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ANTIBIOTICS
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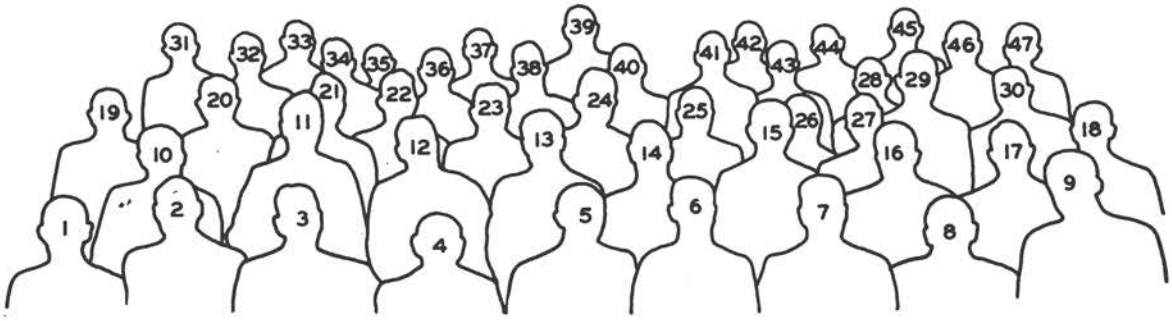

KEY TO GROUP PHOTOGRAPH

Front Row

1. L. A. Maynard
2. W. E. Krauss
3. J. McGinnis
4. C. F. Huffman
5. T. C. Byerly
6. H. I. Cole
7. T. J. Cunha
8. F. Ruiz-Sanchez
9. R. L. Squibb

Second Row

10. J. Schole
11. J. Tiews
12. J. Brueggemann
13. H. Zucker
14. H. R. Bird
15. C. M. Christensen
16. R. Braude
17. G. Briggs
18. J. J. Christensen

Third Row

19. H. Clausen
20. A. C. Francois
21. C. A. Baumann
22. T. D. Luckey
23. F. E. Deatherage
24. L. Anigstein
25. S. K. Kon
26. M. Finland
27. K. R. Johansson
28. O. L. Kline
29. K. Breirem
30. G. L. Romoser

Fourth Row

31. J. G. Horsfall
32. W. L. Smith
33. W. J. Zaumeyer
34. H. D. Branion
35. B. M. Duggar
36. L. C. Norris
37. S. Dyrendahl
38. H. Yacowitz
39. E. Freerksen
40. J. Duckworth
41. W. S. Gordon
42. E. Mrak
43. J. K. Loosli
44. H. L. A. Tarr
45. H. E. O. Heineman
46. D. Coles
47. D. Catron

PROCEEDINGS
FIRST INTERNATIONAL CONFERENCE
ON THE

USE OF ANTIBIOTICS IN AGRICULTURE

held under the auspices of
THE AGRICULTURAL BOARD AND THE
AGRICULTURAL RESEARCH INSTITUTE
NATIONAL ACADEMY OF SCIENCES—
NATIONAL RESEARCH COUNCIL

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FOREWORD

With the discovery in 1949 that an increase in growth rate in young pigs and chicks, first attributed to vitamin B_{12}, was actually due to an antibiotic (Aureomycin), a new field of research and development in animal production was opened and rapidly exploited. Reports dealing with studies of various antibiotics, species and body functions appeared in greatly increased numbers each year. As might have been expected, some of the results reported, both here and abroad, were contradictory and only served to confuse the questions at issue.

By early 1955 many of the scientists working in this field felt it was time to analyze critically their concepts, methodology, results and conclusions, and to discuss lines of future research. The National Academy of Sciences—National Research Council agreed to sponsor such a round-table discussion on the use of antibiotics in agriculture. Because of the probable future importance of antibiotics in the control of plant diseases and in the preservation of food and because of a wide variety of opinion in regard to the public health aspects of possible antibiotic residues in foods, the conference was broadened to include these three subjects.

It is hoped that as a result of the conference, new methods of attack on the problems discussed will be developed, and that accelerated research will be initiated which will lead to a clearer understanding of the mode of action of antibiotics in animal nutrition, plant protection and food preservation.

L. A. MAYNARD

THE NATIONAL ACADEMY OF SCIENCES—NATIONAL RESEARCH COUNCIL

A Private Non-Profit Organization of Scientists Dedicated to the Furtherance of Science and to Its Use for the General Welfare

The National Academy of Sciences was established in 1863 under a Congressional charter signed by President Lincoln. Empowered to provide for all activities appropriate to academies of science, it was also required by its charter to act as an adviser to the Federal Government in scientific matters. This provision accounts for the close ties that have always existed between the Academy and the Government, although the Academy is not a governmental agency.

The National Research Council was established by the Academy in 1916, at the request of President Wilson, to enable scientists generally to associate their efforts with those of the limited membership of the Academy in service to the nation, to society, and to science at home and abroad. Members of the National Research Council receive their appointments from the President of the Academy. They include representatives nominated by the major scientific and technical societies, representatives of the Federal Government designated by the President of the United States, and a number of members-at-large. In addition, several thousand scientists and engineers take part in the activities of the Research Council through membership on its various boards and committees.

Receiving funds from both public and private sources, by contribution, grant or contract, the Academy and its Research Council thus work to stimulate research and its applications, to survey the broad possibilities of science, to promote effective utilization of the scientific and technical resources of the country, to serve the Government, and to further the general interests of science.

CONTENTS

FOURTH SESSION

A. Crop Usage

J. G. Horsfall, *Presiding*

B. Food Preservation

J. G. Horsfall, *Presiding*

FIFTH SESSION

Public Health Aspects

W. W. Spink, *Presiding*

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ORGANIZATION AND ACTIVITIES

In February 1955, the National Academy of Sciences—National Research Council, through its Agricultural Board and Agricultural Research Institute, explored the possibility of holding an international conference on the uses of antibiotics in agriculture. By the spring of 1955 support for such a conference was assured and the Academy-Research Council agreed to sponsor the conference. Originally the Chas. Pfizer & Co. offered to finance the whole conference for the Academy-Research Council, but it soon became apparent that participation of other companies was desirable. Finally, four companies contributed funds: American Cyanamid Co., Merck & Co., Chas. Pfizer & Co. and E. R. Squibb & Sons. These companies also generously financed the postconference tour for the invited participants.

Organization.—A program committee under the chairmanship of Dr. T. C. Byerly was nominated by the Agricultural Board in February 1955 to determine the time, place and type of conference to be held, the subjects to be discussed, and the scientists to be invited to participate.

The program committee decided that the conference should be named "The First International Conference on the Use of Antibiotics in Agriculture" and should be held in Washington, D. C. about October 1955. It also decided that a "panel-of-experts" type of conference was best suited to the subject. The committee proceeded to name the leading scientists in this field throughout the world and from this list invited as many persons as funds would allow, from as many countries as possible, to participate in the conference. Invitations and a tentative program were sent out in June 1955. Arrangements made with the Department of State to allow the foreign guests to enter the United States under the Visitor's Exchange Program greatly facilitated their travel arrangements.

Notices of the forthcoming conference were placed in *Science, Chemical and Engineering News,* and many other journals related to this subject. Except for these notices, no special invitations to attend the conference were sent out except to the Washington Embassies and Legations of all countries and to the State Agricultural Experiment Stations in the United States and its Territories. Two press releases were distributed widely to press and radio outlets. During the conference summaries of all papers presented were released to the press. The U. S. Information Agency interviewed many of the guests and made tape recordings for Voice of America. Many radio farm programs commented on various subjects presented at the conference. The public relations firm of Walker and Crenshaw through Mr. John C. Davis, Vice President in charge of their Washington office, generously donated services and advice on the public relations aspect of the conference.

The local committee with Dr. George Briggs as chairman arranged for the tours, luncheons, dinner, transportation, etc. Dr. G. L. Romoser was in charge of the Beltsville tour; Dr. O. L. Kline took charge of the Mt. Vernon tour and also was entirely responsible for the very efficient management of the scientific sessions. The registration and information desk was very capably handled by Mrs. E. C. Harrison.

The U. S. Department of Agriculture allowed the conference to use the Jefferson Memorial Auditorium without charge and the Botanic Garden furnished plants and palms for decoration.

Many others too numerous to mention generously provided assistance, advice, and guidance in organizing the conference. Representatives of the four supporting companies cooperated wholeheartedly with the Academy-Research Council before and during the conference.

Activities.—On Tuesday morning, 18 October, Secretary of Agriculture Ezra Taft Benson welcomed the participants in his conference room at the U. S. Department of Agriculture. Secretary Benson pointed out that the broiler industry in the United States owes a substantial part of its success to antibiotics. Just beginning in 1935, this industry produced a billion broilers in 1954! He also spoke of the contribution antibiotics are making to the more efficient production of beef, milk, and other livestock products. Concurrently, according to Dr. Henry Welch, antibiotic production in the United States increased from 29 pounds in 1929 to 2,284,000 pounds in 1954, 490,000 pounds of which was used in feeds!

On Wednesday morning, 19 October, the participants were welcomed with short addresses by the Chairman, Division of Biology and Agriculture of the Academy-Research Council, Dr. L. A. Maynard; the Director of the Division of Antibiotics, Food and Drug Administration, Dr. Henry Welch (speaking for the Secretary of Health, Education, and Welfare); the Chairman of the Agricultural Board, Dr. W. E. Krauss; and the President of the Agricultural Research Institute, Dr. H. E. O. Heineman. After the speeches of welcome, the guests were served luncheon at the Academy. Their photograph was taken on the steps of the Academy before they were taken by bus to the opening scientific session in the Jefferson Memorial Auditorium, U. S. Department of Agriculture.

Dr. George W. Corner, Vice President of National Academy of Sciences—National Research Council, gave a welcoming address on Thursday, 20 October at the morning scientific session.

Other official functions included:

A luncheon and briefing for moderators and summarizers with the chairman of the scientific sessions, Dr. T. C. Byerly, at the National Academy 18 October;

A luncheon for invited participants at the National Academy 19 October;

A conference dinner at the Hotel Statler 21 October with Dr. René J. Dubos as guest speaker. (His talk is included in these Proceedings);

A tour of Beltsville and University of Maryland on 22 October. Demonstrations of the effect of antibiotics on crops and animals were given. Luncheon was served in the Terrapin Room, headquarters of the University of Maryland football team;

A sightseeing tour to Mt. Vernon, Arlington Cemetery, Jefferson and Lincoln Memorials, ending with a luncheon at the National Academy of Sciences, 23 October.

Besides these official functions, Chas. Pfizer & Co. entertained at a cocktail party at the Hotel Statler on 18 October, and Merck and Co. held a reception and dinner for invited guests at the Mayflower Hotel on 19 October. At the dinner, Dr. William H. Sebrell, former Director of the National Institutes of Health spoke on "Medical Research and World Peace."

The Soviet Embassy invited representatives of the four supporting companies and several of the participants to a dinner at the Embassy to meet the delegates sent over from the Academy of Sciences, USSR. Color films on an agricultural fair held at Moscow and on USSR national dances were shown after the dinner.

The total registration for the conference was 431.

Postconference Tour.—The Postconference Tour was arranged to give the invited participants an opportunity to visit industrial plants and research laboratories as well as Federal and State institutions doing work on the production, testing, and application of established and new antibiotics. Transportation, lodging, meals, and entertainment were provided by the four supporting companies. The group (33 persons) left Washington, D. C. by chartered plane on 23 October, arriving in Terre Haute, Indiana in time for a late dinner. The next day was spent at the Chas. Pfizer & Co. Animal Research Center near Terre Haute. In the morning several members of the Pfizer research staff presented papers. At lunch a talk on the potential expansion of the feed industry was given by Mr. Diamond, President of the American Feed Manufacturers Association. In the afternoon a panel of nutritionists from various feed manufacturing companies discussed some of their problems in regard to the application of antibiotics to livestock production. This was followed by a tour of the laboratories and animal farm.

On Tuesday the group arrived by chartered bus at Purdue University, Lafayette, Indiana, in time for lunch. The President of Purdue University, Dr. F. L. Hovde, gave an interesting talk which was followed by three short talks on antibiotic use in their countries by Dr. Coles of South Africa, Dr. Clausen of Denmark, and Dr. Gordon of England. After lunch, the group visited the Purdue Farms and witnessed demonstrations of the use of antibiotics in farm animals and poultry. Late in the afternoon, two planes flew the participants to Peoria, Illinois where the next morning was spent visiting the United States Department of Agriculture Northern Utilization Research Laboratory. The demonstrations were well arranged and of great interest to everyone. After lunch a chartered plane flew the group to Newark, New Jersey.

Thursday morning was spent visiting the Squibb Institute and pilot plants, and the new microbiological laboratory at Rutgers University, New Brunswick, New Jersey where Dr. Selman Waksman welcomed the group and gave a talk on antibiotics.

In the afternoon Merck & Company's Branchburg Farm was visited, and short résumés of some of their research work were presented by members of the research staff. After tea, busses took the group to Baltusrol Country Club for cocktails and dinner. Thursday and Friday nights were spent at the Dellwood Country Club near Pearl River, New York. Friday morning and part of the afternoon were devoted to scientific papers by the research staff of Lederle Laboratories, American Cyanamid Co., followed by a tour of the laboratories and plant.

Saturday, as guests of American Cyanamid Co., the delegates visited West Point, had luncheon at the Thayer Hotel and then saw the Army football team play Colgate. After the game, the group was taken to New York City by chartered bus. The guests were lavish in their praise of the hospitality of the four companies and of the tour as a fitting climax to the scientific sessions, filling in as it did, the other aspects of the antibiotics picture not enlarged upon at the Washington sessions, such as research pilot plants; manufacture of antibiotics; composition of feeds; animal, laboratory, and farm experimentation; and marketing of feeds.

HOWARD I. COLE

February 15, 1956
Washington, D. C.

Dinner Address

RENÉ J. DUBOS
Member, Rockefeller Institute for Medical Research
New York, N. Y.

RETROSPECTIVES AND PROSPECTIVES

Among scientific subjects, few are better suited to table conservation and after dinner speeches than the field of antibiotics. The method of discovery of these drugs involves no abstract concept; their application to the treatment of disease has strong emotional appeal; the illusion that they will bring about the final conquest of infection is still widespread. Finally, the introduction of antibiotics in medicine has a picturesque history made up of quaint reports of ancient folk remedies, and of romantic episodes in the life of modern scientists. Indeed, the whole subject evokes the mysterious atmosphere of alchemy or even magic, rather than the cold discipline of experimental science. Note how often the word "miracle" is used in popular writings on antibiotics, and the tendency to regard as magicians those concerned with their discovery and use.

Unfortunately, these tales of the Thousand and One Nights of antibiotic lore have been told so often that they have become somewhat boring. Moreover, anyone familiar with the field of microbial diseases of man is more likely to be impressed by the problems that remain to be solved than by accounts of the miraculous effects of new drugs. And I suspect that workers in animal and plant pathology have similar feelings. I have judged it safer, therefore, to refrain from story telling and to attempt instead a critical discussion of the relations of antibiotics to the much broader field of antimicrobial therapy. Nevertheless, I shall start by narrating a historical incident. It bears on our subject only through formal and very distant analogies, but it shows how venerable is the mode of thinking which has guided bacteriologists in their search for antibiotics.

The story concerns aconite, a plant alkaloid, also known as love poison, much used in ancient times to dispose of unwanted persons. It is told that Guy di Vigevano, physician to the court of France, performed in 1335 an experiment which he believed to have produced an antidote to the aconite poison. Inspecting closely the aconite plant, he found its leaves covered with worms and slugs which were feeding on them. He collected the worms and slugs, compounded them into a medicine, and fed both poison and drug to various animals, some of which survived. Convinced by the results of his tests that the medicine was an effective antidote against aconite, Guy di Vigevano repeated the experiment on himself. He took some of the poison and ingested the worm-based concoction as soon as he began to feel its effects. According to his own account, he fully recovered—although not without vomiting a number of times.

If this Congress were not a serious occasion, I would comment on the fact that the extract of worms and slugs cured the disease caused by aconite at the cost of creating severe nausea in the patient—much as treatment with antibiotics not uncommonly creates a new disease while curing the one for which it was intended. More pertinent to our discussion is the method of discovery of the aconite antagonist. The faith that a worm feeding on the leaves of the aconite plant should provide a cure for the disease caused by the aconite poison is very close indeed to the theory that a microorganism antagonizing another one on an agar plate should yield a drug useful in the treatment of disease. In this faith, we recognize the universal if vague awareness that there exists in the biological world many balancing forces, which assure some sort of ecological equilibrium between living things. It is not without interest that this concept, so unsophisticated as to pertain almost to primitive philosophy, should have led to some of the largest practical achievements of modern medicine. But while great discoveries can come out of primitive concepts, it is true nevertheless that science proceeds further when guided by a rational theory. Fortunately, as we shall presently see, there are enough facts based on rational concepts to provide a sound basis for research on chemotherapy.

It is customary to trace to Pasteur one of the most striking early statements concerning the potentialities of antimicrobial therapy. In 1873 he had recognized that certain microorganisms commonly present in soil can affect the anthrax bacillus in such a manner as to render it unable to establish disease in animals. On the basis of this observation, he suggested that saprophytic organisms might someday be used to combat infectious agents. "These facts," he said, "perhaps justify the highest hopes for therapeutics."

I should like to call to your attention a much earlier statement also made by Pasteur, which I regard as having greater theoretical interest than his remark just quoted. In his very first publication on the germ theory, the "Mémoire sur la Fermentation appelée lactique," published in 1857, Pasteur pointed out that onion juice did inhibit the growth of the lactic acid ferment while it was without effect on the activities of certain other microorganisms. Thus Pasteur clearly recognized that it was possible to inhibit selectively certain types of microbial growths by the use of the proper chemical substances devoid of any inhibitory effect on other microbial species. Although Pasteur utilized selective inhibition repeatedly in his own experimental work, he never developed the technique and concepts involved sufficiently to integrate them into a recognized scientific doctrine. This was achieved singlehandedly by Paul Ehrlich, and constitutes unquestionably the greatest intellectual creation of the science of antimicrobial therapy.

Ehrlich, as you know, conceived the thought that effective antimicrobial drugs would be found among substances possessing a high and rather selective chemical affinity for some exposed cellular constituents of the parasite to be attacked—the cellular receptors as he called them. It was this point of view that he summarized in the two famous sentences, "Only such substances can be anchored at any particular part of the organism which fit into the molecule of the recipient combination as a piece of mosaic fits into a certain pattern . . ." and "Antibacterial substances are, so to speak, charmed bullets which strike only those objects for whose destruction they have been produced."

As early as 1898, Ehrlich put forward in a letter to Carl Weigert the suggestion that drugs might act by competing with some of the essential metabolic processes of the parasite and hence by interfering with its nutrition. This remarkable conceptual scheme involving the reaction between metabolic antagonists and cellular receptors is fully developed in the Herben lectures that Ehrlich delivered in London before the Royal Society in 1907. He proposed his concept as a guide for development of drugs effective not only against bacteria and protozoa, but also against cancer.

Unfortunately, the knowledge concerning the hypothetical "specific cellular receptors" has not progressed very far. Nevertheless, this rational approach led Ehrlich to the discovery of several useful drugs, salvarsan being the most famous, but only one of them. Even more interesting evidence of the scientific validity of Ehrlich's vision is the fact that his doctrine continued to expand and eventually received a precise formulation in the form of the Woods-Fildes theory of metabolic antagonism, which accounted for the antibacterial activity of sulfonamides. It is this theory in turn which led to the synthesis of chemical compounds specifically designed to act as antagonists against folic acid. Among substances prepared with this object in view one need mention only pyrimethamine, a highly valuable antimalarial drug, and some compounds that inhibit preferentially the growth of neoplastic tissue and thus offer some hope of therapeutic attack against cancer.

For the purpose of our discussion, it is important to realize that there is a fundamental breach between Ehrlich's doctrine and some of the present trends in antimicrobial therapy. According to Ehrlich, as we have seen, the ideal drug was one developed to act *selectively* on a cellular constituent—a charmed bullet, tailor-made for a specific receptor in a given type of cell. During recent years, in contrast, the emphasis has been increasingly on drugs capable of attacking several different types of unrelated microorganisms. Gun-shot therapy with the so-called broad spectrum antibiotics has become the ideal of many physicians, and the goal of chemists and bacteriologists engaged in the development of new drugs. The implications of this chemotherapeutic philosophy are not yet all recognized. Some of them, however, have begun to appear in a clearer light as a result of unexpected effects of the use of antimicrobial drugs in medicine and in agriculture.

One of these unexpected effects has to do with peculiar forms of failure of chemotherapy. It had been thought at first that—outside of toxic and allergic reactions—the development of drug resistance on the part of the microorganisms would prove the largest stumbling block in the way of successful antimicrobial therapy. Then another cause of therapeutic failure was recognized in the pathologic character of the lesion which often provides for the infective organism a biochemical environment in which the drug is ineffective. But still another unexpected source of difficulty is now becoming apparent, one with a direct bearing on our discussion.

It has been repeatedly observed that antimicrobial treatment, in particular with broad spectrum drugs, may induce the multiplication of unusual types of microorganisms in various tissues of the treated individuals. The new microbial flora seems to take possession of the ground formerly occupied by the flora eliminated as a result of drug treatment. It is known, for example, that vigorous chemotherapy may bring about an almost complete elimination of the normal intestinal flora which

is then soon replaced by other kinds of bacteria and fungi. In man the gastroenteritis caused by staphylococci, and a variety of fungus infections, are probably the most frequent consequences of these accidents of chemotherapy. Of special interest is the fact that microorganisms not ordinarily able to multiply in the normal individual can cause disease states under these conditions. There is evidence, for example, that the toxicity of penicillin and bacitracin for guinea pigs is due to the fact that the drugs eliminate the normal flora of these animals which is predominantly gram positive and thereby allows unrestricted multiplication of coliform bacilli in the gut. Many manifestations of the same fundamental phenomenon have been recently produced at will in experimental animals. The most dramatic, perhaps, is the demonstration that guinea pigs, which are normally resistant to infection with cholera vibrios, become unusually susceptible to even small doses of these organisms if their intestinal flora is first drastically reduced by treatment with streptomycin. In other words, chemotherapy can become the cause of man-made diseases by permitting the multiplication of certain microbial agents which cannot compete with the autochthonous flora of the tissues in the normal untreated individual.

The other unexpected result of chemotherapy bearing on this problem is one which has been widely discussed in your congress—namely the fact that the feeding of antimicrobial drugs increases under certain conditions the rate of growth of farm animals and renders more efficient their utilization of food. Although the mechanism of these effects is not fully understood, there is ground for the view that the drugs enhance growth by inhibiting certain microbial activities which—in some way or another—exert a deleterious effect on the economy of the host.

All these new unexpected phenomena came as a surprise when first recognized. Yet, one can find a clear anticipation of them in some of Metchnikoff's writings more than fifty years ago.

On the basis of peculiar philosophical concepts Metchnikoff came to believe that civilization exerts on modern man some deleterious influence which may not necessarily express itself in overt disease, but prevents his life from being long enough to bring into play the instinct of death. With these thoughts in mind, Metchnikoff reached the conclusion that the intestinal flora of civilized man is responsible for a slow intoxication causing or accelerating certain degenerative diseases. He pointed out that parrots and certain other birds which defecate at very frequent intervals because they have a short intestinal tract, usually live much longer than would be expected from their size. The remarkable longevity of Bulgarian peasants was due—according to him—to the fact that they consumed large amounts of fermented milk rich in lactic acid bacteria antagonistic to the microbial activities responsible for intestinal intoxication. Control of the intestinal flora by therapy or by diet should, in his view, help in preventing degenerative diseases or at least in retarding their course.

Metchnikoff also believed, on the other hand, that the proper intestinal flora might protect man against the infections caused by certain enteric pathogens. It is known, for example, that exposure to cholera vibrios does not always result in clinical disease. Metchnikoff suggested that this resistance to infection might be due to the presence in the intestinal tract of microorganisms which prevent the multiplication of the cholera vibrios.

It is my opinion that the phenomena of enhancement of infections caused by chemotherapy, and of improvement of growth caused by feeding antimicrobial drugs, as well indeed as Metchnikoff's prophetic if vague imaginings, will all eventually be incorporated in a single biological doctrine. Allow me to attempt a very crude and tentative formulation of a theory which might account for some of these complex phenomena.

All living things—man included—have achieved through the process of evolutionary adaptation some sort of equilibrium with the microbial species which are ubiquitous in their environment, in particular with the microorganisms which they constantly harbor in their tissues. This equilibrium does not necessarily result in the best possible state of health. Only Professor Pangloss was optimistic enough to assure Candide that everything happens for the best in the best of the possible worlds. The evolutionary equilibrium corresponds to a compromise between many conflicting necessities. On the one hand, for example, the presence in the tissues of large numbers of relatively innocuous microorganisms helps in controlling the multiplication of other species with great pathogenic potentialities—from fungi to cholera vibrios. As we have seen, elimination of the autochthonous flora by drug treatment leaves the field opened to many kinds of infection of either endogenous or exogenous origin. On the other hand, many of the microorganisms normally present in the tissues have mild deleterious effects which, although compatible with a normal life, do interfere somewhat with the optimum performance of metabolic functions. Any therapeutic procedure which eliminates, or merely attenuates, these deleterious influences thereby brings about indirectly greater metabolic efficiency.

