussion of dental caries susceptibility.

Toverud (1927) studied the relation of calcium deficiency during pregnancy to the structure and composition of the incisor in the white rat. He observed that the control females which had never been pregnant had incisors of almost normal structure and composition. The incisors of those rats which had borne one litter showed a slight incisal softening, a reduced pigment formation and a slight decrease in the percentage of ash, calcium and phosphorus. More pronounced changes were observed in those rats which had borne two litters. All three groups had received the calcium-deficient ration for the same length of time. No studies were made to determine if there was a reduction in the inorganic, calcium or phosphorus content of the fully erupted molars.

Deakins (1943) and Deakins and Looby (1943) compared the specific gravity of the dentin of teeth from pregnant women with that of other individuals as a measure of the mineral content and the degree of calcification. One hundred and fifty-two dentin samples of 29 carious teeth from 20 women in the later months of pregnancy had an average specific gravity of 2.12 with a range of 2.03 to 2.21. Forty dentin samples from 14 carious teeth of nonpregnant individuals had an average specific gravity of 2.08 with a range of 1.82 to 2.22. It was concluded that no demineralization of sound dentin occurred during pregnancy and that neither gestation *per se* nor demineralization of teeth was associated with carious lesions in these samples.

Dragiff and Karshan (1943) made analyses of the ash, calcium and phosphorus content of the root dentin of 31 teeth of pregnant women and 21 teeth of nonpregnant women. They observed no differences in these components of the root dentin.

Biro (1898) evaluated the dental caries experience of 103 primiparae, 70 secundiparae and 27 multiparous women, taking their age also into consideration. He found that, respectively, 21.21, 23.97 and 28.60 per cent of their teeth were carious. By age grouping and comparison with 100 nulliparae, however, he demonstrated that the dental caries increment was related to the age of the subjects and not to the number of pregnancies.

Gerson (1921) examined the teeth of 50 nulliparae and 50 women in the second to fourth month of pregnancy. All women were in the third decade and were from the same social stratum. Similar rates of dental caries incidence were observed at the first examination. Six months later, however, the ratio between the incidence of superficial carious lesions in the pregnant and nonpregnant was observed to be 89:42, the ratio of deep carious lesions with pulpitis 43:16 and the ratio of the frequency of extractions 12:2.

Ziskin (1926) compared the dental caries experience of two groups of women attending dental clinics in New York, both being from the same social stratum, of comparable

financial means and within the same age group. The first group consisted of 599 pregnant women and the second of 205 never-pregnant women. Ziskin stated that for the most part the oral examinations were made during the latter months of pregnancy. The data indicated that the dental caries experience of the pregnant women increased with age at a rate similar to that of the nulliparae. No relation of dental caries to pregnancy nor to the number of pregnancies was observed. If anything, the dental caries rate appeared to be slightly higher in the never-pregnant control group.

In Minsk, Russia, Starobinsky (1929) determined the dental caries incidence in 216 women in the ninth month of pregnancy and compared them with the dental caries incidence in 150 nonpregnant women of comparable age groups. These data indicated that dental caries incidence increased in both pregnant and nonpregnant in relation to their age but with no definite relation to pregnancy or the number of pregnancies.

Toverud and Toverud (1930a, b, 1931) observed a definite negative calcium and phosphorus balance during pregnancy in 43 Norwegian women, even though their diets contained a relatively adequate amount of calcium and phosphorus. A quart of milk daily or cod liver oil or both improved the balance.

Turkheim (quoted by Toverud and Toverud, 1931) stated that probably there was no increased susceptibility to dental caries during pregnancy. However, Turkheim presented some experimental data by Kirsch which indicated that when calcium salts were given to pregnant women there was a decrease in the dental caries incidence below that observed in suitable pregnant controls.

Drain, Plass and Oberst (1933) studied the effect of a diet similar to those fed diabetic children of 5 primigravadas. These women (1 diabetic, 1 luetic and 3 normal) were in the last 20 weeks of gestation and had active caries. In all 5 cases a consistent decrease in the caries activity was observed soon after the beginning of dietary control.