There is no doubt that the equilibrium between body and microorganisms can be readily upset for better or for worse as a result of the administration of drugs. As is true for any complex ecological situation, on the other hand, it is extremely difficult, if not impossible, to foresee the ultimate consequences of any intervention even when it consists in the use of a selective drug effective against only one or a very few components of the ecological system. Broad spectrum drugs are even more likely to exert unpredictable secondary effects, since they are capable by definition of attacking many kinds of cells. In my opinion, the ideal therapeutic approach requires a precise delimitation of the goals to be reached and the use of techniques designed for pin point attack on these goals. It demands the use of selective drugs or procedures, instead of gun shot treatments applied to conditions of ill defined etiology.

I realize that this attitude is a counsel of perfection not always compatible with practical exigencies. In many ways, the problem of antimicrobial therapy epitomizes a dilemma that all scientists encounter in their professional life, and which is particularly acute for those engaged in problems pertaining to medicine and agriculture. Because we are eager to conduct our studies according to rigorous logic, we would like to apply rational methods to the production and use of drugs specifically designed for each particular pathogen and for each type of pathological situation. But the art is long and life is short, and we often need to resort to any technique—rational or not—that gives hope of rapidly yielding results of practical usefulness. Fortunately, the rational and the empirical attitudes are not necessarily incompatible.

FIRST SESSION

GROWTH RESPONSE IN ANIMALS

October 19, 1955

C. F. HUFFMAN, *Presiding*

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RESPONSE OF POULTRY TO ANTIBIOTIC SUPPLEMENTATION OF FEEDS

Since the initial discovery in 1946(15) that antibiotics stimulate chick growth and the discovery in 1949(21) that supplements which were produced primarily as vitamin B_{12} concentrates contained some factor in addition to vitamin B_{12}, and the subsequent identification of this factor as an antibiotic, a tremendous amount of work has been conducted with poultry on the effects of antibiotics in feeds. Following the publication of Stokstad and Jukes in 1950(22) showing Aureomycin to have a growth promoting effect on chicks, work has been conducted with other species and with other antibiotics. As a result of the extensive studies which have been conducted on antibiotics for poultry throughout the world, we now have a much better understanding of the role of antibiotics in poultry nutrition. For example, Coates *et al.*(8) showed in 1951 that environment had an important influence on the response of chicks to antibiotic supplementation of the feed. It had been speculated by many that antibiotics had an effect on growth and feed efficiency through action on microorganisms in the intestinal tract. The work of Coates *et al.* (8) provided excellent circumstantial evidence for this proposal. In our laboratories at Washington State College(16), we were able to demonstrate that chicks raised in pens with litter floor were benefited to a greater extent by antibiotic supplementation than comparable chicks raised in electrically heated battery brooders with raised wire floors. A similar effect was demonstrated when wire floors were simply removed from the battery brooders and replaced with a litter floor. Kratzer *et al.*(13) at the University of California showed that the addition of feces to the diet of chicks reduced the growth rate; and Aureomycin improved the growth rate of feces-fed chicks. In our laboratories, we showed that the addition of a mixed culture of organisms from fresh chicken feces to the diet of poults was effective in increasing the growth response(18). When penicillin was added to the culture medium, microbial growth was markedly reduced as evidenced by cell volume, odor, gas production, etc., and a response in poults was obtained when this mixed culture was added to the diet, probably because of the residual penicillin in the culture medium.

Following the indications mentioned above that the type of environment has marked influence on the response of chicks to antibiotic supplementation, two pieces of work have appeared substantiating these conclusions. The results of work at the University of Notre Dame(14) where chicks and turkeys were raised germfree

showed that such animals did not respond to antibiotic supplementation of the diet. A second piece of work published by Jukes *et al.*(12) showed that the injection of an antibiotic into the developing embryo which could be considered to be a "germ-free" animal failed to bring about an improvement in embryo growth. This information provides at least a basis for explaining the variability which has been obtained with antibiotics from experiment to experiment and from laboratory to laboratory.

Response of Mature Poultry to Antibiotics.—The response of mature poultry such as laying and breeding chickens and breeding turkeys to antibiotic supplementation has been characterized by much greater variability than is the case for young chicks and turkeys. In our laboratories, both at the State College of Washington and at the Western Washington Experiment Station(3) we have obtained little or no response in egg production through antibiotic supplementation of the diet of hens. Different antibiotics were used in these studies and were fed at different levels. In one experiment, nutritional levels comparable to those which are effective in promoting growth in chicks had no effect on egg production or livability in hens. In another trial (unpublished) where two strains of chickens were used, one of which was highly susceptible to leukosis, the feeding of 160 grams of Terramycin per ton of feed from day of age through one year of egg production had no effect on egg production or leukosis mortality. However, total mortality was reduced somewhat by the antibiotic feeding, since it appeared that this level was effective in suppressing coccidiosis. In another trial(2) in which Aureomycin was added to two different feeds at levels up to 100 grams per ton, there was no significant response when the results obtained in the entire experiment were considered. However, when egg production during the last two periods of the experiment was considered separately, it appeared that the antibiotic increased egg production and feed efficiency slightly. Others have reported responses in egg production to antibiotic supplementation. Elam *et al.*(10) found that the addition of penicillin to the diet of laying hens improved egg production. Similarly, Carlson *et al.*(6) have reported improvements in egg production through antibiotic supplementation. It should be pointed out that in both of these instances, egg production was extremely low in the control groups and that even after antibiotic supplementation the level of egg production obtained would be considered unsatisfactory. It appears, therefore, that mature chickens are benefited much less universally by antibiotic supplementation than are young chicks. The reasons why this is the case are not clear. It is possible that environmental or genetic factors or both are operating. In Carlson's work, some breeds or strains of birds appeared to be benefited to a greater extent than others. Differences in the presence of undefined diseases are probably an important factor.

Very little work has been conducted on the response of mature turkeys to antibiotic supplementation. Unpublished results obtained in our laboratory showed that the addition of antibiotic-containing supplements to the diet of turkey breeders had no effect on egg production or hatchability. Carlson *et al.*(7) reported a slight improvement in both hatchability and egg production by Aureomycin added to a vitamin B_{12} deficient diet. Slinger *et al.*(19) have reported that feeding of penicillin to turkey breeders slightly retarded subsequent growth of poults at eight weeks of age.

Response of Ducks and Geese.—Branion and Anderson(5) reported that the supplementation of the diets of ducklings had no effect on growth or feed efficiency. Goslings(20) were found to be benefited by antibiotic supplementation during the first four weeks of growth. However, when they were given green feed the antibiotic was no longer effective in improving growth.

Environmental Factors Influencing Response to Antibiotics.—Following the demonstration by Coates *et al.*(8) that environmental factors have an important influence on the response of chicks to antibiotics a number of other reports have appeared confirming this observation. In addition to confirming Coates' finding that "new" environments are not conducive to antibiotic responses in chicks, it has been observed that old environments can be cleaned so that an antibiotic response is not obtained. In our laboratories following a thorough clean-up of the chick battery rooms and depopulation for about 30 days, a period of more than one year elapsed before significant responses could again be obtained to antibiotic supplementation. At the present time, responses similar to those obtained in our early studies can be produced. Whether or not the clean-up of the premises or the depopulation of all birds was responsible is, of course, unknown. At the University of Wisconsin(1), it has been observed that a response to antibiotic supplementation simply disappeared spontaneously and returned after a period of time.

Nutritional Factors Influencing Response to Antibiotics.—Coates *et al.*(9) showed that vitamin deficiencies acted as stress factors to increase the response to antibiotics. Simliar results have been reported by Biely *et al.*(4). Stokstad(23) made a rather extensive study of the effect of different vitamin deficiencies on response to antibiotics and concluded that the response was increased particularly by a vitamin B_{12} deficiency. When all of the results of this type are considered, there appears to be little consistency in the effect of a vitamin deficiency on response to antibiotics. Similar results have been obtained showing that either a protein or energy deficiency in the diet has a tendency to increase the response of antibiotic supplementation. It would appear possible, therefore, that nutritional deficiencies could act as a stress factor that would weaken the young animals and in turn render them more susceptible to certain types of undefined microbial infections.

The response of turkeys to antibiotic supplements may be markedly conditioned by the composition of the diet fed. Scott and Jensen(17) showed that the addition of grass juice to the diet gave the same degree of growth response as Aureomycin and that the combination of the two did not give an improvement over either one alone. The results have been interpreted to mean that the grass juice contained an unidentified growth factor which was spared by the antibiotic supplement. It is also possible that the grass juice contained an antibiotic substance which was effective in promoting growth.

Presence of Disease As a Factor Influencing Response to Antibiotics.—The relation of disease to antibiotic response has been postulated and widely discussed by many. It should be pointed out that the type of disease involved is, for the most part, unknown. The correlation of antibiotic growth response in poultry with specific diseases of known etiology is very limited. White-Stevens and Ziebel(24) have presented information showing that responses of young chicks to antibiotics are greater when chronic respiratory disease is present in the flock. Undoubtedly, the

presence of any disease in poultry caused by an agent susceptible to an antibiotic would tend to increase the response to the antibiotic providing a sufficiently high level was used.

Hill and Kelly(11) have presented some interesting results showing that they failed to obtain a response to low levels of Aureomycin in a new laboratory but did produce a growth response by adding 100 ppm of this antibiotic. This would suggest that the growth response to antibiotics in poultry is influenced by some microorganisms that are susceptible to low levels of antibiotics such as 2 to 10 grams per ton and others that are susceptible only to much higher levels in the neighborhood of 100 grams per ton of feed.

New Research Needs.—It would appear that the following are some of the more urgent problems in connection with the use of antibiotics in poultry production and for a better understanding of their mode of action.

1. Continue the investigations on correlation of growth response in poultry to antibiotics with intestinal microflora changes and include studies in which known organisms are added to the diets.

2. Investigate more fully the nonspecific diseases such as enteritis, diarrhea, etc., which have been associated with antibiotic responses in poultry, in order to obtain more complete knowledge on their etiology.

3. Study more extensively the influence of individual feed ingredients on response to antibiotics.

4. Investigate the relationship between genetics and antibiotic growth responses.

5. Expand the studies on chickens and turkeys kept for egg production and reproduction purposes in order to explain the discrepancies which are now in the literature.

6. Continue long-time studies with both chickens and turkeys in order to obtain information on the effect of continuous use of antibiotics throughout the life cycle of poultry.

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ANTIBIOTICS FOR SWINE, BEEF CATTLE, SHEEP, AND DAIRY CATTLE

This report draws heavily on review papers on antibiotics by Braude, Wallace and Cunha(2) with swine, Knodt(9) with ruminants, Lassiter(10) with dairy cattle and on general antibiotic review papers by Jukes and Williams(8), Cunha and Wallace (6), Stokstad(15) and Braude, Kon and Porter(3). Moreover, many other summaries and papers on antibiotics by other workers in this country were used in arriving at review data presented in this paper.

The antibiotic field is still relatively new and has developed rapidly. The first paper showing the beneficial effect of including an antibiotic in the ration of animals was that of Moore *et al.*(12). No one followed up this first work and it was not until 1949 that antibiotic feeding work started in earnest. In 1949, the first papers appeared reporting that products from Aureomycin (chlortetracycline) fermentation promoted growth in chicks (Stokstad and Jukes, April 1949) (14) and in pigs (Cunha *et al.* September 1949)(5). Shortly after that time it was found that what was then called animal protein factor (APF) supplements contained antibiotics and B_{12}. The first published scientific paper showing the value of crystalline Aureomycin in swine feeding, by Jukes *et al.*(7), appeared in April 1950. Many other investigators soon published similar results from experiments with pigs. These included: D. V. Catron (Iowa State), R. W. Luecke (Michigan State), J. L. Krider and S. W. Terrill (Illinois), W. M. Beeson and C. M. Vestal (Purdue), L. E. Hanson (Nebraska), L. E. Carpenter (Hormel Institute), E. R. Barrick (North Carolina) and many others. Since then antibiotics has become a household word in the feed world. The first published papers on antibiotic supplements concerned poultry (Stokstad and Jukes)(14) and swine (Cunha *et al.*)(5) and were followed closely by papers on dairy calves (Rusoff and Haq, Williams and Knodt, and Loosli and Wallace) (13), (17), (11). Shortly thereafter, reports on ruminants appeared (Colby *et al.* with sheep and Bell *et al.* with steers)(4), (1) but disappointing results were obtained. In the last few years, however, information on beef cattle and sheep indicates that antibiotics have an increasingly important place in the feeding of these animals.

ANTIBIOTICS FOR SWINE

Following is a brief summary of the information available to date (October, 1955) on antibiotics in swine nutrition:

1. Antibiotics increase growth rate an average of 10-20 per cent. The resulting earlier marketing saves labor and other costs. The better the over-all nutritive value of the ration, in the absence of disease level effect, the less the improvement in growth due to antibiotic additions. However, antibiotics are of benefit even with high quality well-balanced rations. It has been shown that Aureomycin (chlortetracycline) and Terramycin (oxytetracycline) are more effective than penicillin, bacitracin and streptomycin for swine.

2. They increase efficiency of feed utilization about 5 per cent, a saving of about 20 pounds of feed per 100 pounds of gain. The antibiotic response is confined to the growth rate and does not affect the final size of the animal. While the saving in feed may appear small, it results in a considerable saving to the swine industry. Under conditions of stress, such as poor sanitation and low quality rations, the improvement in efficiency of feed utilization is much greater. Since conditions of stress are abundant under average farm operations, it must be kept in mind that antibiotic response may be greater there than under Experiment Station conditions. This *must* be kept in mind in applying the Experiment Station antibiotic data to average farm conditions.

3. Antibiotics increase bloom and appearance of the animal.

4. They help control certain types of nonspecific enteritis (scours). This is of considerable importance since enteritis causes excessive loss to the farmer. Most investigators are convinced that in swine nutrition the degree of effectiveness of antibiotic feeding is largely determined by the disease level. The disease level is defined as the degree of feed lot infection with bacterial and/or virus diseases which cause scouring or diarrhea in pigs. Thus, the theory (first proposed by Dr. Damon Catron of Iowa State) is that the greater the disease level, the more response the animal will receive from antibiotic supplementation and the higher the level of antibiotic required.

5. Antibiotics reduce the number of runts and thus make pigs within litters grow more uniformly in size. Runts or young pigs suffering from scours respond to antibiotic feeding more markedly than normal pigs. In twelve comparisons an average growth rate stimulation of 82.2 per cent was obtained. Runts or "poor doers" in a herd of pigs are undesirable. Considerable feed is wasted with runt pigs. Moreover, they are more susceptible to disease infection and may spread trouble to the rest of the herd. Thus, the beneficial effect of antibiotics with runt pigs is of great economic importance to the swine producer.

6. It has been found that antibiotics tend to reduce the amount of protein supplement needed in the ration for swine. Protein is a costly item and a saving, no matter how small, would have major economic significance. It is not yet clear how much of the effect in reducing protein needs is due to the antibiotic and how much to a more adequate ration fortified with B_{12}, riboflavin, niacin, and pantothenic acid. Evidently protein requirements have been high in the past because protein supplements were supplying factors other than amino acids.

7. The greatest beneficial effect from antibiotics is observed during the early growth period. Older animals are benefited by antibiotic supplementation, but the improvement in gain is not as great as for younger animals. When antibiotics are fed to a weight of 75-125 pounds and then dropped from the diet, the pigs generally

slow down in rate of gain. Thus, for continued maximum growth it is necessary to feed antibiotics from weaning to market weight.

8. Creep-feeding antibiotics to pigs during the suckling period will increase the weaning weight of pigs five to ten pounds at eight weeks of age. This extra weight enables these pigs to do better after weaning.

9. At present there are insufficient data to indicate that combinations of antibiotics are more useful than the most effective antibiotic fed alone at the appropriate level even though a combination of bacitracin and penicillin seems to have had some advantage in a few trials. The Illinois Station has also shown that a combination of Terramycin, penicillin, bacitracin and in some cases, streptomycin, exhibited a growth stimulus which was superior to Terramycin alone when fed at equivalent levels. This question, however, needs further exploration.

10. A level of 5 mg of antibiotic per pound of total ration (10 grams per ton of feed) or 25 mg per pound of a protein supplement (50 grams per ton of feed), which is intended to be fed free-choice with grain, is generally considered sufficient, based on Experiment Station data available to date. This level may vary somewhat depending on the antibiotic fed, the weight of the pig, the ration used and also on environmental conditions that may be related to disease level and other stress factors to which pigs are normally subjected, such as weaning, castration, vaccination, temperature fluctuations and others. Under some of these circumstances a higher level may be necessary.

11. Therapeutic levels, or high level antibiotic feeding, may mean supplying as much as 10-50 times (and possibly higher occasionally) the present feeding levels. These high levels are used as an aid in overcoming infections and for periods of nutritional stress. High level antibiotic feeding aids in the prevention and treatment of swine dysentery, scours, enteritis and other forms of nonspecific digestive disorders. Unfortunately, there is very little experimental information on high level antibiotic feeding since the "disease level" at the various Experiment Stations is much lower than that encountered on the average farm. However, experience on farms shows that high level antibiotic feeding has a definite place in swine feeding.

12. Several antibiotics such as neomycin, subtilin, rimocidin, polymyxin, and Chloromycetin have been tested and have not been of any benefit.

13. Nineteen comparisons conducted on pasture produced an average improvement in growth of 13.7 per cent and a corresponding saving of 3.8 per cent in feed as a result of antibiotic supplementation. Thus, it appears that antibiotics are effective on pasture as well as in dry lot. However, they increase growth rate more under dry lot feeding conditions.

14. Several studies have been specifically designed to determine the role of vitamin B_{12} as it relates to the antibiotic effect. In these tests the average growth response was 15.6 per cent for rations containing B_{12} and 11.0 per cent when B_{12} was not added. From this evidence, it is apparent that the antibiotic stimulated growth regardless of B_{12} supplementation. However, the best results were obtained when both were added. Additional experiments are needed to clarify the relationship of antibiotic response to other B-complex vitamins. However, there are indications that antibiotics may spare certain B-complex vitamins such as B_{12}, pantothenic acid, niacin and riboflavin.

15. The antibiotic effect is materially influenced by the type of ration fed. Other factors being equal, pigs fed a ration with better over-all nutrient balance will respond less to antibiotic supplementation than those fed a poorer ration. Even on good rations there is variation in antibiotic response depending on the feeds used in the ration. Thus, the quality of the ration as well as the kind of feeds used will affect antibiotic response.

16. At the present time, the information available on the effect of antibiotics on swine carcasses is not in complete agreement. There are a few reports which indicate that antibiotics increase the depth of backfat and, in general, lower carcass quality. However, there are more reports which show that antibiotics do not interfere with carcass quality when fed in properly balanced rations. The Florida Station showed that Aureomycin, per se, did not have any effect on carcass quality when feed intake was kept the same and the only difference between the rations was Aureomycin. Studies presently under way should add considerably to the knowledge in this field. It is possible that the breeding of the pig may account for some of the variation encountered in the effect of antibiotics on carcass composition.

17. A majority of experiments show that the subcutaneous implantation of baby pigs with antibiotic pellets is not effective in increasing weaning weights or liveability.

18. Antibiotics have been shown to increase appetite. This causes the pig to eat more feed and thus gain faster and with increased efficiency. An exception to this is a recent study by Wallace and Gillespie (1954) (16) which showed that erythromycin fed at levels of 2, 6, 10 and 20 mg per pound of feed in a corn-soybean ration increased feed efficiency (about 7 per cent) even though feed intake was decreased (about 6 per cent). Rate of gain, however, was not increased. Erythromycin combined with penicillin increased the rate of gain with a corn-cottonseed meal ration.

19. Antibiotics do not eliminate the necessity for practicing strict sanitation in swine production. However, they do make possible the production of pigs under high disease level conditions where such was not possible before. The organisms which cause the disease level are likened to weeds in a pasture. They do not kill the pasture but they lower the production rate because of competition for nutrients, etc. Thus, what we have considered as normal growth in the pig has been, in many cases, below normal because undesirable microorganisms in the digestive tract were retarding the animal to a certain extent. Thus, antibiotics may act as policemen to control the undesirable microorganisms. This is a new concept since most pigs, although apparently healthy in appearance, are actually suffering from various degrees of intestinal infection. Most hog producers use their same facilities year after year and in many cases keep crowding more animals into the same area. This means that their disease problem will increase and more attention to disease control and good sanitary practices will be needed. Antibiotics will be of great help in this problem but they should be used as an adjunct to sanitation and not as a substitute for it and good management.

20. As yet, there is no definite evidence which explains the manner in which antibiotics function. Work in England, at Beltsville, at the Texas Station, and elsewhere has shown that the growth stimulating effect of antibiotics could not be duplicated by keeping chicks in isolation in new quarters where chicks had not been kept previously. All of that work would imply, as has been thought by most investigators,

that antibiotics act upon some system connected directly or indirectly to the microbial flora of the host animal. However, exactly what occurs is still unknown. Some of the possibilities are as follows: (1) Increase of microorganisms which produce known or unknown vitamins or other growth stimulating factor(s); (2) Decrease of microorganisms which use up growth factors in the intestinal tract; (3) Decrease of microorganisms which produce toxins or are pathogenic and thus cause slow growth (owing to subclinical infection or disease level effect); (4) Decrease of microorganisms which interfere with absorption of food through intestinal wall; and (5) A combination of some or all of these possibilities or even others.

21. Evidence on the value of antibiotics for gestation and lactation is still inconclusive. A few reports indicate that in some experiments, the antibiotic is of value when fed at levels of 10-15 mg per pound of ration (20-30 grams per ton of feed) or even higher. Some investigators, however, have found no beneficial effect. Sows at the Florida Station have been fed Aureomycin continuously and have produced 5 litters without any harmful effect. Data at the Georgia Station on first and second generation animals have shown no harmful effect and possibly a beneficial effect from including Aureomycin in the ration for sows (see Table 1).

It is apparent, from data obtained to date, that Aureomycin feeding to sows is not harmful and may be of value in certain cases. It is very possible that the beneficial effect is apparent when stress factors such as quality of ration, disease level, sanitary condition, etc. are operating. Under average farm conditions where sanitation is not always too good and the ration is not always well balanced, it is possible that antibiotics may be beneficial for the sow.

22. Data obtained at the Florida Station showed no difference in thiamine, riboflavin, or niacin deposition due to antibiotic feeding. This indicates that the antibiotic did not interfere with the deposition of these vitamins in the carcass of the pig.

23. There is very little, if any, likelihood of harmful effects from feeding the effective antibiotics to swine. Iowa Station workers fed pigs from weaning to 100 pounds (55 days) on a ration containing 500 mg of a combination of antibiotics per pound of total ration. Not only were no ill effects observed but the pigs made very rapid gains of 1.41 pounds daily and required 217 pounds of feed per 100 pounds of gain. This provided an intake of as much as two grams of antibiotics per pig daily.

TABLE 1

COMBINED SUMMARY OF ALL FARROWINGS*

	Without Aureomycin	With Aureomycin †
Sows bred	37	38
Litters farrowed	35	35
Av. No. pigs farrowed alive	8.42	9.28
Av. No. pigs farrowed dead	1.52	0.86
Av. birthweight, lbs.	2.73	2.79
Av. 21 day weight, lbs.	10.75	10.67
Av. No. pigs weaned per litter	5.70	7.29
Av. weaning weight, lbs.	34.6	32.9

* Includes data on all first and second generation animals.
† Data from Dr. R. F. Sewell, Georgia Agr. Exp. Sta., Athens, Georgia.

While a great deal has been learned about antibiotics, more complete information is still needed on the following:

1. Influence of effective level of antibiotic feeding on the other nutrients required by the pig. Certain nutrients such as protein, B_{12}, pantothenic acid, niacin, riboflavin and others may be lowered because of antibiotic feeding; whereas calcium, phosphorus and others may be increased.
2. More complete information on the effect of antibiotics during growth on carcass quality.
3. Effect of combinations of antibiotics versus single effective antibiotic feeding to swine, especially with varying disease level conditions.
4. Effect of high level antibiotic feeding on swine, the levels to use and the length of time they should be fed.
5. Level of antibiotics to use in pre-starter and starter feeds.
6. Effect of antibiotics on the intestinal flora of the pig.
7. Mode of action of antibiotics.

Dairy Animals

The feeding of an antibiotic appears to be justified in the rations of dairy calves under 16 weeks of age although experiments have shown antibiotic supplementation to benefit dairy calves considerably past this age. There appears to be very little advantage, however, in feeding an antibiotic to animals past 12 to 16 weeks of age. No information is available to indicate any advantage to feeding antibiotics to mature lactating animals. Studies at Louisiana, Cornell, and Kansas showed no harmful effects from feeding Aureomycin to lactating cows. Aureomycin supplementation of the ration did not significantly affect milk and fat production, milk composition, bacterial count of milk, appetites of the cows, or rumination. There is no reason yet, however, to include antibiotics in dairy cow feeds. However, antibiotics may be effective against certain diseases and thus may be used therapeutically.