Ziskin and Hotelling (1937) concluded from statistical analyses of their data based on the Bodecker dental caries index, number of pregnancies, age, and pH of the saliva in 324 pregnant and 31 never-pregnant women that "pregnancy in some way tends to prevent decay." Of the 324 cases, 164 were in their first pregnancy, one was in the eighth, with the others intermediate. The mean number of pregnancies was 1.95. The average duration of pregnancy at the time of examination was only 5.76 months, so that the effects of the last 3 months, when the calcium requirement of the fetus was greatest, were not determined in this survey. The possible protective effect of pregnancy on dental caries was not accounted for. Dietary instruction was discounted as a factor in the reduced dental caries incidence, since no instructions were given until the time of this examination. Since the average duration of pregnancy was 5.76 months at the time of this examination, the effect of the last third of pregnancy and of lactation, where dietary requirements were greatest, were not observed in this survey except in the 160 subjects who had had previous pregnancies. It is most unfortunate that only 31 never-pregnant women constituted the control group, since the standard deviation in so small a group would be very large.

Mull and Bill (1933) and Mull, Bill and Kinney (1934) studied the calcium and inorganic phosphorus concentration of the serum in 579 pregnant women in Cleveland, from 28 weeks before delivery to 7 weeks postpartum. A seasonal variation in the serum calcium concentration was observed. The calcium and phosphorus concentration both declined early in pregnancy, rose sharply just before parturition and remained high postpartum. Dental examinations were made on 358 women during pregnancy. Fifty-four of these had definitely active carious lesions. Since the serum calcium and inorganic phosphorus in 49 of the subjects

with actively progressing lesions showed no evidence of deviation from the values of the group as a whole, it was concluded that there was no connection between dental caries and the reduced calcium and inorganic phosphorus concentrations of the serum during pregnancy.

Buckley (1929) stated that a medium-sized human fetus (3,000 to 3,200 gms.) contained 40 to 43 gms. of lime (28 to 31 gms. of calcium).

Hess, Lewis and Roman (1932) studied the calcification of human teeth at birth. The total calcium and phosphorus content of the teeth of both jaws was 212 mg. and 106 mg., respectively, which would be equivalent to about 500 mg. of tricalcium phosphate.

LeFevre, Bale and Hodge (1937) studied the chemical nature of the inorganic portion of the fetal tooth structures. The total weights of the tooth substance from each of two term fetuses was 0.4376 gm. and 0.6260 gm., as compared with 0.2387 for a 7-month fetus. Therefore, two or three times as much calcification occurred during the period from the seventh month to birth as during the previous 7 months. The authors observed an inorganic content of 80.4 $\pm$ 3.2 per cent, calcium 34.5 $\pm$ 2.3 per cent and phosphorus 16.9 $\pm$ 0.5 per cent of the total tooth weight.

Hunscher (1930) studied the calcium and phosphorus utilization of three women during two successive lactation periods. A negative calcium and phosphorus balance was observed during the early weeks of lactation despite the consumption of a diet excellent in regard to calcium and phosphorus. Toward the middle of lactation, the negative calcium and phosphorus balance reached a maximum. In late lactation when less milk was secreted, both calcium and phosphorus were stored in the maternal body. Complete oral examinations with radiographs were made simultaneously with the metabolic balance studies. By the methods used, no demonstrable changes in the caries activity occurred during lactation. The daily output of calcium and phosphorus in the breast milk during the 27th week of lactation varied from 0.5 to 1.3 gms. and 0.2 to 1.4 gms., respectively.

Résumé: The old adage "a tooth for every child" has not been completely disproved, but the preponderance of the evidence in the literature indicates that it is not necessarily true if the proper precautions are taken. Biro (1898), Ziskin (1926), Starobinsky (1929) and Ziskin and Hotelling (1937) have presented data which indicated no increased dental caries activity with pregnancy. Deakins (1943) and Deakins and Looby (1943) observed no change in the specific gravity of the dentin in pregnant women. Dragiff and Karshan (1943) found no difference in the ash, calcium and phosphorus content of the root dentin during pregnancy. However, Gerson (1921) reported, without noting the dietary adequacy, an increased incidence of superficial and deep dental caries during the last 6 months of pregnancy. Toverud and Toverud (1930a, b, 1931) observed a negative calcium and phosphorus balance during pregnancy, which was reduced by milk or cod liver oil or both. Kirsch (quoted by Toverud and Toverud, 1931) observed a reduced dental caries activity during pregnancy when calcium salts were administered. Drain, Plass and Oberst (1933) found that when dental caries activity occurred during pregnancy it could be arrested by the consistent use of adequate diets.

The relation of lactation to dental caries activity has been inadequately studied. In the three subjects studied by Hunscher (1930) during two lactation cycles, no increased dental caries activity was observed. However, the diet of these women was excellent in all described regards and probably more nearly adequate than that commonly used by most lactating women as attested by the superior milk production of these subjects.

Until more studies have been made of the

adequacy of the diet during pregnancy and lactation, no final conclusion can be reached concerning the dental caries activity during these periods of increased nutritional requirement. Present data indicate that dental caries activity during pregnancy can be reduced by dietary means and that increased dental caries activity is not necessarily associated with pregnancy and lactation.

SUMMARY OF CONCLUSIONS

1. Various types of survey data in a wide selection of populations indicate that there is a strong association between diet and the susceptibility to dental caries.

2. Arrest of dental caries activity and reduction in the initiation of new lesions can be accomplished by adherence to a fully adequate dietary regimen. Much critical investigative work is necessary to evaluate how much of the caries resistance thus produced is due to systemic influences, to the oral environment produced by the diet or to the oral environment affected indirectly through systemic influences of the diet.

3. Individual variations in the ability to assimilate and utilize the digested nutrients must be considered in an evaluation of the response to a dietary treatment.

4. It is not known whether there is a relation between the sugar and refined carbohydrate content of an adequate diet and the dental caries susceptibility. In any study of the relation of carbohydrates to dental caries activity, the adequacy of the diet in question must be guaranteed. This has not been done in a large number of experiments where the refined carbohydrate content of the ration has been increased.

5. If the oral and systemic environments of the teeth are favorable to the processes of tooth decay, those teeth which are the most poorly formed and calcified appear to have a higher susceptibility to tooth decay than normal teeth. If the environment is not suitable for decay, there appears to be no evidence that a tooth must become carious because it is structurally imperfect.

6. There is a higher incidence of hypoplasia (gross and microscopic) in children who have had rickets during a period of tooth development than among normal children, but rickets is not the sole cause of hypoplasia.

7. There is evidence of a slightly higher dental caries incidence among those children who have had rickets during infancy than in children who have not had rickets. The validity of the data in numerous of these investigations is obscured because the diagnosis of rickets was attempted years after the active rachitic process had ceased.

8. In general, vitamin D supplements result in a decrease in the dental caries activity. When the vitamin D content of the diet is adequate, no reduction in caries activity with additional vitamin D would be expected. Adequacy of vitamin D in the current diet appears to be of greater importance than past periods of vitamin D deficiency.

9. A direct association between the hours of sunshine per year and the dental caries experience has been reported for some areas.

10. There is an inverse association between the latitude and the dental caries experience, but this relationship is not always dominant, as shown by excellent dental records of many groups living in extreme northern regions.

11. There appears to be no direct evidence that vitamin A reduces the susceptibility to dental caries. In those experiments where cod liver oil was given as the source of vitamin D, vitamin A may have contributed to the positive results.

12. No conclusive evidence of a relation between the deficiency of members of the vitamin B complex and an increased dental caries incidence has been noted. Some surveys indicate that lower susceptibility to tooth decay may occur in single and multiple deficiencies of the B-complex and vitamins A and C. A deficiency of folic acid in the rhesus monkey has been demonstrated to produce severe pathological changes in the supporting tissues of the teeth.