Antibiotic feeding stimulates growth rate as measured by body weight gains and skeletal growth, decreases the incidence and severity of calf scours, increases feed consumption and feed efficiency and improves the condition and appearance of young dairy calves. Growth rate is improved from 10 to 30 per cent and most of the improvement occurs before the calves are eight weeks of age.

Many workers have shown that the mortality rate with young dairy calves is in the range of 15-30 per cent and that calf scours is one of the main causes of these deaths. Since antibiotics reduce the incidence of calf scours, they are very valuable and effective in this respect. This undoubtedly is one of the most important reasons for including antibiotics in dairy calf rations.

As far as has been determined, antibiotic supplementation does not have any detrimental effect on the rumen or fecal flora of the calf. Antibiotic supplementation has been shown to be beneficial with calves fed whole milk as well as milk substitutes.

Based on information available to date and reviewed by Lassiter (1955) (10) it appears that the minimum effective level of antibiotic for young dairy calves is approximately 15-20 mg per 100 lbs. live weight daily. Greater amounts than this may be needed, however, when high level infections exist on the farm. Milk replacement

feeds should contain about 20 mg of antibiotic per pound of feed. Calf starters should include 10-20 mg of antibiotic per pound of feed. Lassiter (1955) also states as follows: "The antibiotics, Aureomycin and Terramycin, are the only ones which have been studied sufficiently to warrant valid conclusions. More research work needs to be conducted with Terramycin before conclusions concerning its use can be accepted with the same degree of confidence as with Aureomycin."

Beef Cattle

The first report on feeding Aureomycin to beef cattle showed that it was harmful. Later, however, it was found that if Aureomycin was fed at lower levels and thoroughly mixed in the ration, no adverse effects were obtained and that beneficial effects may occur. The research information obtained to date indicates the following benefits from Aureomycin feeding to beef cattle: (1) decreases incidence of scours, increases growth rate and reduces liver abscesses in calves; (2) may increase rate of gain and feed efficiency in fattening steers; (3) improves hair coat and causes more bloom in the animals. A level of 10 mg. of Aureomycin per 100 lbs. live weight daily is about optimum for fattening steers and for beef calves. The kind of ration and the ratio of concentrates to roughage may be of some importance in determining antibiotic effect. Antibiotics generally have been beneficial with high roughage rations and not with high-concentrate rations but there have been exceptions to this and the kind of rations benefited by antibiotics is not yet clear. Antibiotics may have their greatest effect when fed to calves or steers with low grade infections brought about by conditions of stress such as shipping exposure, feedlot infection, bad weather, poor management, etc. which usually exist under commercial feeding practices. It must be emphasized that the above information is based on research to date and that more experimental work is needed before all the details are obtained on feeding antibiotics to beef cattle. Indications now, however, are that Aureomycin can be safely fed to beef cattle if used at proper levels, and beneficial effects may be obtained. Very little work has been conducted with other antibiotics to determine their value in beef cattle feeding.

Sheep

An early report on sheep showed that feeding them Aureomycin by capsule was harmful. It was later found, however, that if lower levels of the antibiotic were used and thoroughly mixed in the ration no harmful effects resulted. The research information obtained to date indicates the following benefits from Aureomycin feeding to lambs: (1) Usually there is an increase in rate of gain and efficiency of feed utilization; (2) Lambs are easier to get on full feed; (3) Death losses from enterotoxemia may be reduced; (4) Less trouble occurs with pneumonia and scouring; and (5) A slight improvement in carcass grade and dressing percentage has been obtained in some trials. It appears that Aureomycin improves the health of lambs and thus the biggest reason for its use in lamb rations may be in suppressing feed lot infection. A level of 10 mg of Aureomycin per pound of total ration is about optimum. The type of ration and the ratio of concentrates to roughages may be important in determining whether an antibiotic will benefit sheep, although

benefits have been obtained with many types of rations. In this respect an interesting new approach is the use of self-feeding techniques for fattening lambs. The addition of Aureomycin at recommended levels results in beneficial effect with either pelleted or non-pelleted feeds. The use of pelleted rations appears to offer a definite advantage in increasing rate of gain and feed efficiency and is being widely accepted in feed lot areas. Aureomycin has been beneficial in creep rations for suckling lambs. Good results have been obtained by feeding Aureomycin to cull or scrub lambs. More research information is needed on antibiotic supplementation with lambs before it can be recommended without reservation in all lamb rations. All recent experimental work with appropriate levels of antibiotic mixed in the ration, however, has shown no harmful effect and in most cases a variable but favorable response was obtained. Certainly the over-all picture concerning the use of antibiotics in sheep rations looks much brighter now than it did a few years ago. Most of the sheep work has been conducted with Aureomycin.* Very little work has been reported on the value of the other antibiotics in sheep rations.

Summary

The value of antibiotics in swine and dairy calf feeding is well established. Data on the effectiveness of antibiotics in rations for beef cattle and sheep are accumulating rapidly. Present knowledge indicates that effective antibiotics mixed in the ration at proper levels do not have any harmful effects and are usually beneficial for beef cattle and sheep. More work is needed to determine the value of different antibiotics in rations for sheep and beef cattle. Most of the experimental work to date with these classes of livestock has been with Aureomycin.* It seems that the most marked effects from antibiotics are found with unthrifty animals.

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* However, considerable work has recently been done with Terramycin (see discussion by Dr. Luther, pp. 41–42) (Ed.).

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THE INFLUENCE OF ANTIBIOTICS ON CARCASS QUALITY OF PIGS

A very great number of experiments on the feeding of antibiotics to pigs have been carried out in many countries under very different conditions. A great number of these experiments have dealt only with the effect on growth, feed utilization and health of the animals, but there are also many experiments on the influence of antibiotics on carcass quality, too numerous to mention in this short review. Another difficulty is the evaluation of carcass quality. Not only do methods of measuring and judging carcass qualities differ from country to country, but so do the carcass weight and quality aimed at. As the sex has a great influence on carcass quality, experiments with different numbers of gilts and barrows in each group without sex correction, may contribute to the difficulties in evaluating the results. Finally different breeds (lard or meat type) may not react alike to different kinds of feeding and management. Therefore, a comparison of results from one experiment to another or from one country to another can be very difficult.

In Denmark carcass quality is considered a point of the utmost importance, and no feeding experiments on pigs are carried out without an examination of the carcasses. The following survey is to a rather large extent based upon Danish experiments.

The level of feeding (restricted or ad lib.) at optimal amounts of proteins, minerals and vitamins has a great influence on the carcass quality, especially on the ratio of lean meat to fat. It is impossible to force the pigs to produce meat above the level determined by heredity. Practically speaking a surplus of feed (calories) will, therefore, be converted into fat, and lots fed according to appetite will consequently put on more fat than those fed restrictedly when fed the same kind of feed.

Breirem *et al.*(9) point out "that antibiotics tend to increase the appetite. . . . The reason why the response to antibiotics is less in terms of feed efficiency than in terms of growth, may be that the higher feed consumption leads to greater depositions of fat. In the Scandinavian countries we now recommend restricted feeding in order to obtain leaner pigs. Feeding according to appetite (ad lib.) as mostly practised in the United States and other countries, produces a surplus of low-price lard which it would serve no useful purpose to produce under European conditions."

On the other hand, Lasley *et al.*(17) write ". . . that increases in feed con-

sumption after antibiotics were added to the ration were due to the larger size of the pigs fed Aureomycin and not to an increase in the amount of feed consumed per day per unit of body weight." Therefore, when dealing with the problem of effect on carcass quality when feeding antibiotics, it is necessary to consider this effect both with restricted and with ad lib. feeding.

Restricted Feeding

Influence on Carcass Quality at Optimal Protein Levels.—Ludvigsen and Thorbek(18) found no difference between Aureomycin fed pigs (20 mg daily) and controls with regard to total N retained. The former had a greater respiratory quotient for nitrogen-free substance (and, at the same time, a lower heat production). This seems to indicate that Aureomycin has an unknown effect on the transformation of carbohydrates into fat.

Using procaine penicillin (10 mg per Scandinavian Feed Unit=S.F.U.) Møllgaard(19) found a definite increase in the rate of production of protein and phosphate structures in growing pigs, whereas the influence on the calcium structures was negligible.

Hegener(13) found that streptomycin, procaine penicillin and Aurofac had a favourable effect on the nitrogen balance.

During the last three and a half years my department, in co-operation with the Chemical Department of the National Research Institute on Animal Husbandry and the Danish Meat Research Institute, has carried out more than 100 experiments, comprising more than 3,000 pigs, on feeding antibiotics to growing pigs (20-90 kg live weight). Table 1 gives a summary of some of the results obtained. All in groups A and B have received the same amount of digestible true protein 110-120 grams per S.F.U. at 20 kg, decreasing to 80-90 grams per S.F.U. at 90 kg live weight. Furthermore the supplements of minerals and of the vitamins A, B and D have been up to the known standards and the same for both groups. In consequence, the differences between groups A and B should be due to the effect of the antibiotic supplement.

When feeding 10 mg * procaine penicillin, Terramycin, Aureomycin or Aurofac (the Aurofac administered in amounts corresponding to 10 mg of pure Aureomycin per S.F.U.) there was a significant effect on the daily weight gain and feed consumption per kg live weight gain, as found by other investigators. (Table 1.)

Feeding the above antibiotics had no influence on the body length of the pigs. However, when speaking about carcass quality the most important question is whether the increased daily weight gain, achieved by feeding antibiotics together with restricted rations, can be expected to bring about an increase in the production of fat. Table 1 shows, however, that this is not the case. Feeding of antibiotics has had no effect on thickness of back fat, thickness of streak, weight of leaf fat, number of scores for amount of lean meat in the carcasses, and on the grading of the pigs according to thickness of back fat.

Body length is taken from the front edge of the pubic bone to the atlas joint

* 10 mg of antibiotics per S.F.U. correspond with approximately 10 grams of antibiotics per ton of feed.

TABLE 1

THE INFLUENCE OF PENICILLIN, TERRAMYCIN, AUREOMYCIN AND AUROFAC ON THE QUALITY OF PIG CARCASSES

Restricted Feeding: Growing period 20-90 kg live weight.

Antibiotics	Procaine Penicillin		Terramycin		Aureomycin		Aurofac*	
	A§	B	A	B	A	B	A	B
No. of pigs	722	722	145	145	73	73	53	53
S.F.U. per pig daily†	2.05	2.06	2.02	2.04	2.01	2.02	2.01	2.00
Daily weight gain, g	583	610	576	621	569	593	573	587
S.F.U. per kg live weight gain†	3.52	3.38	3.51	3.29	3.53	3.41	3.50	3.40
Dressing percentage	73.0	73.2	73.1	73.6	73.4	73.5	73.5	73.7
Body length, cm	94.5	94.5	93.8	93.6	94.2	93.9	94.4	93.9
Thickness of back fat, cm‡	3.34	3.36	3.41	3.39	3.43	3.37	3.44	3.41
Weight of leaf fat, kg	1.76	1.75	1.78	1.75	1.79	1.73	1.77	1.73
Thickness of streak	3.21	3.22	3.31	3.32	3.34	3.34	3.33	3.37
Scores (0–15) for:								
Amount of lean meat	12.5	12.5	12.6	12.6	12.6	12.8	12.6	12.7
Colour of lean meat	12.3	12.4	12.1	12.3	12.0	12.4	12.1	12.4
Size of shoulders	11.9	11.9	12.0	11.9	12.2	12.2	12.2	12.4
Size of hams	12.2	12.2	12.1	12.1	12.5	12.5	12.4	12.7
Distribution of back fat	12.7	12.7	12.7	12.5	12.7	12.7	12.6	12.7
Fineness of head, bone, skin	13.0	12.9	13.0	13.0	12.9	12.9	12.8	12.9
Bacon type	12.0	12.0	11.9	11.9	12.2	12.3	12.2	12.2
Firmness of back fat	13.1	13.1	12.8	12.7	12.8	12.8	12.9	13.0
Iodine number (back fat)	59.8	59.6	59.8	60.3	57.2	57.6	57.2	56.6
Per cent pigs in grade I (lean)	83	83	84	81	83	84	82	78
Per cent pigs in grade II (too fat)	15	14	14	18	14	14	15	18
Per cent pigs in grade III (much too fat)	2	3	2	1	3	2	3	4

* Aurofac is given in amounts corresponding to 10 mg pure Aureomycin per S.F.U.
† 1 S.F.U. is the feeding value of 1 kg barley.
‡ Corrected for an equal number of sows and barrows in each group and for 67.0 kg slaughter weight and 94.0 cm body length.
§ Group A = no antibiotics. Group B = 10 mg antibiotics per S.F.U.

As the experiments with Terramycin, Aureomycin and Aurofac did not comprise so many different combinations of feed as did those with penicillin, the table should not be used for comparisons between the effects of the different antibiotics.

while the side is lying flat on a table. Thickness of back fat is measured: A. Shoulder, at the thickest point, B. Mid back, at the thinnest point and C. Loin, 3 measurements made into one average for loin. Average of A, B and C is the thickness of back fat. Thickness of streak is the average of 3 measurements a-c all taken in the row of teats by help of a stiletto: (a) At a point four fingers' width from the sternum, (b) At a point four fingers' width from the ham and (c) At a point exactly between a and b.

Table 2 gives more detailed results from the experiments with procaine penicillin. There is no clear evidence that procaine penicillin has increased fat production at any of the feed combinations used. Only when extr. Soya Bean Meal+extr. Linseed Meal was used as protein supplement can a tendency to increased fat be noticed, but the figures refer only to one experiment with 10 pigs in each group.

To be sure that the pigs had not deposited fat in other parts of the body than the back 92 carcasses were anatomically cut up into bones, fat, and lean meat. The results are given in Table 3. Penicillin- and control pigs had exactly the same amount of lean meat when fed restrictedly.

Chemical analyses of the whole carcasses in connection with antibiotic experiments on rabbits have been made. Procaine penicillin did not change the crude protein, fat, and water content in the carcasses of rabbits fed restrictedly. The procaine penicillin has reduced the percentage of ash and the difference is significant at $0.05>P>0.01$. (Table 4.)

To investigate whether feeding antibiotics results in an increased production of intramuscular fat, samples from eye muscle (*longissimus dorsi*) and tenderloin (*psoas m. major*) were analysed in two experiments. (Table 5.) The antibiotics used did not change the chemical composition of the meat. The results seem to indicate that procaine penicillin and Terramycin reduce the water content of the back fat while the water content is increased by Aureomycin and Aurofac, but the differences are not significant.

The pigs used in the experiments shown in tables 1-5 were fed restrictedly and all of them slaughtered at the same live weight (90 kg). The results indicate that the antibiotics used in these experiments have had no effect on the content of lean meat and fat in the carcasses or on the content of fat in the muscles. Consequently the following conclusion can be drawn: As antibiotics used increase the daily weight gain without changing the chemical composition of the carcass it can be concluded that the daily production of meat as well as that of fat is increased by the use of penicillin, Terramycin and Aureomycin.

Official British experiments(1) showed that procaine penicillin and Terramycin (restricted feeding) given in rations containing animal plus vegetable protein or vegetable protein alone, increased the daily weight gain. The quality of the carcass was not affected by the feeding of these antibiotics.

According to Table 6 different levels of procaine penicillin have no effect on body length, thickness of back fat and streak, weight of leaf fat, firmness of back fat and the grading of the pigs according to thickness of back fat. The dressing percentage will be discussed later.

Table 1 shows that a supplement of 10 mg procaine penicillin, Terramycin, Aureomycin (or Aurofac) per S.F.U. to pigs fed restrictedly and slaughtered at

TABLE 2

THE INFLUENCE OF PENICILLIN ON CARCASS QUALITY

Restricted Feeding

No. of pigs in each group	Protein supplement	Basic fodder	Dressing percentage		Weight of leaf fat kg		Thickness of back fat cm		Thickness of streak cm		Scores for amount of lean meat	
			A§	B§	A	B	A	B	A	B	A	B
84	Milk	Grain	73.8	74.0	1.7	1.7	3.33	3.32	3.27	3.34	12.6	12.6
24	Milk	Grain + lard	74.3	74.1	1.7	1.5	3.48	3.54	3.39	3.41	12.3	12.0
43	Milk	Grain + sugarbeet	72.7	72.7	1.8	1.7	3.37	3.27	3.14	3.11	12.3	12.3
8	Milk	Grain + boiled potatoes	72.6	72.4	1.7	1.7	3.50	3.50	3.18	3.25	12.2	12.6
108	Milk + M & B-Meal*	Grain	72.7	73.2	1.7	1.7	3.48	3.47	3.21	3.22	12.3	12.2
34	Milk + M & B-Meal	Grain + sugarbeet	74.0	73.7	1.8	1.8	3.11	3.13	3.23	3.28	12.4	12.2
48	Milk + M & B-Meal	Grain + boiled potatoes	72.5	72.4	1.5	1.5	3.25	3.29	3.29	3.23	12.6	12.7
12	Milk + M & B-Meal	Grain + raw potatoes	71.8	71.8	1.5	1.7	3.38	3.28	3.29	3.19	12.6	12.7
16	M & B-Meal	Grain	74.0	73.2	1.9	1.8	3.60	3.39	3.36	3.30	12.3	12.8
20	M & B-Meal	Grain + sugarbeet	72.6	72.7	1.9	1.8	3.37	3.22	3.17	3.28	12.5	12.9
138	M & B-Meal + Soya B-Meal†	Grain	73.2	73.8	1.8	1.8	3.33	3.41	3.11	3.15	12.4	12.4
82	M & B-Meal + Soya B-Meal	Grain + sugarbeet	72.5	72.4	1.8	1.8	3.24	3.31	3.15	3.12	12.8	12.6
12	M & B-Meal + Soya B-Meal	Grain + boiled potatoes	72.2	72.2	1.9	1.7	3.36	3.53	3.22	3.17	12.9	12.7
29	Milk + M & B-Meal + Soya B-Meal	Grain	74.1	74.4	2.0	2.0	3.29	3.36	3.32	3.41	12.5	12.7
22	Milk + M & B-Meal + Soya B-Meal	Grain + sugarbeet	71.1	70.9	1.5	1.6	3.10	3.05	3.04	3.00	12.9	12.9
12	Milk + M & B-Meal + Soya B-Meal	Grain + boiled potatoes	71.6	72.5	1.5	1.7	3.46	3.48	3.12	3.26	12.7	12.3
10	Soya B-Meal + L-Meal‡	Grain	73.4	73.8	2.2	2.6	3.20	3.53	3.56	3.60	12.7	12.3
20	Lupin Seed Meal	Grain + boiled potatoes	74.1	73.4	2.1	2.0	3.51	3.49	3.27	3.25	12.7	12.5
Average 722 pigs in each group			73.0	73.2	1.76	1.75	3.34	3.36	3.21	3.22	12.5	12.5

* M & B-Meal = meat and bone meal.
† Soya B-Meal = extr. soya bean meal.
‡ L-Meal = extr. linseed meal.
§ A = no penicillin. B = 10 mg procaine penicillin per S.F.U.

TABLE 3

INFLUENCE OF PROCAINE PENICILLIN ON THE AMOUNT OF MEAT IN PIGS

Restricted Feeding: Anatomical cutting-up of whole carcasses. All pigs slaughtered at 90 kg live weight.

	No Procaine Penicillin		10 mg Procaine Penicillin per S.F.U.	
	No. of pigs	% meat	No. of pigs	% meat
Gilts	21	58.9	22	58.6
Barrows	28	55.3	21	55.6
Average	49	57.1	43	57.1

TABLE 4

INFLUENCE OF PROCAINE PENICILLIN ON CHEMICAL COMPOSITION OF RABBIT CARCASSES

Restricted Feeding

	No. of carcasses	Weight of carcass kg	Crude protein %	Crude fat %	Ash %	Water %
No Procaine Penicillin	5	1.76	20.4	10.3	5.2	64.7
Procaine Penicillin	13	1.82	20.7	10.2	4.6	64.8

TABLE 5

INFLUENCE OF FEEDING ANTIBIOTICS ON CHEMICAL COMPOSITION OF MEAT AND WATER CONTENT OF BACK FAT IN PIGS

Restricted Feeding

	No anti-biotics	Procaine Penicillin	Terra-mycin	Aureo-mycin	Aurofac
No. of pigs	8	7	8	8	7
Eye muscle (*longissimus dorsi*):					
Per cent dry matter	26.5	27.1	26.8	26.0	27.1
Per cent crude fat	1.9	2.4	2.4	1.9	2.5
Per cent fat-free dry matter	24.7	24.8	24.4	24.2	24.7
pH	5.48	5.49	5.48	5.49	5.38
Tenderloin (*psoas m. major*):					
Per cent dry matter	24.8	24.9	24.7	25.0	24.9
Per cent crude fat	1.9*	2.7	2.1	2.1	2.3
Per cent fat-free dry matter	22.5	22.2	22.7	22.9	22.7
pH	6.06	6.04	5.85	5.93	5.79
Per cent water in back fat	8.75	8.33	8.20	9.40	9.60

* One sample not analysed for crude fat (lost). All antibiotics: 10 mg per S.F.U.

90 kg live weight, has had no influence on size of shoulder and hams, distribution of back fat along the side, fineness of head, bone and skin, firmness of back fat or on the general bacon type.

In all the four groups of experiments (Table 1) the antibiotics used have increased the scores for color of lean meat (the meat has become darker). In breaking down the figures we found, however, that the antibiotics only increased the meat

TABLE 6

INFLUENCE OF DIFFERENT LEVELS OF PENICILLIN ON THE QUALITY OF CARCASSES

Restricted Feeding

	mg Procaine Penicillin per S.F.U.				
	0	5	10	20	30
No. of pigs	84	44	84	84	36
Dressing percentage	72.8	73.0	73.2	73.5	74.5
Body length, cm	95.0	95.5	95.2	94.9	94.6
Thickness of back fat, cm*	3.26	3.23	3.27	3.26	3.32
Weight of leaf fat, kg	1.74	1.74	1.72	1.70	1.81
Thickness of streak	3.13	3.11	3.20	3.12	3.21
Scores (0–15) for:					
Firmness of back fat	13.2	13.2	13.2	13.2	13.0
Per cent pigs in grade I (lean)	92	91	88	86	89
Per cent pigs in grade II (too fat)	8	9	9	11	11
Per cent pigs in grade III (much too fat)	0	0	3	3	0

* Corrected for an equal number of sows and barrows in each group and for 67.0 kg slaughter weight and 94.0 cm body length.

color significantly (at the 5 per cent level) for gilts. For barrows there was no effect as can be seen from the figures in Table 7.

Neither carcass quality nor the flavor of meat was influenced by procaine penicillin or Terramycin given to pigs up to a live weight of 60 or 90 kg. (Table 8.)

Influence of Antibiotic Supplements in Rations at Different Protein Levels. (Restricted Feeding)—When insufficient amounts of protein are fed the daily weight gain goes down and the feed consumption goes up. Many investigators have shown that the response is larger when antibiotics are added to rations with low protein levels; this can also be seen from the Danish experiments shown in Table 9. The group with a low protein level plus procaine penicillin had nearly the same daily weight gain and feed consumption per kg weight gain as that at the optimal protein level but without procaine penicillin.

When too little protein is fed the pigs are unable to produce meat up to the level determined by heredity, and consequently they put on more fat. Neither procaine penicillin nor Terramycin has been able to decrease the thick back fat found when

TABLE 7

SCORES (0–15) FOR COLOR OF LEAN MEAT

	Gilts		Barrows	
Antibiotic	0	10 mg per S.F.U.	0	10 mg per S.F.U.
Penicillin	12.36	12.46	12.33	12.31
Terramycin	12.21	12.41	12.14	12.21
Aureomycin	12.04	12.51	12.21	12.25
Aurofac	12.07	12.32	12.25	12.40
Average of all pigs	12.29	12.44	12.28	12.29
	Difference significant at $0.05 > P > 0.01$		Difference not significant	

TABLE 8

INFLUENCE ON CARCASS QUALITY WHEN PENICILLIN AND TERRAMYCIN ARE GIVEN UP TO A LIVE WEIGHT OF 60 AND 90 Kg RESPECTIVELY

Restricted Feeding: All pigs slaughtered at 90 kg live weight.

	No Procaine Penicillin	Procaine* Penicillin up to		No Terra-mycin	Terramycin* up to	
		60 kg	90 kg		60 kg	90 kg
No. of pigs	94	94	94	64	64	64
Dressing percentage	72.7	72.8	72.9	72.6	72.6	73.1
Body length, cm	94.0	94.4	94.6	93.7	93.7	94.0
Thickness of back fat †, cm	3.40	3.37	3.41	3.38	3.31	3.32
Weight of leaf fat, kg	1.75	1.72	1.72	1.78	1.72	1.68
Thickness of streak, cm	3.17	3.17	3.20	3.19	3.22	3.22
Scores (0–15) for:						
Firmness of back fat	13.1	13.0	13.0	12.7	12.3	12.4
Amount of lean meat	12.6	12.6	12.5	12.6	12.7	12.7
Colour of lean meat	12.4	12.3	12.3	12.2	12.2	12.3
Per cent of pigs in grade I (lean)	87	83	83	87	88	90
Per cent of pigs in grade II (too fat)	12	16	15	11	12	8
Per cent of pigs in grade III (much too fat)	1	1	2	2	0	2
No. of pigs	72	72	72	42	42	42
Scores (0–5) for:						
Flavour in fresh pork (cutlet)	3.92	3.96	3.91	3.92	4.00	3.97
Flavour in cured bacon	3.82	3.86	3.85	3.82	3.87	3.85
Colour in cured bacon	4.23	4.32	4.32	4.23	4.41	4.37

* 10 mg antibiotic per S.F.U.
† Corrected for an equal number of sows and barrows in each group and for 67.0 kg slaughter weight and 94.0 cm body length.

TABLE 9

PENICILLIN GIVEN TO RATIONS WITH DIFFERENT PROTEIN LEVELS

Restricted Feeding

	Protein level			
	Low		Optimal	
Protein concentrate* daily, gm	150	150	225	225
Procaine Penicillin, mg per S.F.U.	0	10	0	10
No. of pigs	20	20	20	20
Daily weight gain, gm	590	627	642	651
S.F.U. per kg live weight gain	3.67	3.39	3.33	3.26
Dressing percentage	72.8	72.9	72.4	73.1
Thickness of back fat,† cm	3.44	3.46	3.23	3.37
Thickness of streak, cm	3.20	3.20	3.11	3.12
Weight of leaf fat, kg	1.85	1.79	1.58	1.69

* ⅔ extr. soya bean meal + ⅓ meat and bone meal.
† Corrected for an equal number of sows and barrows in each group and for 67.0 kg carcass weight and 94.0 cm body length.

insufficient amounts of protein were given. (Tables 9 and 10.) This finding shows what severe danger to carcass quality there can be in general pig production, as the farmers are tempted to decrease the amount of the expensive protein supplement and just add antibiotics to the rations. Such a practice may give satisfactory results from the point of view of production but it will give carcasses which are too fat. This risk may be even greater in the case of feeding small amounts of protein with a low biological value (pure vegetable). However, this problem combined with restricted feeding needs further investigation, especially on meat-type pigs with the high protein requirement.

The Influence of Antibiotics on Dressing Percentage.—In the tables 1, 5, 6, 8, and 9 there is a clear tendency for the dressing percentage to increase when pigs are fed antibiotics. By dressing percentage is here understood weight of cold carcass with head, leaf fat and legs 24 hours after slaughtering in percentage of the live weight of the pig, recorded at the experimental station at 8:30 in the morning after the last feeding at 6:30 and before slaughtering at 12:30. A summary is given in Table 11.

As an average of 999 pigs which received no antibiotic supplement, the dressing percentage was 73.07. For the other 999 pigs fed antibiotics this percentage was 73.31. It is, however, noteworthy that the antibiotic had no effect on the dressing percentage when the basic ration consisted of grain plus sugarbeet or of grain plus potatoes. The whole effect comes from the pigs fed grain as their basic ration and here the dressing percentages were 73.42 and 73.84 respectively.

As an average the dressing percentage of all antibiotic-fed pigs, which received grain as their basic ration, was 0.42 better than that of the controls. This difference was significant at $0.05>P>0.01$. For a pig slaughtered at 90 kg live weight this means an increase in slaughter weight by 0.378 kg and this is important in countries where pigs are paid for according to carcass weight.

Considering the fact that all the groups A and B two by two received the same amount of feed per day (restricted feeding) the content of feed in the intestinal

TABLE 10

TERRAMYCIN IN RATIONS AT DIFFERENT PROTEIN LEVELS

Restricted Feeding*

	Protein level					
	Very low		Low		Optimal	
Kg skim milk per pig daily.....	0.5	0.5	1.0	1.0	1.5	1.5
Protein concentrate†	40	40	75	75	110	110
Terramycin per S.F.U., mg.....	0	10	0	10	0	10
No. of pigs.....	10	10	10	10	10	10
Dressing percentage	73.6	73.7	73.3	74.4	73.2	73.3
Thickness of back fat, cm‡.....	3.94	3.82	3.50	3.62	3.43	3.23
Thickness of streak, cm.....	3.55	3.43	3.35	3.39	3.40	3.40
Weight of leaf fat, kg.....	2.10	1.79	1.54	1.77	1.46	1.67

* The experiment just finished. Daily gain and feed consumption not yet evaluated.
† ¾ extr. soya bean meal + ¼ meat and bone meal per pig daily.
‡ Corrected for an equal number of sows and barrows in each group and for 67.0 kg carcass weight and 94.0 cm body length.

TABLE 11

DRESSING PERCENTAGE WHEN ANTIBIOTICS ARE ADDED TO A BASIC RATION CONSISTING OF GRAIN OR GRAIN + ROUGHAGE

	Penicillin‡			Terramycin			Aureomycin			Aurofac			Average		
Basic ration*	No. of pigs†	A§	B	No. of pigs†	A	B	No. of pigs†	A	B	No. of pigs†	A	B	No. of pigs†	A	B
Grain	385	73.30	73.70	101	73.44	73.94	53	73.85	74.22	53	73.85	74.24	592	73.42	73.84
Grain + sugarbeet	201	72.65	72.55	42	72.58	72.88	30	72.39	72.33	10	71.70	71.80	283	72.58	72.55
Grain + steamed potatoes	112	72.59	72.50	12	72.20	72.50	0	—	—	0	—	—	124	72.55	72.50
											Average		999	73.07	73.31

* Protein supplement, see table 2. All groups have received minerals and vitamins according to known standards.
† Number of pigs in each group.
‡ Procaine Penicillin.
§ A = no antibiotics. B = 10 mg antibiotic per S.F.U.

tract at the time of slaughtering can be considered the same for the pigs in both groups. The results, therefore, seem to indicate a weight reduction of 378 grams of the empty entrails when antibiotics are fed. Unfortunately the weights of the entrails and the organs were not recorded in the Danish experiments. Braude(7) found that weight of intestines from pigs fed Aureomycin was 146 grams less than that of the controls. Therefore, this alone can probably not be fully responsible for the above mentioned increase in carcass weight by 378 grams. Braude(7) further reports that British investigators have found that intestines from chicks fed antibiotics are thinner than those from controls.

The data in table 6 indicate that increasing levels of procaine penicillin seem to increase the dressing percentage, and in table 8 that procaine penicillin or Terramycin fed to a live weight of 60 kg has no or a very slight effect on dressing percentage. The effect seems, therefore, mainly to appear at the end of the growing period.

Feeding According to Appetite
(ad libitum)

Optimal Protein Level.—The numerous experiments with antibiotics given to pigs which have been fed ad lib. seem rather confusing as far as carcass quality is concerned. This may be due to different biological values of the rations, to the fiber content of the feed, or to the extent to which bulky feed (roughage) has been used. If the content of net calories per pound of feed is very high, an increase in the appetite caused by antibiotic supplement may result in poor carcass quality. If the content of calories per pound of feed is low, the effect may be negligible. Furthermore, environmental conditions and health may be limiting factors with regard to appetite.

Braude *et al.*(8) giving 20 grams of Aureomycin per ton of feed found no effect on nitrogen retention even though there was an increase in efficiency of food utilization. Teh-Cheng Huang *et al.*(15) using 33 grams Terramycin per ton of feed reported an improvement in growth and feed efficiency. Dry matter and protein digestibility was improved, especially at an early age. No difference was noted in the thickness of back fat, body length, and bone structure. The dressing percentage was significantly greater for the Terramycin-fed pigs.

Catron *et al.*(10) found with self-feeding and a concentrated feed ration consisting of fortified corn-soya bean oil meal that 10 mg Aureomycin per pound of ration did not increase the average daily feed intake, the thickness of back fat or the whole carcass quality, but the authors stress that all of the animals were very fat. The Aureomycin-fed pigs had a higher dressing percentage but the differences were not significant.

Hanson *et al.*(12) have added B_{12} plus Aureomycin, Terramycin or procaine penicillin to a concentrated ration given to self-fed pigs. The average daily feed consumption was increased by approximately 10 per cent, but percentages of moisture, crude protein or fat in the carcasses were not affected significantly by the feeding of any of the antibiotics. Also in these experiments all the pigs were, however, extremely fat. The antibiotic-fed pigs had a higher dressing percentage but the difference was not significant. The percentage of ash in the carcasses decreased significantly in all but one of the lots of pigs fed antibiotics.

Perry *et al.*(20) found with crossbred pigs that the daily feed consumption as well as the thickness of back fat considerably increased when Aureomycin was added to a concentrated ration of corn plus soya bean oil meal. Also in this experiment all the pigs were extremely fat. The dressing percentage was higher for the Aureomycin pigs but not significantly so.

Vestal reported that pigs receiving Aureomycin had back fat which was 10 per cent thicker than that of controls, but the pigs were allowed free choice of selection between shelled corn and a high-protein supplement with the result that the Aureomycin-fed pigs selected a higher proportion of corn than did the controls(21). Consequently the Aureomycin pigs have been fed ad lib. at a lower protein level than have the controls.

Bowland *et al.*(14) found for meat-type pigs (purebred Yorkshire) that Aureomycin and penicillin fed with a pure vegetable ration considerably increased the daily feed intake and also the thickness of back fat. The total Advanced Registry scores for slaughter quality was poorer for the antibiotic-fed pigs. The difference in shrinkage was significant ($P=0.05$) and indicates that the carcasses from pigs fed antibiotics probably contain more water. Other experiments also on purebred Yorkshires carried out by Bowland *et al.*(4) showed that Aureomycin given to rations containing vegetable or animal protein supplement in both cases increased the daily feed consumption and reduced the carcass quality. The authors say "Because of the effect on carcass quality antibiotic supplements cannot be recommended for use in finishing rations."

In the experiments with ad lib. feeding, here chosen as examples, it is difficult to find a clear line. Most of the experiments indicate that antibiotic combined with ad lib. feeding increases the daily feed consumption and brings about an unwanted higher fat production (thicker layer of back fat). Possibly this is less dangerous for the extremely fat pigs (lard-type) than for meat-type pigs. Most ad lib. feeding experiments show a tendency towards higher dressing percentages when antibiotics are added to the feed.

The explanation for the higher dressing percentage can be due to a special effect of the antibiotics (e.g. lower weight of entrails) or, as shown by Clausen(11), that fatter pigs on the average have a higher dressing percentage than leaner ones.

Bohman *et al.*(3) gave 4 different levels of alfalfa meal (0–50 per cent of the ration) with and without Aureomycin (10 mg per lb. of feed). Aureomycin accelerated the growth rate and decreased the feed required per pound of gain even at high levels of alfalfa feeding. Aureomycin had no effect on carcass measurements. With increasing levels of alfalfa, leaner carcasses were produced.

As the carcass quality and especially the meat content of the pigs is of the utmost importance in many countries, more experiments with many pigs are urgently needed. Co-operation between the experimental stations on an international basis would therefore be advisable. In Denmark we have now started a series of experiments based upon the following plans:

All pigs are fed at the optimal protein level with protein of high biological value (skim milk plus meat and bone meal plus extr. soya bean meal plus minerals and vitamins).

Plan I. The effect of antibiotics on daily feed consumption, gain, feed conversion and on carcass quality when added to rations (ad lib.) containing different levels of fiber (ground oat straw) which is added to the rations at increasing amounts.

Plan II. The effect of antibiotics on daily feed consumption, gain, feed conversion and on carcass quality when added to rations (ad lib.) containing high levels of roughage (bulky feed) (sugarbeet, fodder sugarbeet and potatoes).

It would be desirable to have these experiments duplicated in other countries with other breeds (lard-type) at out- or indoor-keeping, and with protein of lower biological value.

Ad lib. feeding experiments show, that antibiotics, if given during the first growing period from weaning up to 75, 100 or 125 lb. of live weight respectively, produce a smaller response to growth and feed consumption per unit of live weight gain and only a limited effect on carcass quality(5), (6), (11), (12), (14). It is, therefore, important to find a ration to which antibiotics can be added during the whole growing period without any effect on carcass quality. (See above.)

Different Protein Levels, ad lib. Feeding.—Generally speaking low protein levels will give carcasses with more fat and less meat. The antibiotics are not able to change these results significantly(2), (10), (14), (22), (23). As, however, antibiotics—also at ad lib. feeding—have a favorable effect on daily weight gain and feed conversion, there is a very great risk that pig producers will feed rations with low protein levels (or protein with a low biological value) supplemented by antibiotics and thus produce a poor carcass quality.

Used the right way antibiotics are of considerable importance in pig production; used wrongly they can be of considerable danger to carcass quality.

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Growth Response in Animals

PANEL DISCUSSION

Moderator

C. F. Huffman

Panel Members

Hjalmar Clausen Douglas Coles T. J. Cunha
John Duckworth James McGinnis

Summarizer

J. K. Loosli

Dr. Huffman: Dr. Cunha, are there any questions you would like to ask the other panel members while we are waiting for questions from the floor?

Dr. Cunha: I would like to ask one question of Dr. Clausen. I don't think we have any argument with the statement that if you feed pigs too low a level of protein they will get fatter. We are not advising that such a low level of protein be fed that carcass quality will be ruined. We are recommending feeding the lowest level of protein possible without sacrificing carcass quality.

I would like to ask Dr. Clausen what level of protein he used when he got increased fat with low protein.

Dr. Clausen: The normal level of protein recommended in Denmark is 115 to 120 grams of digestible true protein per Scandinavian Feed Unit when the pigs weigh 20 kilograms, decreasing to 85 to 90 grams per Feed Unit when the pigs weigh 90 kilograms. It is very difficult to convert this into digestible crude protein per pound of feed as we use skim milk and to a certain extent roughage in the rations. We usually supplement a basic feed consisting of grain or grains plus roughage with 3 kilograms of skim milk daily, and it means about 100 grams of digestible true protein added as supplement to the basic feed. In one of the experiments I showed you this protein supplement was reduced by 33 per cent to 67 grams daily, in another by 67 per cent to only 33 grams digestible true protein daily. To this you of course will have to add the amount of digestible true protein in the basic fodder.

Dr. Cunha: Dr. Huffman, I can't follow that. We can't convert that over to our per cent protein ration, but it looks to me as if he would be feeding less than our recommended levels. I wonder if it would be all right to ask anybody from the audience to make a comment.

Dr. Huffman: Absolutely.

Dr. Cunha: I wonder if Dr. Catron could make a comment. He has expressed levels of 14 per cent and other levels. Let us see if he has obtained any difference when he has lowered the protein level and whether it has affected carcass quality with or without antibiotics.

Dr. Damon Catron: First of all, I want to say that I certainly agree with Professor Clausen in his remarks, as Dr. Cunha has. The paper from Iowa which he

cited was the very first experiment which we conducted on protein levels with and without the antibiotic Aureomycin. Those pigs, as he stated, were all fat, with 1.8 inches of back fat as an average. Of course, as he also stated, as you increase protein you can't go beyond the genetic potentiality of the pig in putting more fat on. Since that report in 1951 we have converted to a leaner meat type pig by a three-way cross of Landrace, Duroc, and Black Poland China. We get only about 1.6 inches of back fat on that cross, and antibiotics have not increased the back fat thickness in feeding tests.

We do agree with him that as you go up in protein you get a leaner carcass. This is not new. I think Dr. Byerly will remember the work of Hankins and his group in this country. However, we also wish to make the statement as far as live weight gains and feed efficiency are concerned, that the feeding of an antibiotic will reduce the protein needed for those gains and feed efficiency by 2 units per cent. That does not necessarily give us the leanest carcass. Naturally, you can't get nitrogen out of antibiotics.

DR. HUFFMAN: Let us switch from meat to chickens. Here is a question from the floor for Dr. McGinnis. "Dr. McGinnis has indicated that CRD is a specific disease which may be treated by antibiotics in the feed. Do you feel that antibiotics might be recommended for layers and breeders in the control of specific diseases and, if so, what disease conditions might be involved?"

DR. MCGINNIS: I believe this is a question which could probably be commented on more intelligently by some of the veterinarians in the group because it is somewhat out of the nutrition field. However, there are a number of reports in the literature dealing both with broilers and laying and breeding chickens where benefits have been derived by adding high levels, from 50 to 200 grams of Aureomycin or Terramycin per ton of feed where CRD is a problem.

As far as specific diseases in laying and breeding flocks, aside from this type of chronic respiratory disease, offhand I don't know of any diseases that we have for which we are recommending this type of treatment or prevention. Perhaps someone in the audience may want to add to that, but the specific diseases like bronchitis, Newcastle disease, and others, we ordinarily don't try to prevent or treat. We cannot. Whether there are any bacterial diseases which in commercial practice we are preventing or treating this way I am not sure. There are some which would respond. There are some reports, for example, that cholera is susceptible to antibiotic treatment in feed if a high enough level is used. The problem that we have when we come to treat specific, known diseases, is the level of antibiotic to use which will work for that particular disease, if it renders itself treatable through antibiotics.

Maybe someone in the audience has some comment on that problem.

FROM THE FLOOR: The question related to scours or diarrhea. Would you have any idea what the disease conditions might encompass?

DR. MCGINNIS: The question was scours or diarrhea in laying flocks of chickens. I didn't interpret that as a specific disease of known etiology. Perhaps I misinterpreted the question. With that type of problem we have seen beneficial responses particularly to intermittent use of antibiotics. I thought you had in mind a specific disease of known etiology.

DR. COLES: I think, Mr. Chairman, we can contribute something on this from

the Union of South Africa. We are in the extremely fortunate position of having no Newcastle disease. We have had it four times and got rid of it four times. We have no infectious bronchitis and no laryngotracheitis. I suppose some of you think I should not be here, living in a paradise like that. But we have plenty of CRD, so our outbreaks are virtually uncomplicated by all these other diseases which so often complicate the disease here in Maryland, where I have had the fortune or misfortune to see all these mixed infections.

Our experience is that the use of antibiotics in the control of CRD is of value only when the birds are over the age of seven or eight weeks. I might say that CRD is an extremely prevalent condition in our broiler flocks. If you try to treat birds which are under the age of seven to eight weeks with these levels of 200 to 400 grams of antibiotic per ton of food, you are wasting your money. You might just as well kill them. But once they have passed this age you will at least succeed in marketing those birds past twelve weeks or fourteen weeks. You probably won't make any profit on them, but you won't lose money on them.

Our feeling is that anybody who tries to control CRD, except occasionally, by the use of antibiotics, is looking for financial trouble, and it will come to him in a big way very soon.

The only way that we know which has been successful in controlling CRD is to depopulate for one month and then repopulate with a clean stock, which is not always easy to come by. In these circumstances we have never had a failure. I am quite convinced that there is no other way of coping with CRD, but I fully realize the extraordinary difficulties confronting those people who have to buy chickens from unreliable sources because, as you know, the infection is passed through the egg.

From the point of view of scours I think we can say definitely there is no known disease of chickens called scours. I suppose the nearest that we can come to it is coccidiosis. It has been our experience that although you cannot control coccidiosis by the use of antibiotics, you can at least lead to the production of a more uniform flock in spite of the coccidiosis, by feeding antibiotics at the usual levels.

We have been asked two or three times this afternoon whether there is any other disease which can be controlled by feeding antibiotics. I don't think this work has yet appeared in print, but I understand it will appear shortly. Here I refer to ornithosis in pigeons and caged birds in California. At the Hooper Foundation, I understand from Dr. Karl Meyer, they are controlling the disease very satisfactorily indeed by soaking the grain in antibiotics before feeding it to these birds. Only two nights ago he told me that at least 98 per cent of the birds could be depended on to be free of infection at the end of the feeding treatment.

There is no other disease that we know of in the whole of veterinary science which will respond to large or at least high levels of antibiotic feeding.

DR. HUFFMAN: Thank you very much, Dr. Coles.

Dr. Cunha, we have a question for you. It says: "Fifteen to 20 milligrams per 100 pounds of live weight of antibiotic for dairy calves was mentioned. Yet Iowa and Louisiana recommend 75 milligrams of Aureomycin per day."

Shall we refer that to Dr. Rusoff?

DR. CUNHA: Yes.

DR. HUFFMAN: Dr. Rusoff?

DR. L. L. RUSOFF: At Louisiana we have been very successful with 75 milligrams. We have not tried the lower levels, but apparently there was some early work in which we did. We feed from the day of birth on.

DR. HUFFMAN: Thanks very much, Dr. Rusoff.

Here is a question for Dr. McGinnis: "What type of response occurs in chicks where antibiotics have been used for a long period of time on farms growing broilers?"

DR. MCGINNIS: I think the point that you have in mind here is similar to what we have heard discussed for swine, and for chickens also. Do we tend to lose the response to antibiotic in quarters where it is used continuously? We have some evidence which would indicate that perhaps we do. Dr. Schaible at Michigan State has reported recently that they had a gradual reduction in the response to antibiotics in their laboratories when they used them continually. They also reported that with a very short period of nonuse they apparently got recovery of the full virulence of the organisms or reinfections of the quarters, and so on.

As far as the use of antibiotics under commercial conditions of broiler production is concerned, I don't know of any results where we have a decreased response. In one case, with which I am familiar, a large broiler farm starting 5,000 chicks each week and which has been running about three years, had actually to go to a higher level to get responses and to prevent some of the problems. For a period of time in the first couple of years of their operation they were feeding about 10 grams of Aureomycin. They began to get failure with this level. By increasing to 50 grams per ton, they are now getting along very well. In this case, instead of disappearing, it looks as if it was a situation which required a higher level in order to produce a response.

In the work at Michigan State, as I remember it, there was not a great difference in growth rate between the birds on the basal diet and the birds with the supplements. It is just that the growth of the birds on the basal diet without antibiotic tended to be more comparable to that of the birds with supplementation.

DR. HUFFMAN: Would any one in the audience like to comment on this question?

DR. COLES: I would like to comment, Mr. Chairman. I presume that where the broiler raiser found it essential to use more antibiotic it was because they were not growing properly.

DR. MCGINNIS: That is right. He was experiencing high mortality and very poor weights at ten weeks. The average weight of birds dropped from around 3.25 pounds in some cases down to as little as 2.6 at ten weeks.

DR. COLES: I would think from our own experience that he was actually dealing with CRD.

DR. MCGINNIS: I think he was, too, but it was interesting that by raising to this level he was able to get along quite well.

DR. HUFFMAN: Here is another question, Dr. McGinnis: "Have the cultures isolated from chick feces been identified? Second, are antibiotics detectable in the meat?"

DR. MCGINNIS: The answer to the first part: The organisms in the mixed culture

responsible for the growth depression have not been isolated and identified. Some efforts were made, but were unsuccessful.

In answer to the second question, "Are antibiotics detectable in the meat?" We have not made analyses of the tissue for antibiotic content in our own laboratories. I have noted some results, which showed that it was impossible to detect antibiotics at the levels which were used for nutritional purposes.

DR. HUFFMAN: Is there anyone else who would like to comment on that?

DR. RUSOFF: Regarding antibiotics in meat, not of poultry but of dairy calves, we have done some studies in Louisiana and have found no trace of antibiotic in the meat. I don't know whether this is true of poultry or not, but we have fed much higher levels than mentioned here and we haven't found it in the muscle.

DR. HUFFMAN: Thank you, Dr. Rusoff. Dr. Jukes, would you care to make some comments along this line?

DR. JUKES: With reference to the question of antibiotic residues in meat, there is a whole paper on that subject on Friday afternoon. The amount found in the meat actually depends, of course, on how much is fed to the animal. At the ordinary levels which are used, depending upon the disease, the amount is less than one-tenth of a microgram per gram. That is a very important problem.

Perhaps comment might be made on the question of appetite. This has been discussed to some extent in another part of the presentations today. I think that one should not necessarily assume that in all cases the effect of antibiotic supplementation is to increase the appetite. In fact, in some animals, as in chickens, we have noticed in the presence of mild CRD that upon adding antibiotic the feeding rate actually is decreased as compared with the unsupplemented controls, while at the same time the growth has increased and the feed efficiency has increased. I suppose that means that antibiotic supplementation improves digestibility. There is some work which shows that it may increase the length of time that the food remains in the intestinal tract.

DR. HUFFMAN: Thank you very much, Dr. Jukes. The question of appetite and the effect upon appetite is of course very important in livestock feeding, and we are vitally interested in it from the standpoint of roughage consumption in cattle. It is too bad we cannot discuss the effect of antibiotics on hay consumption and fiber digestion.

DR. CUNHA: I would like to add something to that. In some of Dr. Beeson's work at Purdue with beef cattle when he controlled feed intake and had a high roughage ration, the feed intake was the same with antibiotic added. He obtained increased efficiency of feed utilization and also increased rate of gain. Therefore increased appetite is not always necessary in order to obtain increased efficiency.