13. There appears to be no adequate evi-

dence of an increased susceptibility to dental caries in ascorbic acid deficiency or of a reduction of the dental caries activity by supplements of crystalline ascorbic acid. When the intake of ascorbic acid is inadequate, however, there is a severe impairment of the supporting structures of the teeth, with a resultant increased loss of teeth and severity of gingival infections. In the guinea pig, ascorbic acid deficiency results in marked malformation of the teeth.

14. The effect of citrus juices on dental caries is still obscure. In one experiment a marked reduction in dental caries activity was observed when one pint of orange juice and the juice of one-half or a whole lemon were given daily to growing children. Three ounces of orange juice were inadequate. The question merits further investigation.

15. The effect of synthetic vitamin E in chewing gum on the incidence of dental caries has been studied in one investigation with inconclusive results and in a second with negative results.

16. There is an apparent seasonal variation in the incidence of dental caries. The period of greatest caries activity has been reported to be during the fall and winter, but the period of greatest caries initiation is unknown because of the difficulties in the diagnosis of early lesions. The causal factor of such a seasonal variation might be ultraviolet light, but the possible role of dietary factors cannot be overlooked.

17. No incontroversial evidence has been found which indicates that diets partially deficient in calcium or phosphorus or both result in an increased susceptibility to dental caries. Individual variations in ability to utilize calcium and phosphorus have been observed to influence the caries susceptibility.

18. A relationship between dental caries activity and retention of calcium and phosphorus seems to have been demonstrated.

19. No correlation between the calcium or phosphorus concentration of the blood and dental caries has been demonstrated adequately by any of the reports reviewed.

20. No adequate evidence seems to have been presented which indicates any relation between the acid-base balance of the diet and the dental caries incidence.

21. There is an association between an increased hardness of the public water supplies and a reduction in the dental caries experiences. This association appears to be at least partially independent of the possible effect of fluorine.

22. The conclusions on the relation of fluorine to dental caries incidence have been presented at the end of the section on Fluorine and Dental Caries.

23. The release of ammonia in the saliva from a dentifrice containing dibasic ammonium phosphate has been reported to reduce the *Lactobacillus acidophilus* count in the saliva, but the effect of this mechanism on the dental caries incidence is unknown at present.

24. Good prenatal care and infant feeding appear to be associated with a reduction in the dental caries susceptibility. Interpretations of war-time caries data have indicated that the reduction in tooth decay observed did not reach a maximum until six or more years of restriction in carbohydrate intake. Thus the reduction in dental caries could have been related to systemic factors acting during tooth development instead of a simple alteration in the oral environment. Experimental studies with rodents strengthen this hypothesis.

25. It appears unlikely that breast feeding results in a higher resistance to dental caries than adequately supplemented artificial feeding. Breast milk of a woman receiving an adequate diet, however, is probably superior to unsupplemented or inadequately supplemented artificial feeding. There is still inadequate basic information to guide "perfect" supplementation of cow's milk in replacement of human milk.

26. The effects of social and economic factors on susceptibility to dental caries have not been established.

27. Tooth form and position appear to be associated with heredity, and the susceptibility to dental caries may be altered by the form and position of the teeth.

28. The evidence concerning a relation of heredity to dental caries susceptibility in human beings is confused by the overlapping factors associated with the family unit and the locality in which it resides. In experimental animals, definite relationships between heredity and caries incidence have been demonstrated.

29. Most evidence seems to indicate that no increase in dental caries activity is necessitated by pregnancy.

30. There commonly is a negative calcium and phosphorus balance during late pregnancy and the first two-thirds of the lactation cycle. The question may be raised as to whether the dental caries activity may be increased during the reproductive cycle because of this negative balance if the ration is grossly inadequate.

31. No extensive survey of the relation of lactation to dental caries has been found during this literature search.

32. Examples of dental caries arrest by dietary means during pregnancy are presented.

33. An inverse relationship between the deposition of calculus and dental caries activity has been postulated but not tested adequately by clinical experimentation.

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