DR. HUFFMAN: Thanks. I have a question here for you, Dr. Cunha: "What about intramuscular injection of antibiotics in dairy calves and in beef cattle?"

DR. CUNHA: I don't know too much about that, or whether much has been done. Maybe it has been done in the case of disease in beef cattle. If antibiotic was to be used under average feed lot conditions I should think it would be better to include it in the feed rather than inject it. However, it may be possible when animals are off feed and their appetite is very poor. Antibiotic might then be injected or put in

the water supply. Sometimes they won't eat but will drink water. Perhaps Dr. Rusoff or someone in the dairy field may have some information on injection versus oral administration in dairy cattle.

DR. HUFFMAN: Thank you very much, Dr. Cunha. Dr. Rusoff would you care to give us an expression along this line?

DR. RUSOFF: Yes, very briefly. We compared intramuscular injection of antibiotic with oral administration, particularly with Aureomycin. Once a week we injected intramuscularly a 400 mg. dose of aluminum chloride complex of Aureomycin in oil. Apparently the oil solution allowed the antibiotic to diffuse out slowly. We got a growth increase similar to that resulting from the oral feeding.

I noticed just recently an article in *Science* that high levels of antibiotic caused the antibiotic to appear in saliva. I think this was reported by Radisson and coworkers. They used 1,000 mg. injection. We used 400 mg. and found none of it in the rumen. We did find it in the bile, and maybe some of it does get into the intestine in that way. In practice it may not be worth while. We were interested in trying to get at the mode of action.

DR. HUFFMAN: Thank you very much, Dr. Rusoff. Here is a question for Dr. McGinnis: "Are there any experiments where live bacterial cultures were fed along with penicillin and which had an added effect in poultry?"

DR. MCGINNIS: We haven't done any of this work, but I believe Dr. Romoser and Dr. Combs and the group at the University of Maryland have done some work indicating that some of the cultures which they isolated from feces and grew have given added growth response. Dr. Romoser is here and perhaps he could comment on that more intelligently.

DR. G. L. ROMOSER: We primarily isolated *E. coli* and *Aerobacter aerogenes,* two non-fastidious organisms, from the fecal contents and reproduced them in large numbers in skim milk. We found there was an additive effect from the organisms of both types. *E. coli* seemed to be a little more effective than the *Aerobacter aerogenes.* However, we were able to increase the response of penicillin by about 80 per cent when we included penicillin and the organisms together. On the other hand, this may help answer the question which was earlier addressed to Dr. McGinnis in regard to the identity of the organism which depressed growth.

These data are not published, but we have grown in large quantities an organism, *Lactus bifidus,* which we earlier thought might be associated with the apparent depression in the growth rate of the birds. We fed these organisms in massive doses orally every other day, with a sterile saline solution as a control. Growth rate was somewhat depressed with these massive doses of *L. bifidus* which, by the way, is a very nutritionally fastidious organism. This work will be repeated.

DR. HUFFMAN: Thank you very much, Dr. Romoser. Would anyone else like to comment along this line? If not, I have a question here for Dr. Clausen: "Will Dr. Clausen supplement his admirable paper by telling us something about the economics of antibiotic feeding?"

DR. CLAUSEN: We found the response by using antibiotic different for different kinds of diets. As an average of all the experiments with restricted feeding, the saving of food was 4 per cent or approximately 10 Scandinavian Feed Units per pig, which means a saving of approximately 5 Danish kroners per pig. To this must

be added the value of 378 grams more carcass weight when antibiotics are added to the ration, or about 1.70 Danish kroners per pig. The total value of saving of feed and the value of higher carcass weight thus being about 6.70 Danish kroners per pig. As the antibiotics necessary per pig cost about 3.5 to 4 Danish kroners, there should be a reasonable profit to the farmers. In some cases with poor environmental conditions or high disease level the profit will be higher. In other cases of optimal environmental conditions, smaller. We of course hope that it will be much cheaper to buy antibiotics in the years to come.

DR. HUFFMAN: Thank you, Dr. Clausen.

We would like to call on Dr. Duckworth for a few remarks, anything he cares to say, or to ask questions of other panel members.

DR. JOHN DUCKWORTH: Mr. Chairman, I was very much impressed by the paper which Dr. Clausen read. In approaching it in the way he did, differentiating between the meat type of pig where the whole pig from the skin down is gone through, as distinct from the lard type of pig, there is a different type of problem in Europe, particularly in Denmark and in the United Kingdom. Under our European conditions there is not very much to choose among those standards. Dr. Clausen's feeding standard for protein, which he was speaking of, is one to which we adhere fairly closely ourselves. That is, the 115–120 grams of digestible true protein per Scandinavian Feed Unit is a ration which is a little over 15 per cent protein but a good proportion of that protein is of high biological value, in Denmark from skim milk, in the United Kingdom from whitefish meal rather than from the cod fish.

Under those circumstances and considering that the oil seed proteins that we use to help us economize in our animal by-products are not soybean meal which is well understood in its processing, but rather sunflower seed meal, peanut meals, and some fish meals which are imported, and of variable protein quality, frequently the farmer buys some mixed ration, and combines it with his own grain and is, therefore, always under the threat of poor quality protein from bad processing, insufficient protein and exposure to that risk of fatness of which Dr. Clausen spoke.

It is, I should say, perhaps at least for most of us the one point that makes us most cautious not from the standpoint of antibiotics but most cautious in use from the standpoint of our protein quality. That is a little different outlook which develops because of our different background of feeding systems.

DR. HUFFMAN: We appreciate your remarks very much, Dr. Duckworth. One objective of a symposium like this is to get the point of view and philosophy of our neighbors across the sea. Dr. Coles, I wonder if you have any further comment which you would like to make.

DR. COLES: Mr. Chairman, there is one thing I would like to know more about. We hoped, perhaps too hopefully, that we would be able to stimulate the appetite of our broilers in the hot weather by feeding up to 15 grams of antibiotics per ton and it seems that we have failed. I would very much like to know whether in this country, which experiences in many areas much greater heat than we do, any observations have been made on improving the growth of the birds in hot weather.

DR. HUFFMAN: That is a very fine question. We have a southern country over here, and a very prominent man from that country—Dr. Couch, of Texas. Would

you please come down and answer the question about growing chickens in the Deep South where it gets hot?

DR. JAMES RUSSELL COUCH: The only work that I know of which has been done in this regard was done by Dr. Heywang at Glendale, Arizona. He did find a beneficial effect from antibiotics during hot weather which was over and above that observed in cooler weather. A higher level of antibiotic was required to get this response. I don't think that we have any information from our station in that regard.

I would like to comment briefly, if I might while I am on my feet, in regard to the egg production question. This is certainly a controversial question as Dr. McGinnis pointed out and as we were reminded by one of the speakers when he said if he split the audience in two halves, one half would be in favor of adding antibiotic to egg laying feeds and the other half would be opposed to it.

I think in this case if we would think about it in terms of practical conditions which several of the speakers mentioned during the afternoon we would find possibly that antibiotics would be of more benefit in laying rations than in broiler rations. I am convinced of that myself because of a number of experiments which we have conducted during the past two years. The question often comes up: when do we need antibiotics in egg laying rations? The answer to that question is: when the egg production drops. If the egg production drops, there is something wrong with the hen. She may have CRD, she may have a dozen other things, but she is not laying eggs.

One experiment that I conducted during the past 90 days might be of interest. We had a group of about 500 hens laying at the rate of 65 per cent. Suddenly something happened to the birds. We separated them into four equal groups. We had one group which was getting 100 grams per ton, another group getting 50, another group getting 10, and another group getting none. At the end of 60 or 75 days following, in the group getting none the egg production dropped to 19 per cent average for the last 75 days, and in the group getting 100 grams of antibiotic the egg production was 45 per cent, indicating the place of antibiotic when there is something wrong with the birds. Obviously if the birds are laying at a rate of 65 to 85 per cent they do not need supplementation, and we do have some down in Texas which lay at the rate of 85 per cent. In this regard I think we used the antibiotics where we needed them, and I think we need them in poultry flocks.

DR. HUFFMAN: Thank you, Dr. Couch. Have you any other remarks, Dr. Coles, which you would care to make?

DR. COLES: Maybe, Mr. Chairman, we are really encroaching on a later program. I am not sure, but one of the major causes of failure in egg production is what is known as bluecomb in this country and as visceral gout in my own country. We hear that Terramycin has an extremely useful effect on this disease. The disease appears out of the blue and does all the damage it is going to do and vanishes equally mysteriously. I really and truly don't think that we have ever solved the etiology of that disease. It may be that there is somebody here who can give us a great deal more information on this point that might help to explain the beneficial role of antibiotics in boosting egg production.

We naturally know that CRD, according to the latest Canadian figures, occurs in approximately 48 per cent of all breeding flocks, and we must suspect that CRD

will reduce egg production. I am thinking now particularly of this disease bluecomb or, as we call it, visceral gout.

DR. HUFFMAN: Thank you very much, Dr. Coles. Dr. Cunha, we have a question for you: "Will you discuss (1) liver abscesses in cattle; (2) the cause of same and how antibiotics may prevent it?"

DR. CUNHA: Some preliminary work has been done at the Nebraska station by Dr. Matsushima. I think it is on a preliminary basis because he doesn't have too much data on it. He mentioned in his paper, as I recall, that 4 to 5 per cent of the livers of beef cattle in this country are abscessed. His theory was that the microorganism which causes these liver abscesses was prevented from getting into the body system by the antibiotic.

DR. HUFFMAN: Thank you, Dr. Cunha. Now we have a question for Dr. McGinnis: "Do pheasants always grow faster with antibiotics? What number of birds were used for these data?"

DR. MCGINNIS: This is a problem on which we don't have too much experimental data, but Dr. Scott at Cornell University has a nice article published on this problem. In several different experiments he got responses as high as 30 per cent to antibiotic supplementation of his pheasant experimental rations. In the work that I referred to at Washington State we had about 600 birds on each of the experimental diets. As I said, we did not get any specific data on them because the fellow had turned them loose or at least most of the ones that were on the antibiotic supplementation.

DR. HUFFMAN: Thank you very much, Dr. McGinnis. Here is a question: "Do you have any data on the effects of antibiotics on rumen microorganisms, cellulose digestion, and initial feed intake of ruminants?"

We have been doing a little work at Michigan State on the effect of antibiotics on the rate of passage of dry matter through the rumen of animals and have shown in 900-pound steers fed a high roughage ration that the use of 0.5 gram of Aureomycin stimulated the passage from the rumen of dry matter and crude fiber. Quite a bit of work has been done on rumen bacteriology. Dr. Doetsch of the University of Maryland has written a beautiful review on this subject, published in the Journal of Dairy Science. Is Dr. Doetsch here today? Is Dr. Bryant from Beltsville here today? Dr. Bryant is one of the foremost rumen bacteriologists in the country. We would like to have your comments on this question, Dr. Bryant: "Do you have any data on the effects of antibiotics on rumen microorganisms, cellulose digestion, and initial feed intake of ruminants?"

DR. MARVIN P. BRYANT: We have no data of our own and I don't believe that there are any really good data on the effect of antibiotics on rumen bacteria. I am thinking particularly of cellulolytic organisms. There are some data from the University of Maryland on inactivation of antibiotics in the rumen. However, they obtained very little inactivation, and the rumen fluid or content which they used came from animals which were not fed antibiotics. It is quite possible that if the animals had been fed antibiotics there would have been an adaptation of the flora which would have changed the results.

DR. HUFFMAN: Thank you very much, Dr. Bryant. Dr. Luther?

DR. HERBERT G. LUTHER: I should like to try to add something to that last

question. I believe Perry of Purdue indicated that there was some initial depression in feed consumption when antibiotics were added at levels of about 75 mg per head per day. We have been studying this effect and obtained an initial depression for about 4 days with penicillin and Aureomycin and Terramycin.

We have been studying for sometime the effect of antibiotics on rumen microorganisms by means of cellulose digestion determinations and have found that there is an adaptation period when the microorganisms adjust themselves to the presence of the antibiotic. I believe Dr. Lorraine Gall, who has been doing similar work has also attempted a study of the organisms involved. The initial depression of cellulose digestion by antibiotics is more marked for some than for others, but the effect is transient.

While I am on my feet, if I might for a second comment on some of Dr. Cunha's discussion on the effect of antibiotics in ruminants, I should like to add with reference to the statement that there are not sufficient data on Terramycin, that the Rome symposium in May, 1955 included, I believe, a summary of ten experiments on Terramycin, including three tests at Texas, one at Purdue, and several at our research center (Animal Research Center, Chas. Pfizer & Co., Terre Haute).

With reference to comparison of antibiotics Owens and his associates at Iowa have recently completed a comparative study, as have Voelker and Perry. These tests cover calves and beef animals. The authors have found in all three cases that under the same test conditions they had essentially the same responses to broad-spectrum antibiotics.

DR. WILLIAM M. MCKAY: My comment is not on that subject, but if I could revert to the one raised by Dr. Coles of South Africa, I would like to tell you something of our experience in England.

Second possibly to avian monocytosis or partial paralysis, this so-called pullets disease is probably our most serious economic disease of adult broilers and is responsible, and increasingly so, for a considerable reduction in the egg yields in our country. It so happens that recently we had an outbreak of so-called pullets disease in a flock belonging to a very important lady in England which was confirmed as being paralysis as near as we could get to it. Speaking from memory now, there was a drop from about 76 per cent to about 42 per cent in the egg production. We tackled this by feeding a level of Aureomycin of 100 grams per ton for 3 days, followed by 5 days at 36 grams per ton, after which it was stopped. Within 10 days the egg production had risen to approximately what it had been before the outbreak.

DR. HUFFMAN: Thank you very much. Is there anyone else who would like to make a few comments? If not, we are ready to listen to our summary by Dr. Loosli. Dr. Loosli, will you please take over and give us a summary of our afternoon's program?

SUMMARY OF THE FIRST SESSION

DR. J. K. LOOSLI: I am sure you are all aware, that we have been told this afternoon that we do get increases in the rate of gain and decreases in the amount of feed required to make a pound of gain when certain antibiotics are added to the ration of animals.

We have heard that this type of response occurs with almost all species, but not with ducks as far as the question has been studied. We don't know, I believe, why this bird differs from other animal species.

We have been told that with chicks and turkeys the most effective antibiotic is probably penicillin, with Aureomycin and Terramycin a very close second, and with the other antibiotics somewhat less effective.

This is quite different from the situation with dairy calves. We were told that with this species Aureomycin and Terramycin are more effective than the others, and that penicillin gives little or no response and may be harmful, that is, actually decrease the rate of gain. Some of the data cited indicates that streptomycin may give slight responses in the growth of dairy calves, but probably not as good as the other antibiotics.

The question arises, then, why we have this species difference. Perhaps this gets into the mode of action of antibiotics, which we should not discuss too much because there is a whole session devoted to it later.

When we look at the question of rate of gain only there are differences in the importance of this for the various species. For broilers and swine which are to be slaughtered the effect is obvious. When we come to dairy calves and heifers which are being grown for replacements, there is a real question as to whether this increased rate of gain has any true value for lifetime level of production of a cow.

The only data we have on this perhaps are the Danish and New Zealand work indicating that there may be some harmful effects from growing heifers too fast or perhaps from getting them too fat during the growing period. This is a question which has not been studied with respect to antibiotics. There are a few experiments in progress now, especially at Ames, I think, with long-time feeding. It is going to be some time before we have much of an answer here.

The data with fattening steers and lambs are very recent. Not much has been published in the scientific literature. Responses to Aureomycin and perhaps Terramycin appear to be effective. That is, there does seem to be some growth response and improvement or decrease in the amount of feed per unit of gain.

Perhaps the greatest benefit of antibiotic feeding for fattening animals is their ability to take full feed faster, with fewer digestive disturbances, and perhaps just general improvement in health. With lambs this seems to be especially true from the reports of decreased death loss from overheating disease.

With swine, calves, and lambs the effect of antibiotics in preventing unhealthy animals is very important from the practical standpoint.

There have been some studies on the effect of antibiotics on the digestibility of feed. Some of these show no effect. Others show increase in digestibility. Perhaps differences in the experiments as they were carried out explain this different response, but as far as I know there are no recent data in which the small or the low level recommended for practice has actually depressed digestibility. The earlier studies mentioned, in which lambs and steers were fed high doses, of course did decrease feed utilization, but apparently that is not true if the animals have time to adapt and if the doses are not too great. Under some conditions there may actually be some improvement in the digestibility of feed by ruminant animals.

The question of carcass quality with swine is of course extremely important. I

think we have heard this afternoon a very fine summary and presentation of these data. It seems to me that we in America have had too little interest, perhaps, in this question until recently. I believe now we are becoming aware of the problem of surplus fat and that emphasis is being placed at least genetically on getting animals of lower fat content.

The data that have been presented show that, on restricted feed intakes, antibiotics have not affected carcass quality, either cut-out value or chemical composition, at least under Danish conditions. On the other hand, with ad lib. feeding the amount of fat in the carcass apparently may be increased under certain conditions, and the protein level would seem to be one factor which might influence this.

It seems to me that this presents quite a challenge to our research workers here, especially in swine nutrition, to get data on the effects of various ratios of protein to energy on carcass quality with antibiotic as a variable.

Studies that Dr. Crampton and associates have published in Canada have shown rather conclusively, I believe, that a ration very high in corn gives fatter animals than one composed primarily of barley or containing a certain amount of oats. It is thought that the bulk or the fiber in the ration which restricts the total caloric intake of pigs on ad lib. feeding is a factor involved. This type of change in ration of course would also decrease the ratio of protein to total available calories; in other words, increase the protein on a relative basis to total available energy. Perhaps this ratio of protein to energy is a factor here. Certainly we need more data.

To get this, we need cut-out value on carcasses and also chemical composition, because from the nutritional standpoint we are interested in the grams of protein which are available for human nutrition. We think that swine along with poultry are the most efficient converters of feed protein into human food protein. We do need to work out the types of rations which will give us the greatest possible efficiency under these conditions.

There are many other things which have been mentioned, but I see my time is up, so I am going to turn the microphone back to our Chairman, Dr. Huffman.

DR. HUFFMAN: Thank you, Dr. Loosli. I want to take this opportunity to thank the panel for helping me out on this afternoon's program, and also the audience, because it is the audience participation which makes a program like this worthwhile.

. . . The meeting adjourned at five twenty-five o'clock. . . .

SECOND SESSION

SPECIAL BIOLOGICAL PROBLEMS

October 20, 1955

DAMON CATRON, *Presiding*

C. A. BAUMANN
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TEST ANIMALS

The test animals under discussion are the rat, the chick, and the guinea pig. Antibiotics in the diet increase the growth rate of many species of animals, but the circumstances necessary for the response to take place vary considerably from species to species. No single proposed mode of action for the antibiotics fits all of the facts, and almost the only point of agreement is that the extra growth observed when antibiotics are eaten is somehow associated with induced changes in the microorganisms of the digestive tract. The proposed mechanisms of action are the following:

(a) the antibiotic suppresses organisms that cause "disease," usually too mild to be recognized as such but sufficient to depress the growth rate below that which would occur in the absence of the disease;

(b) the antibiotic changes the microorganisms in such a way that vitamin synthesis is increased in those parts of the digestive tract in which microbial vitamin can be made available to the host;

(c) the antibiotic suppresses organisms that normally destroy vitamins or other nutrients in the intestine, or that absorb them and thus render them unavailable to the host;

(d) the presence of antibiotics tends to make the intestines thinner than those of control animals. This may actually be a variant of the disease mechanism if the thickened intestine of the conventional animal should prove to be a symptom of mild disease, and the thin intestine of the germfree animal or of that fed antibiotics should prove to be normal. In any event thinner intestines might offer one means by which improved absorption of nutrients or better net usage of nutrients is brought about.

A priori there is no reason why all four mechanisms should not be operating simultaneously in all animals, and in most situations one can assume the effective operation of at least two of them. In this respect the differences between species are probably quantitative rather than qualitative, and it is only under comparatively special circumstances that one mechanism predominates to the virtual exclusion of the others. One example of this latter situation is the very spectacular increase in growth sometimes observed when the appropriate antibiotic is fed to diseased pigs. In this case the cure of the disease is infinitely more important to the animal than any fractional increase in vitamin synthesis or availability. An inverse variant of

this phenomenon is the development of fatal infections in guinea pigs fed Aureomycin in spite of the possibility of a simultaneous improved vitamin synthesis or absorption.

Rat

The advantages of the rat as a test animal include a similar growth rate for different individuals (in contrast to the wide variations within groups usually encountered with chicks), a relative resistance to infection, and the fact that the nutritive requirements of the rat appear to be fairly well known. Antibiotics added to the complete semisynthetic diets now available usually fail to elicit any additional growth response(12), (21), (29). Thus in the better strains of the conventional laboratory rat there does not appear to be any "normal infection" that holds down the rate of growth, as is so often the case with the chick, and hence the rat is well suited for the study of other mechanisms by which antibiotics might stimulate growth.

When the basal diet is limiting in an essential nutrient, the addition of an appropriate antibiotic to the diet increases the rate of growth significantly(12), (20), (21), (29). This is true for many nutrients and for quite a few antibiotics; it is particularly true for deficiencies—or relative inadequacies of the B vitamins combined with the presence of penicillin, Aureomycin or Terramycin; occasional increases in the growth of rats have also been recorded for other antibiotics. It follows that, under the right circumstances, an incomplete diet may be improved either by adding the missing vitamin or by adding an appropriate antibiotic. The implication of the added growth response is that the antibiotic enables the animal to make better use of limiting amounts of vitamin within the digestive tract, either by improving absorption or stability of the limiting vitamin or by diminishing diversion to competing organisms; and almost certainly the presence of antibiotic enables an increased synthesis of many of the vitamins to take place.

When antibiotics were first added to diets to stimulate growth, the rations used happened to be low in vitamin B_{12} and methionine, and the amount of study devoted to this particular combination gave rise to the impression that antibiotics may be peculiarly effective in sparing vitamin B_{12}. However, the improvement observed when an antibiotic is added to a diet suboptimal in vitamin B_{12} is but one example of a very general phenomenon, and any specific association between vitamin B_{12} and antibiotics must be regarded as a historical phenomenon rather than a physiological one. Had antibiotics been generally available in the days when nutritional investigators were focusing their attention on pantothenic acid, we would probably have heard about a specific interrelationship between antibiotics and pantothenic acid.

As one might expect, there are quantitative differences between antibiotics in the magnitude of the growth response they bring forth when added to various deficient diets. One combination which is particularly effective is a low-thiamine diet to which penicillin has been added(20), (29). If the level of thiamine is so adjusted that the control rats just barely continue to grow, the presence of penicillin may increase growth by 200 per cent. When the level of thiamine in the basal diet permits half-optimal growth, the increase in growth due to penicillin is roughly 25 to 50 per cent, and this effect of penicillin decreases further as the thiamine content of the diet is

increased(17). Penicillin fails to increase the growth of rats fed a nutritionally complete diet, as it also fails when the basal diet is completely devoid of thiamine. This latter failure may, however, be more apparent than real, in the sense that the thiamine-deficient rats eat so little food, that any increased synthesis of thiamine by the intestinal microorganisms would probably be insufficient to alter the course of the deficiency.

Other antibiotics such as Aureomycin, also increase the growth of the low-thiamine rat, but usually not as effectively as penicillin. Yet in inadequacies of riboflavin, pyridoxine, or pantothenic acid, Aureomycin often is a better growth stimulator than penicillin. Results from different laboratories agree quite well on the fact that a number of quite different antibiotics stimulate the growth of rats receiving inadequate amounts of any of the B vitamins required in the diet as such; but the agreement between laboratories is less good as to the magnitude of the response, or the relative merits of different antibiotics in any given vitamin deficiency—whether e.g., penicillin or Aureomycin is most effective when riboflavin is limiting(12), (20), (28). And results with certain of the antibiotics, e.g., streptomycin, may range all the way from substantial responses to none at all.

When one considers the complexity of the situation, it is not surprising that there is variation in result between laboratories, or even between different experiments in the same laboratory. Relatively low concentrations of antibiotic are added to the alimentary canal in which numerous species of competing organisms exist in increasing numbers as one passes downward. The concentration of ingested antibiotic, on the other hand tends to decrease due to absorption and destruction as the numbers of organisms are increasing. One thus has a changing concentration of antibiotic acting upon increasing concentrations of organisms. Each species of organism is affected by the antibiotic to an extent that is characteristic of the organism, the antibiotic, the concentrations of each, and to the sum total of favorable or adverse ecological influences arising from the other organisms present. Even under the relatively simple situation of a complete synthetic diet and no antibiotic, the numbers in one of the large classes of organisms such as the lactics or coliforms can change by a factor of 10 from one day to the next(13). The changes within a single species, or within strains of that species, are at least as great as those within the large classes of organisms ordinarily measured. Thus it is less surprising that the nutritional effects of dietary antibiotics are sometimes variable, than that they are as consistent as they are.

That the microorganisms within the digestive tract synthesize vitamins has been recognized for a long time; and under ordinary circumstances the rat may derive a major portion of its requirements for vitamin K, biotin, and folic acid from this source. Balance experiments suggest that not only these vitamins but others such as pantothenic acid are synthesized as well, though not in sufficient amounts to prevent deficiency symptoms from developing. In this connection it is of interest that the addition of penicillin or Aureomycin to a diet devoid of pantothenic acid stimulates growth significantly(20), (29) whereas in our hands at least, these antibiotics were virtually without effect when added to diets devoid of thiamine, riboflavin or vitamin B_6.

Most investigators seem to agree that one mechanism by which antibiotics increase

the growth of animals is by increasing the synthesis of intestinal microorganisms. The data on chicks are quite convincing on this point(24), and results with rats are not inconsistent with this assumption, e.g., the total amounts of limiting vitamin are higher in the blood and liver of rats receiving antibiotic than in the controls even though the amounts excreted are increased(12), (30). Increases in urinary vitamin likewise suggest that more vitamin has been available to the rat. Another observation consistent with the mechanism of increased intestinal synthesis is the fact that the increase in growth due to antibiotic is greatest at intermediate levels of limiting vitamin. Presumably on a complete diet the amount of vitamin present is so great that it does not matter to the host whether the organisms manufacture an additional increment or not; whereas, in the face of severe deficiency with its attendant complications, the small change in synthesis caused by the antibiotic does not materially affect the health of the host. It is at intermediate intakes of limiting vitamin that alterations in intestinal synthesis would be expected to exert the greatest effect on growth, and this is the range at which the addition of antibiotic to the diet of the rat is most effective in practice.

Nevertheless, these facts do not establish whether increased vitamin synthesis is the sole or even the major mechanism by which antibiotics increase growth. An alternate suggestion stems from the fact that organisms of the lactic type can absorb very large amounts of vitamin B_6 from the surrounding medium(32) and that by incorporating the vitamin into their cells these organisms could effectively remove a portion of the vitamin from the fluids of the digestive tract and thereby render it unavailable to the host. Competition of this sort for vitamin B_1 has been demonstrated by live yeast cells consumed by human subjects(10). In the case of the lactics, pyridoxal is absorbed more efficiently than pyridoxamine which in turn is absorbed better than pyridoxine. The growth of rats fed limiting amounts of these three forms of the vitamin is inversely proportional to this absorbability by the organisms, that is, growth is best on pyridoxine, and poorest on pyridoxal(23). Yet when the three forms are injected, their activities are approximately equal(28), and growth is also approximately equal when the three forms of vitamin B_6 are fed in diets containing Aureomycin(21). The mechanism suggested by this series of events is that the antibiotic brings about a change in the microorganisms such that strains that normally divert or "steal" vitamins away from the digestive contents are prevented from doing so, thus leaving more of the limiting vitamin available to the host. This mechanism has also been invoked to explain the beneficial action of Aureomycin in poor quality protein diets that cause hepatic necrosis(14).

Attempts to distinguish between the mechanism of vitamin sparing from that of vitamin synthesis have recently been made by Schendel and Johnson(30), and by Jones in our laboratory(17). The technique employed was to administer the limiting vitamin in two different ways, by incorporation into the diet or by subcutaneous injection. In each case antibiotic was added to the diet of half of the animals. Since the rats receiving limiting vitamin by injection were on a diet free from the vitamin in question, the presence of antibiotic in the diet could hardly operate by affecting vitamin destruction or diversion, since the amounts of the injected dose that would reach the digestive tract would be practically negligible. On the other hand the mechanism of vitamin sparing could be exerting its full effect in the group receiving

antibiotic and limiting vitamin in the diet. Schendel and Johnson studied two combinations, penicillin-thiamine and pantothenate-Aureomycin, and in both cases antibiotic in the diet produced substantial increases in growth in the animals receiving limited vitamin by injection. Since the vitamin-sparing mechanism could not have been operating to any substantial degree under these conditions, they concluded that antibiotics stimulate growth by promoting vitamin synthesis.

In the Wisconsin experiments(17) four vitamin inadequacies were studied with each of three antibiotics, and the dosages of vitamin varied until growth in the injected controls equalled that in the control rats receiving limiting vitamin in the diet. This enabled quantitative comparisons to be made in the effectiveness of the antibiotic under the two conditions. For most of the combinations tested antibiotic in the diet stimulated growth whether the limiting vitamin was in the diet or given parenterally, from which it appeared that antibiotics stimulate growth by the mechanism of intestinal synthesis at least to the extent that growth increases when the dosage of limiting vitamin is administered parenterally.

This latter stimulation, however, was usually less than that observed when the dose of limiting vitamin was administered in the diet, and for the 18 comparisons made, the relative effectiveness of penicillin and Aureomycin in the parenteral experiments was approximately one-half that in the oral experiments. The data of Schendel and Johnson(30) indicate Aureomycin to have been 68 per cent as effective when pantothenic acid was injected as when it was fed.

One possible explanation for two observed levels of effectiveness for any given antibiotic is that both the mechanism of vitamin synthesis and that of diminished vitamin diversion may be operative when the antibiotic and the limiting vitamin are present in the digestive tract together, whereas the administration of limiting vitamin by injection permits the antibiotic to operate only by the mechanism of vitamin synthesis. According to this concept the discrepancy between the results of the two types of procedure might be a measure of the alteration in vitamin diversion that normally takes place when antibiotic is added to the diet.

Quite another possibility is that the effectiveness of an antibiotic upon sensitive organisms may vary with the completeness of the medium in which that organism is growing(19). When the limiting vitamin is present in the diet, the medium (intestinal contents) is more nearly optimal for the organism than when a B vitamin is omitted from the diet. This latter situation may explain why streptomycin was frequently more effective when a vitamin was missing from the digestive tract (injection experiment) than when it was present in an amount suboptimal for the rat.

Chick

Several types of observations with chicks point toward the cure of disease as a major mechanism by which ingested antibiotics increase the rate of growth. Numerous attempts to abolish this increase by improving the nutritional quality of the diet have failed(34), and the supplements used have included mixtures of alleged crude sources of virtually every unidentified growth factor postulated during the last two decades. Dietary antibiotics did not stimulate the growth of germfree chicks(11), nor are they effective when added to complete diets of chicks which are

kept in an environment where there had been no previous contact with chickens(2), (5), (6), (16). And in at least one case chicks in an "old" environment progressively lost the ability to respond to antibiotics for a period of nearly a year(35) and then gradually regained it. Growth in the control groups was often, though not always, relatively high during the period of unresponsiveness to antibiotic.

Ingested antibiotics also apparently increase the growth of chicks by nutritional means. On diets inadequate in the ordinary B vitamins the addition of antibiotic produces larger increments of growth than on complete diets(1), (36). This phenomenon can be studied with more vitamins in the chick than in the rat, and includes limiting niacin or folic acid. Much evidence suggests that antibiotics act by stimulating the intestinal synthesis of vitamins in the chick(24), and this phenomenon is fairly well known. It has also been suggested that the amounts of necessary unidentified factors produced by the microorganisms might be increased by the presence of antibiotics within the digestive tract. The good growth observed in germfree chicks(11), however, raises some doubt as to the importance of this particular mechanism.

More recently, emphasis has been placed on the changes in the weight of the small intestine which occur in chicks fed antibiotics. Thus, Coates *et al.*(7) reported that procaine penicillin added to normal mash accelerated the growth of chicks but reduced the weight and to a lesser extent the length of the small intestine. But chicks in isolation units showed no decrease in the weight of the gut nor any increase in growth when penicillin was fed. Decreased intestinal weights have also been reported when other antibiotics were fed(18), (25). The implication is that the intestine responds to certain microorganisms by thickening, and that it tends to diminish in thickness when such organisms are suppressed. The question of importance in nutrition is whether the thin intestine is a better organ of absorption than the thick one. An improved utilization of nutrients in general or of protein has frequently been reported for animals fed antibiotics(9) and in the chick there is a significant improvement in gm. gained per unit weight of food consumed. But in many cases it is not clear whether this is a direct effect, or merely a result of the high rate of growth caused by other mechanisms(31). A direct effect is suggested by evidence that antibiotics improve the absorption of calcium(8), (22) or of vitamin A(3), (7), nutrients that are not likely to be synthesized or diverted by intestinal microorganisms. Higher levels of blood glucose(4), (33) in swine or calves fed antibiotics likewise point toward improvement in absorption, although other factors may also be involved. Our most recent experiments have dealt with the diffusion of xylose through isolated intestines, and thus far the results suggest a more rapid diffusion through intestines of rats or chicks fed antibiotics than through control intestines. To this extent at least, intestinal thickness appears to have nutritional significance.

Guinea Pigs

Perhaps the most striking example of species specificity is the sensitivity of the guinea pig to dietary Aureomycin. In contrast to most other species, which grow better when Aureomycin is present in the diet, the guinea pig rapidly loses weight when as little as 25 mg. of Aureomycin is included in the diet. This so-called

"toxicity" was first reported by Heilman in 1948(15) ; it has been confirmed in at least seven other laboratories, and has been the subject of a revealing series of experiments by Roine and associates(26), (27).

The affected animals look drowsy, a kind of opthalmia develops, and some animals have convulsions or turn in circles. The animals stop eating almost completely within a few days, and also drink very little. Weight loss is severe, there is a fall in body temperature and death usually ensues within 7–15 days. A few animals recover spontaneously and then continue to grow in the presence of Aureomycin. Blood changes include increase in neutrophils, decreases in lymphocytes, and irregularities in monocytes, without, however, any distinct increase in these latter cells.

These effects are essentially the same in older animals as in young ones, and have been observed both on crude diets (clover, timothy, oats, rye, etc.) and on a suitably supplemented purified diet. Internally the most obvious change is a greatly enlarged cecum, and there may be pale-grey areas 1–1.5 mm. of focal necrosis of the liver. The spleens were decreased in size.

Terramycin in the diet appears to be as toxic as Aureomycin and others have reported toxic effects due to penicillin and to streptomycin, although there is some disagreement as to these latter effects.

The symptoms are almost certainly mediated through a change in the intestinal microorganisms, since comparable amounts of Aureomycin injected subcutaneously fail to produce any adverse effects whatsoever.

Bacteriological investigations showed distinct differences between the cecal flora of control animals and of those receiving Aureomycin. After only one day most of the organism types normal to the guinea pig were suppressed; Aureomycin almost completely eliminated the cells of Oscillospira, Fusobacterium, and Borrelia, while the number of lactobacilli was greatly reduced. Sarcina and Listeria cells, on the other hand increased, and after three days there was also a marked increase in Proteus.

The authors place particular emphasis on Listeria, which occurs as a parasite in both wild and domestic animals and birds including the guinea pig. Listeria infection has been reported in sheep, cattle, pigs, chickens, horses and mink, with symptoms that in general resemble those observed in guinea pigs fed Aureomycin. Cultures isolated from such guinea pigs induced the symptoms and death when inoculated into guinea pigs not receiving any Aureomycin.

Thus the experience with guinea pigs seems to involve the "infection mechanism" of antibiotic action, that is so useful in explaining the favorable responses of swine and poultry to the ingestion of antibiotics. In all three species many kinds of intestinal microorganisms are suppressed, and others replace them. In swine, and in poultry from "infected hen-houses" the organisms suppressed are presumed to be mildly deleterious, while those that take their places are innocuous or slightly beneficial. In the guinea pig on the other hand, the organisms suppressed seem to be relatively unimportant, and what matters is that a highly toxic species, normally present in small numbers, increases to the point where it can cause the death of the host.

It so happens that Listeria is much more sensitive to penicillin than to Aureomycin, and also somewhat more sensitive to chloramphenicol. Thus it was possible

to save a certain proportion of the guinea pigs by administering these other antibiotics either before or after the feeding of Aureomycin, but in no case was multiple therapy completely successful.

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HIGH LEVEL USE OF ANTIBIOTICS

The use of antibiotics at low levels in feeds for chickens, turkeys, swine, young calves and lambs has become an accepted practice for general nutritional benefit and promotion of growth. Many workers have observed that growth response was influenced by the degree of contamination in the environment.

Recently the high level feeding of antibiotics has been used in the prevention and treatment of certain diseases of animals and poultry. The antibiotics that are used in this manner are chlortetracycline (Aureomycin ®), oxytetracycline (Terramycin ®), bacitracin, penicillin, streptomycin, dihydrostreptomycin and chloramphenicol. The meaning of high level feeding varies from one area to another and from one species of animal to another, but the minimum level appears to be 50 gm. and the maximum 200 to 1000 gm. per ton of feed. If the antibiotic is used in the drinking water the range of high level use is from 25 parts per million (p.p.m.) to 250 p.p.m. or approximately 100 mg. to 1000 mg. per gallon.

Many investigators believe the degree of effectiveness of antibiotic feeding is determined by the disease level in the animals and poultry. The disease level may be defined as the degree of infection with bacterial and/or viral diseases which cause respiratory, digestive or other disorders. The term, disease level or disease burden, is used then to indicate the amount of infection, clinical or subclinical, in a flock. Another term, stress factor, or trigger mechanism, has been widely used to indicate the effect of environmental factors and other diseases on the occurrence or the severity of the disease. It is the play of these terms, "disease level," "stress factors," with high level feeding of antibiotics that has caused considerable confusion and differences of opinion. It is the purpose of this paper to review the information that is available on the effectiveness of high level feeding of antibiotics in chickens, turkeys, swine, young calves and lambs.

Poultry

Respiratory Infections

Virus Diseases.—There are a variety of infectious diseases of poultry. The antibiotics have no effect on certain of the virus diseases that commonly affect poultry such as Newcastle disease, infectious bronchitis, infectious laryngotracheitis, fowl pox, avian leukosis complex, avian encephalomyelitis, equine encephalomyelitis and

fowl plague(5). These diseases usually produce characteristic symptoms and lesions and may be identified by isolation of the virus, serological tests, and histopathology. Some outbreaks of these virus diseases may not be apparent and may go unrecognized except by laboratory procedures. The apparent benefit of the practical antibiotics in true viral infections would be to control secondary invaders and to stimulate feed consumption. Under practical farm conditions poultry may be infected with many species of bacteria that under certain conditions may cause detrimental effect. The effect of mixed infections on mortality may be greater than any single disease. White-Stevens(83) reported that in a Newcastle disease outbreak the accumulative mortality in a chlortetracycline-treated group (30 gm. per ton) was markedly reduced compared with an untreated control group. He also reported similar results in an outbreak of infectious laryngotracheitis.

Ornithosis, Psittacosis, Pneumonitis Group of Viruses.—Recent reports(21), (57) have shown the importance of this group of large viruses to disease losses in poultry and caged birds. Loosli(53) used the mouse pneumonitis virus (MPV) infection for the study of the use of antibiotics and sulfonamides for the treatment and prevention of diseases caused by the psittacosis group of agents. Under the conditions of the experiments chlortetracycline, oxytetracycline, chloramphenicol and erythromycin were highly effective in the prevention and treatment of MPV infection in mice. Penicillin prevented the development of lesions but did not sterilize the lungs. Streptomycin(43) shows little activity against this group of viruses. There is insufficient information concerning effect of bacitracin and other antibiotics on this group. Davis and Delaplane(21) reported chlortetracycline in the feed at 400 gm. or more per ton prevented mortality in poults and eliminated the carriers. Ten gm. per ton had no effect on the course of the disease, while 100 and 200 gm. prevented mortality but did not eliminate the carriers. Meyer(57) reviewed the problem in parakeets and turkeys and reported on the successful use of tetracycline in the feed to control psittacosis and to eliminate the latent, carrier-stage of infection.

Pasteurellosis (fowl cholera).—Pasteurella infection of poultry caused by *Pasteurella multocida* has been a problem in many areas for years. Acute and chronic forms of fowl cholera can cause serious mortality and morbidity losses in poultry flocks. McNeil and Hinshaw(56) reported that streptomycin showed in vitro and in vivo activity against the fowl cholera isolates. Little(52) reported chlortetracycline had activity against experimental infection of fowl cholera. Prior(64) also reported favorable results. Dorsey(22) found that high levels (1000 gm. per ton) of chlortetracycline and oxytetracycline in the feed were necessary to reduce the mortality in experimental and field outbreaks. Kiser and deMello(47) reported that Pasteurella resembles the gram positive bacteria in its response to antibiotic therapy.

Infectious Coryza.—Infectious coryza of chickens is caused by *Hemophilus gallinarum.* This disease produces symptoms similar to chronic respiratory disease. Several investigators(7), (18), (33), (68) have reported on the beneficial effect of streptomycin as a treatment for infectious coryza. Bornstein and Samberg(8) found that streptomycin was effective in experimental studies on *H. gallinarum.* No trials were conducted using streptomycin in the feed or water, but parenteral use indicated 50 mg. per pound of body weight was necessary to obtain bactericidal effect. Inadequate doses may result in the development of streptomycin-fast strains. The

authors also concluded that practical doses of penicillin do not produce desirable results. The same authors(9) found that intramuscular injection of dihydrostreptomycin sulfate in hens affected with infectious coryza caused a remarkable clinical recovery and strikingly quick return of egg production. In affected growing stock it caused a clinical cure and a quick return of appetite and rate of gain.

Chronic Respiratory Disease (CRD) *and Infectious Sinusitis* (IS).—Considerable confusion exists concerning the prevention and treatment of chronic respiratory disease of chickens and infectious sinusitis of turkeys. The causative agent of chronic respiratory disease and infectious sinusitis is considered to be an organism having characteristics of the pleuro-pneumonia-like group (PPLO)(55), (78), (79). Secondary infections appear to complicate chronic respiratory diseases and infectious sinusitis and the term "air sac" disease covers the clinical picture as seen in field outbreaks(81). Organisms from several genera such as *Escherichia, Proteus, Pseudomonas* and *Micrococcus* have been recovered from birds with air sac lesions. Also it is not uncommon to have concurrent diseases diagnosed in flocks infected with CRD(30), (54). Many stress factors appear to increase the severity of CRD. Fahey and Crawley(27) have presented evidence that there is a viral agent associated with CRD in chickens. The clinical infection, "air sac" disease, may be a combination of CRD virus, PPLO and other secondary bacterial infections. The CRD virus is not susceptible to antibiotics. In vitro studies in ova and test tube indicated that the agent (PPLO) causing infectious sinusitis of turkeys and chronic respiratory disease of chickens was susceptible to various antibiotics such as magnamycin®, streptomycin, chlortetracycline, tetracycline, oxytetracycline, chloramphenicol and erythromycin. The agent was resistant to penicillin(20), (42), (88), (90). Various field trials on air sac infection of poultry have shown encouraging results with antibiotics added to the feed on a preventative or therapeutic level. The results are based on reduction of mortality, less culls, and improvement of feed conversion. In contrast to encouraging field trials, controlled laboratory studies have not been as promising(78), (79).

ANTIBIOTICS COMMONLY USED IN THE PREVENTION AND TREATMENT OF CHRONIC RESPIRATORY DISEASE OF CHICKENS AND INFECTIOUS SINUSITIS OF TURKEYS

Chlortetracycline.—The summary of information presented by White-Stevens *et al.*(82), (83), (84), (85) indicated beneficial results in infected flocks. In broiler flocks controlled field trials indicated that high level feeding on a continuing basis was more effective than spot treatment during an outbreak. Continuous feeding of from 50 to 200 gm. gave better results than controls receiving no antibiotic and groups receiving from 50 to 400 gm. per ton of feed only at the time of an outbreak. In laying and breeding flocks that show depressed egg production from CRD, the use of 100 or more gm. per ton of feed resulted in increased production. Breeding flocks showing depressed egg production, fertility and hatchability responded to increased levels of 100 to 200 gm. per ton. Balloun(2) reported that under conditions unfavorable to high egg production the addition of 100 gm. per ton of chlortetracycline may improve egg production. Carlson and Kohlmeyer(13) reported some beneficial effect from feeding 100 gm. per ton to laying flocks. Heywang(39) also

found beneficial effect in laying chickens using 50 and 100 gm. per ton of feed. Sherwood and Milby(72) reported that a mixture of oxytetracycline-chlortetracycline at 200 gm. per ton of laying ration failed to produce any improvement in egg production, feed efficiency or hatchability. The same authors(73) compared caged layers as well as birds housed in the conventional manner on 100 gm. per ton of chlortetracycline and found the antibiotic had no effect on egg production. Sizemore *et al.*(76) found that feeding 40 gm. per ton of chlortetracycline to growing pullets had no effect on mortality or date of sexual maturity. Bearse and Berg(3) reported no significant differences in hatchability, mortality, egg shell quality, albumin quality or in blood spot incidence between pens of chickens fed on two levels of chlortetracycline and no antibiotic. There was, however, a significant increase in egg production at the end of the laying year in the antibiotic treated groups. Carlson *et al.*(15) have summarized four years' work on the effect of several antibiotics on the reproductive performance of chickens, and concluded that antibiotics in breeder diets have not shown consistent effects with respect to relative progeny growth performances. Several investigators(32), (37), (40), (65) have reported on the beneficial effect of chlortetracycline in infectious sinusitis of turkeys. Food and drug regulations(31) provide that chlortetracycline may be used in poultry feed for the prevention of chronic respiratory disease at not less than 50 gm. per ton as long as the labeling bears adequate directions and warnings for such use. If it is intended for use as a treatment for chronic respiratory disease, the feed must contain not less than 100 gm. per ton as long as labeling bears adequate directions and warnings for such use.

Penicillin.—The information concerning high level use of penicillin for chronic respiratory disease is very limited. In vitro tests indicated that penicillin had no effect on the agent causing CRD. Klussendorf(48) reported that penicillin may reduce secondary infections, particularly those caused by gram positive organisms, improve the appetite, reduce severity of the disease and shorten its course. Elam *et al.*(24) reported that prolonged feeding of penicillin at 33 gm. per ton to laying hens resulted in increased egg production and hatchability. King(46) concluded that the use of penicillin at 25 gm. per ton did not improve growth rate and that more work was needed on the effect of "infection" on rate of growth.

Food and drug regulations provide that in the prevention of CRD, poultry feed must contain the equivalent of not less than 60 gm. of penicillin G master standard per ton and in the treatment feed must contain the equivalent of not less than 120 gm. of penicillin G master standard per ton provided labeling bears adequate directions and warnings for such use(31).

Bacitracin.—As in the case of penicillin, information concerning the high level use of bacitracin is very limited. In vitro tests indicated bacitracin has little or no effect on the agent of IS and CRD(20), (88). The antibiotic does control some of the secondary infections, improves the appetite and reduces the severity of the disease(48).

The recommended level for use in the prevention of CRD is 100 gm. or more and in the treatment 200 gm. or more per ton of feed, provided the labeling bears adequate directions and warnings for use(31). Elam *et al.*(24) reported that prolonged feeding of bacitracin in laying hens resulted in increased egg production and hatchability.

Streptomycin.—Streptomycin and dihydrostreptomycin are very active in in vitro and in vivo studies against the organism associated with CRD and IS. Also when used parenterally in chickens and turkeys with the lower respiratory form of CRD the drug has given encouraging results(28), (36), (70). Wong(89) found that streptomycin stimulated feed intake and lowered mortality. Cover(19) reported in laying flocks that streptomycin had little effect in reducing the mortality to CRD. Lee(50) found that streptomycin fed to chicks at the rate of 30 gm. per ton did lower mortality and stimulate growth. Carlson *et al.*(14), (15) reported that streptomycin at 60 gm. per ton of feed in laying hens improved egg production, feed efficiency and hatchability.

Oxytetracycline.—This antibiotic has been shown to have in vitro and in vivo effect against the organisms of CRD in chickens, IS in turkeys. Carlson and Kohlmeyer(13), (15) found that oxytetracycline at 100 gm. per ton of feed for laying hens produced beneficial effect on feed efficiency. Several investigators(16), (36), (38), (61), (67) have reported on the beneficial effect of high level feeding and parenteral use of oxytetracycline. Other investigators(19), (28) have reported little effect on the mortality in natural outbreaks.

Food and Drug regulations provide that oxytetracycline may be used in poultry feed for the prevention of chronic respiratory disease at not less than 50 gm. per ton and when intended for use as a treatment, it is used at not less than 100 gm. per ton(31).

ENTERIC INFECTIONS

Salmonella Infections.—Pullorum disease, fowl typhoid, paratyphoid infections and Arizona paracolon infections may cause serious losses in young chicks and poults(5). Benson(4) reported that streptomycin when used parenterally was effective in reducing mortality and morbidity in chicks experimentally infected with *Salmonella pullorum.* In vitro studies with *S. pullorum* indicated that streptomycin had bactericidal action(17).

Smith(77) found that chloramphenicol at 0.5 per cent level in feed reduced the mortality but did not eliminate the carriers of *S. pullorum* in experimental trials. Kiser and deMillo(47) found that streptomycin, neomycin, magnamycin ®, chlortetracycline, oxytetracycline, tetracycline, and chloramphenicol were active against *S. gallinarum.* Penicillin and erythromycin showed no activity. Prior and Alberts(66) found that chlortetracycline was more effective in vitro than streptomycin and penicillin against *S. gallinarum.* In experimentally infected chickens the drug at the level of 1000 gm. per ton had some prophylactic value. Glantz and Gordeuk(34) found that chloramphenicol and chlortetracycline at 1000 to 2000 gm. per ton were necessary to reduce mortality to *S. gallinarum* infection. Controlled studies have not indicated the practical use of antibiotics in the prevention or treatment of salmonellosis of poultry.

Nonspecific Enteritis.—Because the specific causative agents of pullet disease of chickens and bluecomb disease of turkeys have not been identified, considerable confusion exists concerning these diseases. Numerous names have been used to describe the conditions in chickens and turkeys. The following antibiotics have been used in the prevention and treatment of bluecomb (nonspecific infectious enteritis,

mud fever): penicillin, oxytetracycline, chlortetracycline, bacitracin, streptomycin and dihydrostreptomycin. The minimum levels are the same as described for chronic respiratory disease(31). Peterson and Hymas(62) first reported effective use of antibiotics in the treatment of bluecomb disease of chickens and turkeys. Pomeroy and Sieburth(63) reported the disease affected turkeys of all ages. In young poults levels of 0.5 to 1.0 gm. of penicillin, streptomycin, oxytetracycline, chlortetracycline per gallon of drinking water reduced the mortality 50 to 75 per cent as compared to the control groups. The antibiotics had to be used at high levels in the feed, 500 gm. per ton to be effective. In older turkeys lower levels (100 to 200 gm. per ton) under field conditions have given encouraging results. Sieburth and Pomeroy(74) have studied a catarrhal enteritis of chicks similar to bluecomb disease of poults. Penicillin, tetracycline and streptomycin at 1 gm. per gallon were effective in reducing the mortality 50 to 85 per cent as compared to control groups.

Hexamitiasis.—It is a disease of young poults and is caused by a protozoan, *Hexamita meleagridis.* It causes an acute catarrhal enteritis and must be differentiated from bluecomb disease of poults(5), (63). Almquist and Johnson(1) reported encouraging results with chlortetracycline, oxytetracycline, and penicillin in experimental trials. Streptomycin was of no value. Chlortetracycline and oxytetracycline may be used in the prevention and treatment of hexamitiasis if used at minimum levels recommended for chronic respiratory disease(31).

Synovitis.—The causative agent of this disease has not been characterized but experimental studies indicate it is an infectious disease(58), (59), (86), (87). Several investigations(6), (60), (87) indicate that chlortetracycline and oxytetracycline were effective in the prevention and treatment when used at levels of 100 gm. or more per ton of feed. Penicillin, streptomycin and erythromycin administered in the feed from 100 to 400 gm. per ton had little effect.

Staphylococcal Arthritis in Turkeys.—Fahey(29) reported effective use of a combination of antibiotics when used parenterally and when oxytetracycline was used at the same time at 200 gm. per ton of feed. Browness and Fahey(12) reported favorable results in a trial using chlortetracycline in the feed and a combination of antibiotics parenterally.

Swine

ENTERIC INFECTIONS

The Virus Diseases; i.e., *Transmissible Gastroenteritis and Hog Cholera.*—Antibiotics have little value in the prevention and treatment of these diseases, but may aid in the control of secondary infections(32), (44).

Bacterial Diseases.—The principal primary and secondary etiological agents of enteric infections belong to the gram negative group of organisms. Salmonella, vibrio and related enteric organisms are commonly encountered.

Swine Dysentery.—The causative agent of this disease is considered to be vibrio. Doyle(23) Salisbury *et al.*(69) have found that streptomycin, oxytetracycline and chlortetracycline were effective in the treatment of swine under controlled field trials. Bacitracin has been found beneficial in the treatment of swine dysentery(35).

Salmonella Infections.—Various species of Salmonella have been encountered in swine. *S. choleraesuis* is commonly associated with acute and chronic forms of

enteritis. Unfortunately there is very little data available on the use of antibiotics in experimental salmonella infections of swine.

Nonspecific Diarrhea (Scours).—Numerous investigators(45) in their studies on the effect of antibiotics as growth stimulants in baby pigs, and in growing and fattening swine, observed that antibiotics had significant effect in reducing the incidence of scours. No attempts were made to determine the etiological agent of the scours. Certain antibiotics, chlortetracycline, oxytetracycline, and bacitracin may be used in animal feeds in the prevention (50 gm. per ton or more) and as a treatment (100 gm. per ton or more) for infectious swine enteritis as long as the labeling bears adequate directions and warnings for such use(31).

Calves

ENTERIC INFECTIONS

Various etiological agents have been associated with calf scours. Members of the Enterabacteriacae family, vibrio, clostridium, rickettsial and viral agents have been considered primary agents by various investigators. Numerous investigators(26), (45) in their studies on the effect of antibiotics as growth stimulants in young calves reported that antibiotics had a beneficial effect in reducing the incidence of scours and colds. In most of the observations no attempts were made to identify the causative agent or agents. Encouraging results have been obtained in the treatment of infectious enteritis in calves with streptomycin, chlortetracycline and oxytetracycline.

Salmonella Infections.—Salmonella dublin infections in calves responded to the oral use of chlortetracycline. A dosage of 12 to 16 mg. per pound of body weight per day gave excellent results. Bacitracin and streptomycin were not as effective(10). In an outbreak of *S. bredeny* infection, chloramphenicol gave better results than streptomycin(41). Neomycin has been observed to be effective in the treatment of *S. dublin* infection(11).

Lambs

Losses from enteric and respiratory infections occur in young lambs. In clinical trials antibiotics have been effective in the treatment of certain of the systemic infections such as pneumonia of lambs involving Pasteurella as a primary or secondary invader. One of the common problems in lambs is the condition referred to as enterotoxemia which is caused by *Clostridium perfringens.* In a summary of the use of antibiotics in the feed of lambs Elliott and Maddock(25) reported that chlortetracycline had beneficial effects in reducing the deaths from enterotoxemia and pneumonia and that less scouring occurred. A level of 10 milligrams per pound of total ration was suggested for continuous feeding.

The antibiotics that are commonly used in the treatment of lambs with dysentery are streptomycin, chlortetracycline and oxytetracycline.

Discussion

Poultry.—In the concentrated poultry raising areas of this country, respiratory infections have been responsible for serious losses. It is estimated that the annual

cost of Newcastle disease and other respiratory infections to the poultry industry is over 40 million dollars. Chronic respiratory disease has become a major problem in broiler production because of its relationship to increased mortality, reduction in feed efficiency, and occurrence of more culls. In laying flocks the disease has an adverse effect on egg production and causes increased mortality.

The high level use of antibiotics in poultry feeds and water as an aid in the prevention and treatment of chronic respiratory disease was stimulated by numerous controlled field and laboratory investigations. The oral use of effective antibiotics has proven to be of value in stimulating feed intake, maintaining normal weight gains, reducing mortality, and increasing egg production in laying flocks. Oral feeding at levels commonly used (50 to 200 gm. per ton) will not completely prevent or cure the disease on a flock basis.

There is considerable controversy over the high level use of antibiotics in commercial poultry feeding programs. The high level use of antibiotics in the first two weeks of the brooding period may reduce early chick and poult mortality. Also high level use during periods of stress such as vaccination and environmental changes may be desirable. This use should not be a substitution for a sound sanitation and management program on a chicken or turkey farm.

The continuous feeding of high levels of antibiotics to chickens and turkeys from day of age to market or in the laying flock under all conditions does not appear to be justified. In the presence of certain diseases such as chronic respiratory disease, infectious sinusitis, bluecomb, hexamitiasis and nonspecific infectious enteritis, the beneficial effects of high level oral use of antibiotics do justify the increased cost of antibiotic supplementation. In laying flocks that have below normal production, high level feeding may have beneficial effect on egg production and mortality.

The confusion that exists in the high level use of antibiotics in some of the obscure diseases of poultry should stimulate research workers to specifically identify those unknown infectious agents and eliminate the free use of the terms, "disease level" or "disease burden."

Swine, Cattle and Sheep.—Losses from animal diseases are high. The estimated annual loss in calves and lambs is equally as staggering.

The high level oral and parenteral use of antibiotics in young pigs, calves and lambs has been an aid in reducing the loss in these animals from respiratory and enteric infections. Because research has not resolved the etiology of some of these respiratory and enteric disorders of young animals, confusion does exist concerning the specific use of the antibiotics. They are recommended for use on such all inclusive conditions as pneumonia, infectious scours, shipping fever, and necro. The high level use is not a substitute for a sound sanitation and management program on our livestock farms.

Under field conditions many of the respiratory and enteric infections in our livestock and poultry are complicated by concurrent infections and the effects of secondary infections. Because the disease problems are becoming more complex, the necessity for an accurate diagnosis becomes more important if an objective approach is going to be made to control and reduce the disease hazards on our farms.

The development of bacteria resistant to antibiotics has been reported by many investigators. The use of antibiotics has an effect on the bacterial flora of the in-

testinal tract and the number of certain species are reduced while other species become established. Certain mycotic infections are occasionally encountered in poultry and these organisms are widely distributed in nature. Sieburth and Roth(75) studied the effect of high level antibiotics on the establishment of *Candida albicans* in the intestinal tract of chicks and poults. The lesions involving the crop were more extensive in the experimental birds on high levels of antibiotics. However, these birds did not show symptoms of mycotic infection as compared to inoculated birds on basal ration free of antibiotics. There is no clinical evidence that the use of antibiotics has resulted in an increased incidence of any disease of animals or poultry. Waibel *et al.*(80) found that at 10–35 gm. per ton level, good growth responses were consistently obtained. However, these responses eventually disappeared entirely for some time. It was observed that groups without antibiotics grew as well as groups with antibiotics. The authors believed that if resistance had been built up to the antibiotics, then growth with antibiotics would have declined to the level of growth without antibiotics. Libby and Schaible(51) have made similar observations. The potential hazard of the development of resistant bacteria, spirochetes, rickettsiae and certain of the viruses must, however, always be considered.

Conclusions

1. Oral use of certain of the antibiotics as an aid in the prevention and treatment of certain of the infectious diseases of poultry and livestock is indicated.
2. The addition of these antibiotics at high levels to commercial feeds for livestock and poultry has become an established practice.
3. Medicated feeds have a place in the control of infectious and parasitic diseases, but should be used as a supplement to a sound sanitation and management program on our livestock and poultry farms.
4. The use of antibiotics at high levels in the feed for livestock and poultry under all conditions is not indicated at the present time. There is no substitution for an accurate diagnosis and rational use of the antibiotics as treatment for specific diseases.

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ANTIBIOTICS IN REPRODUCTION

The use of antibiotics in animal feeds has become widespread for all classes of livestock. For the most part, usage has developed for purposes of feeding growing animals rather than mature animals. This implies, of course, that much less antibiotic products are used during the reproduction cycle than during the growth period. By the same token, it means that much shorter periods of time are ordinarily involved. Greatest usage has been for the short-term growth period rather than in the interests of longevity or lifetime and successive generation effects.

Experimental data on the reproductive effects are rather limited. Certain pilot studies have been conducted with laboratory animals such as the rat and the guinea pig. However, there have been a fair number of studies with hens, including the breeding flock, and a few studies with swine.

In reviewing the subject of antibiotics in reproduction, what effects do we look for? For this purpose, we can outline the steps about as follows:

(1) the body size attained;
(2) the mortality and the life span;
(3) the age at sexual maturity;
(4) fertility and fecundity—(In the case of poultry, this refers particularly to the hatchability of the eggs, while in animals such as swine the number of ova produced and fertilized is important.);
(5) the embryonic survival;
(6) the newborn survival.

Body Size.—In general, experimental work has shown that animals ordinarily gain mature weight somewhat earlier when suitable antibiotics are used in the diet. The mature weight, however, is not necessarily increased. Nevertheless, it is probable that the elimination of runty animals does help to increase the average mature weight in an unselected population. In the case of swine herds, at least, these runty animals would ordinarily be discarded. Certainly, body size has not been much of a factor in consideration of effects of antibiotics on mature animals.

Mortality and Longevity.—Information on mortality is rather limited. To a certain extent there are conflicting reports with some species as to longevity effects. For example, the guinea pig is very susceptible to certain toxic manifestations from the feeding of antibiotics. Of the numerous reports relating to effects of antibiotics on guinea pigs, the work by the Finnish group (Roine and associates)(4) stands

out. They have demonstrated that the guinea pig succumbs to Aureomycin feeding. It appears that this and other antibiotics affect the intestinal flora and permit certain organisms to flourish that obviously produce toxins. The organisms blamed are the listeria and sarcina groups. It has been demonstrated that certain other antibiotics, penicillin for example, can be used to counteract the condition presumably by destroying these groups of organisms.

So far as other species of animals are concerned, there is very little to indicate that antibiotics have any detrimental effects on mortality or longevity. It is conceivable, of course, that some very abnormal situation might develop in a given species analagous to that just cited in the guinea pig where harmful effects might result. Thus far, nothing like this of any consequence has developed. Experiments with laying flocks have not shown any marked differences in mortality between control groups and test groups receiving antibiotics. The few studies with swine likewise have shown that the death losses among the antibiotic-fed animals were fully as low if not lower than among controls.

There are a few longevity studies with rats. In a recent report, French and coworkers at Pennsylvania State University(5) described results with high levels of streptomycin and Terramycin (0.02 and 0.04 per cent) in which the average life span of albino rats was decreased about 10 per cent. The high levels were used in an attempt to control chronic pneumonia and it is not clear whether this circumstance influenced the results.

In the case of livestock and poultry, there is little if any evidence on which to judge longevity effects and for that matter the duration of the fertility span.

Sexual Maturity.—As far as sexual maturity is concerned, it would appear that the antibiotics, by encouraging more rapid growth rate in the young animals, do tend to encourage the attainment of sexual maturity at a slightly earlier age. Actually, tests with rats have not supported this contention in a very convincing way. There are some experiments with swine conducted at the Texas Agricultural Experiment Station(2) that rather indicate gilts do reach puberty slightly earlier.

Fertility.—In the field of fertility, the most convincing data are perhaps in the hatchability of poultry eggs. Tabulation of data from about 15 reports shows no improvement in hatchability in most cases, although there are a few reports showing such an effect. This improvement is most likely to occur on plant source diets low in vitamin B_{12}. It is noteworthy that some rather high hatchability rates have been achieved in recent years using best available information on nutritive requirements of hens.

In the case of swine, there were some doubts early in the experimentation on antibiotics whether estrus might be delayed particularly in gilts. Presumably the suspicions arose through rather high rates of breeding failures experienced in herds that were receiving antibiotics. It was true that proper controls were not necessarily provided to these groups. Experiments at the Texas Station(2), the Purdue Station(6), and at Beltsville(1) have failed to demonstrate any disturbance to estrus. If anything, the females have been more regular.

As to number of ova shed, likewise there have been no very significant differences through various experimental treatments. If ovulation rates have been somewhat

high in some animals, it is more than likely associated with a somewhat lower number of normal embryos. Thus, the net results in terms of live births is about the same.

The Beltsville experiments have been along two lines. In one(1), serious attempt has been made to create extreme conditions through the use of very high levels of an antibiotic in the reproduction ration. Actually, 5 to 10 times the average normal level of Aureomycin was used (10 milligrams per pound of feed). It was thought that these high levels might conceivably show effects on estrus and survival of embryos if there were any unusual effects. Results of these tests failed to show any significant difference over controls in any of the characteristics observed. Of course, there is a tendency to save more pigs up to weaning time and to obtain heavier weaning weights. In the strict sense, however, there was little difference in the effects on numbers of embryos.

Embryonic and Newborn Survival.—The other phase of the Beltsville experiment has dealt with the value of an average level of antibiotic in the gestation ration in comparison with the possible improvements from adjustments in other nutrients, such as protein. Here the number of live pigs at birth and of mortality of pigs in the first few days following birth have been the main criteria. Generally speaking, the addition of an Aureomycin-vitamin B_{12} combination to a gestation ration has greatly reduced the first week's mortality. The use of the Aureomycin has not effected the number of embryos carried to full term. Neither has it affected the birth weights of the pigs. The gestation ration containing 15 per cent of protein and Aureomycin generally increased the number of weaned pigs by one to two per litter, or to a total of approximately 8 pigs. About the same results have been obtained by raising the protein content to 17.5 per cent and 20 per cent.

Several reports indicate some beneficial carry-over effects in chicks hatched from eggs produced by hens fed on a diet containing antibiotics.

In summary then, the use of the more common antibiotics has been tested in reproduction rations to a limited extent in the case of poultry and swine. There is little or nothing on this phase of the life cycle in the case of ruminants where most of the experimental work is still concerned with the growing animal. The usage for poultry and swine has progressed at least to the point where we are reasonably certain that there are no more harmful effects in the reproductive cycle than there are in the growth cycle. This, of course, means in the usual case that there are no harmful effects in the case of poultry and swine, but rather some advantages and some improvements, particularly as measured in terms of survival of the young in the first few days of life whether it be newly hatched chicks or newborn pigs. Presumably, survival and life expectancy are generally improved.

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MUTAGENIC EFFECTS OF ANTIBIOTICS

The nature and importance of genetic changes and of phenotypic variability in microorganisms induced by antibiotics is not only of great scientific and academic interest, but is also of considerable practical importance in connection with medicine, taxonomy, biochemical synthesis, industrial mycology, epiphytotics, breeding disease-resistant varieties of crop plants, and other disease control practices. The growing interest in this field of work is indicated by the appearance of many recent publications on the antibiotic-induced variations in bacteria(12), (14). Most of the studies were not made to ascertain whether antibiotics could serve as useful or effective "tools" for the production of diverse types of mutants, but rather to determine whether antibiotics induced resistance in pathogenic bacteria that cause human and animal diseases. Only a few studies have been made with the use of antibiotics as general mutagenic agents in microorganisms.

It is common knowledge(11), (13), (14) that antibiotics have a pronounced effect on the stability of microorganisms and that phenotypic or genetic changes occur readily when microorganisms are grown on a substrate containing a sub-lethal dose of an antibiotic or of other toxic substances.

Variants resistant to antibiotics can easily be obtained by growing a culture in the presence of an antibiotic. Passing a culture through a series of increasing concentrations of the antibiotic is the common method used in obtaining in vitro an antibiotic resistant line. Similarly, antibiotic resistant lines can be isolated from patients who have previously been treated with an antibiotic(7), (21). There are no reports that antibiotics induced genetic variation in microorganisms by a sojourn on a host plant which has been treated with antibiotics. Also, there is no evidence that antibiotics in the soil induce genetic changes in microorganisms.

The nature and scope of the changes induced by antibiotics are not definitely known. Some attribute them to mixed population, others to adaptation, and still others to mutation and selection. Virtually no attention has been paid to the possible role of segregation and heterocaryosis in the origin of new antibiotic resistant lines of microorganisms. Most of the work seems to indicate that mutation plus selection are the two primary agents involved in development of antibiotic resistant lines of microorganisms.

Mixed Populations.—There is good evidence that mixed populations are a source from which lines can be isolated that are resistant to antibiotics. It is well established

that in bacteria and fungi a species may consist of numerous dynamic biological entities or biotypes(6), (24). For instance, in *Ustilago zeae* (Beckm.) Ung., the causal organism of common corn smut, thousands of biotypes have been isolated and studied in the laboratories at Minnesota(24). Like higher plants, races and biotypes of given species of microorganisms differ in nutritional requirements. Certain substances, whether they be in the plant or animal, or in artificial media, may stimulate one biotype and inhibit another. Therefore, biotypes of bacteria most capable of utilizing or tolerating inhibitory substances like an antibiotic, in a particular environment have a much better chance of survival, providing other factors for their development are favorable. Mixed populations could definitely be an important source for the origin of lines resistant to antibiotics. Obviously the degree to which a resistant line could then be developed would depend on the heterogeneity of the original population and also on the antibiotic involved. This may be the primary reason why certain cultures can be modified much more readily than others, when the organism is grown in association with certain antibiotics.

Adaptation.—Although there is considerable evidence that "adaptation" occurs in certain microorganisms, the validity of many of the conclusions has been questioned. In literature, the term "adaptation" is sometimes used loosely, whereas, in other cases it is used in a rather restricted sense. In a general sense it applies to any change, whether of phenotypic or genetic origin; it is an ecological term which implies that an organism has adapted itself by some unknown manner to a new environment. In a stricter sense, the term "adaptation" means an acquired ability, a protoplasmic alteration, a temporary change, but one not involving an alteration of the genic complement. This implies that an organism may acquire an ability to do something it either could not do originally or could not do as well, although no genic change was involved. It also implies reversible changes when the stimulus is removed. In this paper the term is used in the restricted sense.

A number of investigators(1), (2), (12), (16), (17) have concluded that bacteria causing human and animal diseases readily acquire the ability to tolerate antibiotics. They contend that while the organism is growing in contact with the antibiotic it develops the ability to resist the drug through some fundamental changes in the biochemical properties of the cell. Such biochemical changes, however, do not involve genic changes. Many investigators(1), (4), (11) have pointed out that resistance in bacteria is a gradual buildup in a population of individuals that are resistant to antibiotics as a result of exposure to successive increases in the concentration of antibiotics. This is not always true, as occasionally a resistant bacterial line can be isolated after a single exposure to an antibiotic. A resistant line may remain relatively constant when returned to a nontoxic substrate, whereas others revert(9), (23), (25). Some appear to revert to the original type after a few transfers, while others require a much longer period. In a few cases such changes in resistance also were associated with alteration in other characters such as virulence and color(18), (19), (22). It has been suggested that these additional changes in character are indicative of adaptive changes. Obviously, mutation is not necessarily restricted to a single gene change. Moreover, characters are the expression of the interaction of genes plus environment.

Recent publications by Christensen and Daly(10) and Christensen and DeVay(9)

indicate much of the so-called adaptation in microorganisms, especially those that cause plant diseases, can be explained on the basis of mixed cultures of biotypes, mutation, heterocaryosis or else the data were too meager to prove or disprove the adaptation phenomenon. Similar conclusions can be reached in regard to adaptation to antibiotics.

Mutation.—Extensive studies on mutation in microorganisms have established principles that are important in interpreting adaptive and genetic changes in microorganisms. Mutants frequently cause mixed populations in lines, even in cultures that are considered to be stable biotypes. Frequently such cultures appear to be pure macroscopically but they may actually contain many different biotypes which are not evident without special methods of detection. Thus, with an ever increasing number of studies on genetic variation in microorganisms, it is becoming more and more apparent that genetic purity of an isolate, notwithstanding its derivation from a single spore or cell, is a concept and not a reality.

Although Demerec(11) and many others(15), (17), (20), (23) in bacteriological science believe that mutation plays an important role in the development of lines of bacteria resistant to antibiotics, they believe that there is little or no direct evidence that the antibiotics act as mutagenic agents but rather act as selective agents for isolating resistant lines. Naturally, mutation may occur by chance in a medium containing antibiotics as well as in nontoxic substrates. Since there are varying degrees of resistance, Demerec(11) suggests that several genes may be involved in the genetic changes for resistance to antibiotics and that these genes differ in their potency for resistance to certain antibiotic substances. In contrast, Eriksen(13) and others(1), (22) believe that lines of bacteria resistant to antibiotics are actually induced by contact with the antibiotic. He also points out that the facts are not sufficient to support the view that changes are primarily the result of selection from mixed populations or adaptation.

Usually the investigator working with bacteria does not make inoculations with individual cells but with a population. With fungi, however, it is relatively easy to inoculate the Petri plates and flasks or the host, or at least a plant host, with a single spore. Thus one can expect more precise genetic information than when mass inoculations are made.

There are many experimental results with fungi which indicate that the number of mutants can be markedly increased by growing the organisms on a toxic substrate. During the past twenty-five years, Stakman and Christensen(24) and some of their associates at Minnesota have demonstrated repeatedly that plant pathogens can readily be induced to mutate if grown on a nutrient medium containing ethyl mercury phosphate, mercury bichloride, malachite green and many other toxic substances including a by-product from bacteria as well as with certain known antibiotics.

In 1937, Christensen and Davies(7) found that an unidentified antibiotic produced by *Bacillus mesentericus* Trev. greatly increased the frequency of genetic variation in certain races of an important cereal pathogen, *Helminthosporium sativum* P. K. & B. when added to potato dextrose agar in very small quantities. A similar antibiotic has been isolated by Cercos in Argentina(5). Later Christensen

and Davies(8) used this antibiotic extensively as an effective mutagenic agent in fungi.

Many of the mutants induced in *H. sativum* by this bacterial substance differed from their parents in many characters: color, type of growth, kind and amount of pigment, abundance of sporulation, reaction to dyes and toxic substances, tendency to sector, pathogenicity, and to some extent in morphology. It is rather significant that the mutants differed not only in cultural character but in pathogenicity. A few were much more virulent on wheat and barley than their parents. Most of them, however, were less virulent and two appeared to be almost nonpathogenic.

Usually the most conspicuous mutants were very tolerant to antibiotics. However, when random samples of variants were isolated, the range in tolerance to the antibiotics was very great. Most of them had about the same degree of tolerance as their parents, while some were much more and some were much less sensitive to the antibiotic than their parents. Naturally, there was a general tendency to select lines that were more tolerant to the antibiotics because they tended to outgrow the other mutants and their parents on the substrate on which they originated.

The mutants of *Helminthosporium sativum* were most frequent when the antibiotic retarded growth about 50 per cent its normal rate; apparently there is a definite optimum concentration of an antibiotic for general production of mutants. The constitution and pH of the basic medium to which the antibiotic was added had a marked effect on the frequency of mutation also. In general, a medium of acidic or neutral reaction gave the best results.

It is noteworthy that not only did different species of Helminthosporium differ in response to the antibiotic, but races and mutants of the same species differed greatly in their response to the toxic substances. Tests with many species of fungi, both saprophytic and parasitic, indicated that an antibiotic is rather specific in its mutagenic action.

At present, extensive studies are being made on the mutagenic effect of the following known antibiotics: actidione, agri-mycin, bacitracin, candicidin, Chloromycetin, filipin, mycostatin, pleocidin, streptomycin, and Terramycin. Their mutagenic effects are being studied on the following fungi: *Colletotrichum linicolum* Pethyb. & Loff., *Diplodia zeae* (Schw.) Lev., *Eremothecium ashbyii* Guill., *Fusarium moniliforme* Sheld., *Helminthosporium sativum* P. K. & B. (haploid), *Ustilago avenae* (Pers.) Rostr. (haploid), and *Ustilago zeae* (Beckm.) Ung. (haploid). These studies are being done by the shake culture method and by direct plating of single spores onto nutrient media in Petri plates and flasks containing the desired concentrations. The results being obtained are very similar to those given for the unknown antibiotic derived from *Bacillus mesentericus*. The frequency of mutation depends on the fungus involved as well as on the concentration and kind of antibiotic used. Obviously, antibiotics differ greatly in their mutagenic effects; some increase the frequency of mutation, whereas others have no apparent effect. Moreover, a species responding to one antibiotic substance by increased rate of mutation does not necessarily respond in this way to another substance. The purpose of these studies is not primarily to ascertain whether the antibiotics directly influence the ability to develop mutants that grow on a medium containing a specific antibiotic, but rather to ascertain whether or not they are effective mutagenic agents.

Because of the wide range in characters of mutants produced, it appears that certain antibiotics may become useful and effective "tools" in studying mutation in microorganisms, particularly in fungi. Moreover, antibiotics are relatively cheap and can be used in any laboratory and do not require expensive and elaborate equipment.

Conclusions

Because of the extensive use of antibiotics in medicine and their potential value in agriculture more basic information is needed on the effect of antibiotics on microorganisms. Extensive studies should be made on antibiotics, regardless of their practical importance, to determine their effectiveness in inducing genetic and/or temporary variation in microorganisms. Detailed information is needed on the nature and scope of the changes that occur in populations of microorganisms exposed to antibiotics. Attempts should be made to determine the nature of the biochemical changes that occur in antibiotic-induced mutants such as color, rate of growth, metabolic deficiencies and especially pathogenicity. Morphological, physiological and ecological studies should be made of the mutants that have increased survival ability as these factors may have a bearing on their place in evolution as well as for their practical importance. Also, more consideration should be given the biological and scientific significance of antibiotic-induced changes particularly in their usefulness in technologic fields related to medicine, industry and agriculture. There are good indications that certain antibiotics may be cheap and useful mutagenic agents for studying genetic variation in microorganisms.

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Special Biological Problems

PANEL DISCUSSION

Moderator
Damon Catron

Panel Members

C. A. Baumann, J. J. Christensen, A. de Juana Sardon, N. R. Ellis, B. S. Pomeroy, A. C. Francois

Summarizer
T. C. Byerly

Dr. Catron: This matter of high level feeding bothers me considerably, Dr. Pomeroy because I find there is a great deal of misunderstanding not only in this country but abroad. Some people think that perhaps the development of fastness on our low level feeding is causing us to go to high level feeding. I think this should be corrected in concept and in thinking. What you are talking of as high level feeding is therapy level feeding. It is not the usually recommended low level feeding to stimulate or promote growth and improve feed efficiency.

One other thing which always bothers me—and I work very closely with the people in our School of Veterinary Medicine at Iowa State College—is that we just do not yet seem to have the tools in bacteriology to differentiate and follow through the etiology which causes these different diseases. With that in mind sometimes many of us have thought that by using good judgement we could go ahead and reap the values of the use of antibiotics in animal feeding. I agree with the statement that there is no substitute for a rational diagnosis in the selection of the correct therapeutic agent.

The statement which was made concerning the use of antibiotics to supplement a sound sanitation program is certainly valuable. I personally want to stress this point. The average farmer in this country who is producing livestock and poultry is continually waiting for the nutritionist to come up with something that can be put in the feed bag and he is also waiting for the veterinary research man to produce a pill or needleful of medicine which can be injected into animals to substitute for good management. That is a pathetic situation. A good many of our livestock people are like this. We cannot substitute for good, sound management programs.

Our next speaker is Dr. Ellis, of the U. S. Department of Agriculture. . . . Dr. Ellis presented his paper on the reproductive effects of antibiotic feeding. . . .

Dr. Catron: Thank you very much, Dr. Ellis.

As Dr. Ellis has ably pointed out, we have seen no ill effects on conception from the use of antibiotics. One of the things which is worrying a good many people, Dr. Ellis, is the possible problem of antibiotics interfering with selective breeding. Perhaps that can be commented on at a later time. Frankly, a runt pig is always smaller, whether he receives antibiotics or not.

One of the things which I think is worth noting is the recent interest in high level antibiotic feeding 3 to 5 days prior to, and after parturition. In this connection many hog producers have felt that the incidence of post-parturition infection has been reduced. A recent experiment at the Nebraska Station indicates that antibiotics increase pig survival.

Our next speaker is Dr. Christensen of the University of Minnesota. . . . Dr. J. J. Christensen presented his paper entitled "Mutagenic Effects of Antibiotics.". . .

DR. CATRON: While the questions are coming forward I would like to ask you, Dr. Baumann, whether Dr. Christensen's remarks change your mind any on the mode of action as far as antibiotics are concerned in animal feeding?

DR. BAUMANN: He gave a marvelous illustration of the diversity of forms possible under the influence of antibiotics. Of course, the proposed modes of action without exception depend on altered microbial forms in the presence of antibiotics. This is the sort of basic information which is needed before a real explanation can be offered to the growth-promoting effects which have been observed.

DR. CATRON: Dr. Baumann, we have a question for you from the floor. It states: "What are the effects of antibiotics on the usefulness of assay animals, including the chick embryo? Or for vaccine production?"

DR. BAUMANN: I really know very little about chick embryos or vaccine production. If you try to conduct a bioassay for a vitamin and your known preparations contain some antibiotic, you may get improved growth which you ascribe to the vitamin for which you are assaying, when in actual fact this extra growth comes from the antibiotic which is present and unknown. So the growth-promoting effect of antibiotics may seriously upset a bioassay for a vitamin as far as quantitative results are concerned. I think my comment will have to stop there.

DR. CATRON: Dr. Pomeroy has three questions from the floor.

DR. POMEROY: The first question is: "Would you conclude that antibiotics act primarily in combatting respiratory diseases by indirectly effecting a more healthful intestinal environment and increased appetite rather than by directly combatting respiratory organisms?"

I don't know whether I can arrive at that conclusion as to the mode of action of the antibiotics, but we do know, certainly, that the antibiotics which are used in many of our respiratory infections do have a direct effect on the organism involved. In other words, some of the antibiotics do directly reduce the bacterial population in the bird or sterilize the gut of the bird, and there is a beneficial effect. In turn, we have antibiotics which apparently are used and have been shown to have value but have no direct effect on the infection. This may be an explanation. I would not want to say.

Another question is: "Are there any results on the control of Salmonella with synnematin, which has been shown to have value in humans?"

All I can say, if I am correct in this, is that we have not used that product in birds and animals. Maybe I have not interpreted or read correctly the question. I have seen no reports of its use and we have not used it in poultry, where Salmonella are of great importance.

"Do antibiotics have any anthelmintic effects?"

I assume that the question refers to effects on parasitism of round worms or tape worms. I am not aware of any effect from that standpoint. Again, someone else may be in a better position to comment on that.

"There are considerable field data on the effectiveness of antibiotics against nonspecific enteritis in adult birds at levels of 80 to 100 parts per million. Would you comment on the possible reasons why pullets disease (young birds) requires higher levels (500 parts)?"

The only reason is that the 500 parts per million that we were using in our experimental work gave us some beneficial effect in young birds, whereas 100 or lower levels did not. This is probably because in young birds, in poults, you must have a high level rapidly. If any of you have had experience with turkeys you know that any upset condition will throw them off feed or water consumption. It would appear that maybe it is not that a high level of 500 parts is required to be effective against the agent, but is due to the fact that with the high level we get the antibiotic in the bird more rapidly than at a lower level. Certainly in older birds the lower levels of 50 to 100 parts per million have shown very encouraging results under field conditions in the so-called pullet disease or nonspecific enteritis.

DR. CATRON: Dr. Baumann, you have some questions addressed to you.

DR. BAUMANN: There are three here which are somewhat related, and I will read them:

"What histological changes have been noted in walls of intestines harvested from animals fed antibiotics versus those not receiving antibiotic substances?"

The next one: "Has any histological work been done to indicate the specific site of thickening in the intestine of antibiotic-fed animals?"

The third is simply: "Dr. Rusoff of Louisiana State University has some data on thickness of intestinal walls of calves fed Aureomycin."

We ourselves have not done any histological work. We certainly intend to. I would suggest that perhaps Dr. Kon or Dr. Cuthbertson or others of the British group who initially published papers on thickening of intestines in chickens might be the people to comment on this question; and perhaps Dr. Rusoff.

DR. CATRON: Dr. Rusoff?

DR. RUSOFF: I would like to say that over a period of two years or longer we have been actually measuring the thickness of the small intestines of calves. I think at about the time Dr. Luckey and his group at Notre Dame mentioned the possible differences in the intestinal walls we became interested in our program. Besides weighing the organs and various tissues, we use microcalipers and measure specific sections of the intestine, the upper region, the middle region, and the lower region. We noticed that even in the control animals there is a thinning of the wall in the middle region, but in the antibiotic-fed animals it is even thinner. We now are in the process of analyzing our data, and results should be out fairly soon.

DR. CATRON: Would Dr. Kon please comment on that?

DR. S. K. KON: Very briefly, we have not done yet but very much intend to do histological studies that justify gross chemical composition analysis. We do not see any change in composition, and it seems to us that what is happening is an actual thinning of the intestine of all the elements more or less equally. For the time being this is still a very rough picture, and we are working on it.

DR. CATRON: Thank you, Dr. Kon. Dr. Luckey?

DR. THOMAS LUCKEY: I would like to mention that the impression of Dr. Gordon at Notre Dame University, after his histological studies, seems to be that there is definitely a marked lack of collagen tissue in the wall proper. The muscle structure he thinks is relatively the same in quantity, but the connective tissue is definitely less.

DR. CATRON: Thank you very much, Dr. Luckey. Dr. Christensen, you have some questions from the floor?

DR. CHRISTENSEN: The first one is: "Which mechanism do you feel gave rise to most of the variants you observed; (1) Mutation followed by selection and overgrowth in the presence of antibiotic or (2) the antibiotic actually induced change at a particular locus on the chromosome?"

The ones we are convinced of and have observed are the genetic change and the mutation. But I want to point out, since there are many foreigners here, that "mutation" as used in the United States, at least out west of the Mississippi River, means not necessarily a gene change, but a chromosome aberration of a diverse sort. I know because I spent a year in New York, and it is my impression that there mutation is a question of a point change.

"Would Dr. Christensen comment on the possible mechanisms of antibiotic motivated mutations in fungi and the significance, if any, in higher plants and animals?"

As far as I assume antibiotics do not have any effect in inducing mutation in higher plants and higher animals, all I can say is that I don't know of any experiments which have been made or tried to that effect.

DR. CATRON: Thank you, Dr. Christensen. Dr. Baumann, do you have some more questions?

DR. BAUMANN: "Can you administer a high enough level of thiamine to prevent a response to antibiotics? If not, how can you conclude that antibiotics spare B_1?"

In rats you most certainly can. If you feed a complete vitamin mixture, adding antibiotics to the diet does not give any further increase in growth. In the case of the chicken, if you happen to be in a period in the laboratory when the chickens are not responding to antibiotics on a complete diet, in effect this is a situation in which the diet is adequate in B_1.

The second question: "What would 2 grams of penicillin in one ton of medium do to the growth of organisms? If nothing, how can one believe that antibiotics modify the intestinal flora?"

I don't know about this particular dosage, but certainly when very low doses of penicillin are fed to rats and chicks there are changes in the types of microorganisms which can be measured. The total number may not differ much, but the distribution of species and types of species does change even under the influence of very low levels of antibiotics, even penicillin, which may not survive as such very far below the stomach.

DR. CATRON: Dr. Johnson?

DR. B. CONNOR JOHNSON: With regard to that first question of Dr. Baumann's on whether high levels of vitamins prevent a response to antibiotics, I would just like to say that we have studied, using the rat, the quantitative requirements for thiamine as affected by the antibiotic penicillin. If there is increased absorption or increased synthesis or decreased tying up, you would expect any of these to make

more thiamine available. It should decrease the requirements. Actually, however, we found no change in the requirements. The slopes of the growth rates of animals below the requirement were of course very different with penicillin. It was much less steep without it. They both reached optimum at approximately the same level. This makes it somewhat more difficult, perhaps, to assign a mode of action.

DR. CATRON: Thank you, Dr. Johnson. We will turn to Dr. Ellis' questions next.

DR. ELLIS: There is one question here: "Does the panel have any information pertaining to the use and efficacy of antibiotics in the diets of animals such as the rabbit and mink?"

The answer is yes, and the effects are quite analogous to what they are with poultry and swine. Antibiotics have been shown to have positive effects in rabbits. It has been used in the reproduction cycle, and has given improvement in the growth rate of the young animal. The same thing applies pretty much in the case of mink. We have used it both in the diet of the young growing animal and through the reproductive period with positive and beneficial results.

Another question: "In the Beltsville work where antibiotic-vitamin B_{12} supplement decreased baby pig mortality, is it clear that this is not a vitamin B_{12} effect?"

I think the answer is quite clear that it was not a B_{12} effect, because we had control diets and found it pretty difficult to show any great effect from B_{12} deprivation even on soybean-corn diet, whereas the test which I was describing used a much better diet which carried a good deal of B_{12} naturally.

Another question: "Is there any danger from the use of antibiotics in swine to be used for breeding purposes because the runts are no longer recognizable?"

If they are no longer recognizable, why worry about them? How about that?

DR. CATRON: I can still say that such a pig is a runt after he receives antibiotics.

DR. ELLIS: Here is another question: "Effects of antibiotics on breeding. Can anyone of the panel please list any definite undesirable effects on breeding stock, sow or boar, which would suggest that their use should be limited?"

I am afraid I cannot put anything down which is definite in that direction. Both Dr. Catron and I referred to the worries, suppositions, and so forth, heard several years ago, but which have not been substantiated. Would you care to comment further?

DR. CATRON: I believe not, except just to say that we have fed antibiotics in our sow herds since 1950, and the number of pigs per litter and their livability has increased, not decreased. Back in 1950 we were weaning approximately 7.5 to 8 pigs per litter. A year ago it was 8.3. Now it is 11.5. In the month of August in a multiple farrowing system, farrowing 30 to 40 sows every six weeks, 12.2 pigs were saved at the end of a week. If there were detrimental effects from feeding antibiotics to sows or boars, it is certainly not showing up in our 180 sows on a multiple farrowing program.

DR. ELLIS: With the number of pigs going up, it might become a detriment to try to raise them all.

DR. CATRON: They can probably get enough milk to keep them alive so we can early wean them by one to two weeks of age.

The next comment. Do you have any more, Dr. Pomeroy? Dr. Christensen, do you have any others? Dr. Baumann?

If not, I should like to call on our other two panel members for brief comments. Dr. Francois would like to speak to you in French momentarily, and then we will have a translation by Dr. Fribourg.

DR. FRANCOIS (Through the Interpreter, Dr. Fribourg): The very interesting papers which have just been presented have suggested some comments to me.

The paper of Dr. Baumann in effect presents a subject which interests me especially, that is, the mode of action of antibiotics. Even though our personal experience specifically concerns swine, I would like to indicate briefly in what way the work carried on in my laboratory by Michel and by myself permitted us to solve certain specific biological problems arising in connection with the utilization of antibiotics. While it is a question notably of the problems of vitamins, I will also cite certain more general results which we have obtained through our research work.

In his excellent paper Dr. Baumann recalls the hypotheses which were formulated to interpret the saving of vitamins through antibiotics. These were (a) the increase of synthesis of vitamins by the microorganisms in the digestive tract and (b) the suppression of the organisms which absorb these vitamins.

I think that one can also think of a third hypothesis, that is, the role of the antibiotics in the direct saving at the intestinal level of certain vitamins in the ration. In effect, in my laboratory Michel has shown recently that the degradation of choline by the digestive tract microorganisms is inhibited by certain antibiotics. The experimental technique was the following:

Through differential centrifugation one can isolate the total flora of the swine intestine. After having put it back in suspension in a convenient medium, it is put in the presence of a choline solution. After a pre-determined time the trimethylamine which is formed is measured. In effect, the results obtained show that certain antibiotics inhibit in vitro the degradation of choline. Aureomycin and Terramycin present in this effect the maximum efficiency. At the rate of 10 micrograms per liter they inhibit almost totally after 16 hours of incubation the degradation of the choline. Penicillin is less efficient, and Chloromycetin is practically inactive. Thus we think that this mechanism of direct saving of certain antibiotics towards the vitamins of the B group should be taken into consideration.

It is possible that other vitamins of this group can also be protected by certain antibiotics. Work concerning this problem is at the present time being carried out in my laboratory. We shall have, however, to verify in vivo that the amount of choline absorbed in the intestine is higher when the animal itself takes in antibiotics.

In his paper Dr. Baumann recalls that the blood level of a limiting vitamin can be increased through the ingestion of antibiotics. We have not, however, actually any personal experience on this problem with respect to choline.

On the other hand, this mechanism of direct saving allows us to understand the fact that diets which completely lack the limiting vitamin are not bettered by the antibiotics, while diets which contain an average level of the limiting vitamin can be bettered.

In the latter case, in effect, the antibiotic allows us to conserve a sufficient amount of vitamin to cover the needs of the animal, but at present we have not studied this for choline, and with respect to other vitamins we limit ourselves to hypotheses.

However, we think that this mechanism is possible, for we have previously shown that antibiotics modify the content of certain substances in the blood of the portal vein, that is, that they modify the amounts absorbed in the intestine. For example, we have shown with Michel that the complex flora of the swine intestine easily deaminates the amino-acids. We have also found that antibiotics, and particularly the tetracyclines, inhibit these deaminations in vitro. It thus results that the amount of ammonia which is liberated in the intestine could be smaller in the animal which is receiving an antibiotic.

We have also been able to verify this fact experimentally, studying samples of blood from the portal vein of young pigs. The amount of ammonia decreased greatly when the Aureomycin treatment was considered compared to the control treatment.

I come back, then, to Dr. Baumann's paper, and I think that the suppression of a chronic toxicosis due to ammonia could be one of the mechanisms of action of antibiotics on growth—underlining also that there results from this action a certain saving of amino acids which could explain the possibility of decreasing the amount of proteins in the ration when this ration contains antibiotics.

Dr. Ellis' paper presents such a possibility. However, this mechanism is not entirely clear for one knows that the raw nitrogen balance cannot be improved through antibiotics. However, we have verified that the blood in the portal vein is richer in amino acids when the animal ingests some Aureomycin.

Finally, a last fact which supports the hypotheses which we present: We have in effect established a relationship between the growth index of an antibiotic and its aptitude to inhibit arginine deamination. The indices of growth have been established by Braude, Wallace, and Cunha as a result of a compilation of a large number of experiments. We have found that there exists a direct relationship between the growth index attributable to each antibiotic and its aptitude to inhibit arginine deamination. Terramycin and Aureomycin show indices which are a little higher and strongly inhibit deaminations. On the other hand, chloramphenicol does not inhibit deaminations and shows a very small growth index. Streptomycin, bacitracin, and penicillin indicate average indices for growth and for deamination inhibition.

Thus the modification by the antibiotics of the biochemical properties of the flora would bring about an action of direct saving of certain substances—amino acids, choline, et cetera,—and a decrease of the production of toxic substances such as ammonia. Deaminations are probably not the only biochemical modifications in the action. In this country Melnykowycz and Johansson have shown in the rat that decarboxylations can be modified by antibiotics.

Finally, we have attempted to increase this notion of substances allowing a saving of nutritive amounts in the intestine. We have studied substances which are capable of stimulating growth, such as 3-nitro-4-phenylarsonic acid and copper sulfate, the efficiency of which has recently been shown in Great Britain. We have found that these substances are capable of inhibiting in vitro the deamination of arginine and the degradation of choline. Other substances also possess this property. It is a question principally of tannin 2-4 dinitrophenol and certain metallic salts. Actually we are trying to determine whether these substances are equally capable of stimulating growth.

In summary, we think that the modification of the metabolic properties of the flora of the digestive tract by substances such as antibiotics certainly shows a nutritional significance. However, as has been emphasized by Dr. Baumann, certainly there is not just one mechanism of action, but several. We shall not exclude among these the eventual biochemical modifications which would come up at the blood or cell level.

Our opinion on this subject is reinforced by the fact that we have found that the kinetics of the liberation of ammonia from the blood are modified when the animal has ingested Aureomycin. The different results which have been presented here have been published in the Comptes Rendus de l'Academie des Sciences de Paris and were presented at the Biochemical Congress in Brussels last August.[1] Other papers will be published shortly.

DR. CATRON: Thank you very much. We appreciate those remarks. They confirm our thinking here. Dr. deJuana, do you have any comments? No comments. Are there any other comments from our panel?

I believe there is one comment that should be heard from the floor from Dr. Jukes, on the feeding of antibiotics to guinea pigs. We will entertain that at the present time.

DR. T. H. JUKES: There have been quite a number of publications describing the toxic effects first of penicillin and of Aureomycin, Terramycin, and bacitracin, in guinea pigs. However, there are anomalies in some of the laboratory reports. In one publication from the Mayo Clinic the toxic effects of Aureomycin and Terramycin are found to be overcome by penicillin. In other cases that is not true. Recently Professor Albert Hogan informed us that he had been using Aureomycin in the diet of his guinea pig colony to keep down the incidence of enteritis.

So it seemed that there must be some reason why he found it was not toxic. It appears to be a question of level. In some of our experiments we have found that a level of 10 parts per million in the diet of Aureomycin is tolerated by guinea pigs, although a considerably higher level is toxic.

DR. CATRON: Thank you very much, Dr. Jukes. To conclude this discussion, I present our eminent summarizer, Dr. Byerly.

SUMMARY OF THE SECOND SESSION

DR. T. C. BYERLY: In summary, antibiotics selectively inhibit or stimulate growth of intestinal bacteria. They may thus lead to synthesis of vitamins, to suppression of disease, or in the guinea pig to fatal listeriasis. They may improve the absorption of nutrients and the saving of nutrients.

Antibiotics at levels of 50 to 2,000 grams per ton of feed are selectively effective in controlling many specific diseases, but may not entirely eliminate the infective agent. Antibiotics are also effective against nonspecific enteritis in calves, lambs, pigs, and poultry.

Antibiotics have not been shown to affect reproduction of livestock species per se, but do favorably affect neonatal survival.

With respect to mutagenic effects of antibiotics it has been shown that they do

[1] *See:* Comptes Rendus Acad. Sci. 1955, **240**, 124–126. *Ibid.*, pp. 808–810, "Symposium Européen sur les Antibiotiques," Rome, 1955; "Congrès de Biochimie," Bruxelles, 1955.

induce mutations and that an occasional mutant may be stimulated by specific antibiotics in the medium.

With respect to assay animals it is desirable that the antecedent feeding of the laboratory animal to be used for assay be known.

DR. CATRON: Thank you all very much for your kind attention and participation in this session.

. . . The meeting adjourned at one o'clock. . . .

THIRD SESSION

MODE OF ACTION

October 20, 1955

H. D. BRANION, *Presiding*

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FUNDAMENTALS OF MODE OF ACTION OF ANTIBIOTICS IN ANIMALS*

It has been definitely established that antibiotics cause an increase in the growth rate of young animals. The mode of action of the antibiotics, however, is still a problem. The increase in weight may be the result of several physiological changes. Most authors believe that "structural substances" are formed, i.e. protein, P, and Ca; others(49) consider the increase in weight to be merely an increased retention of water possibly accompanied by a slightly increased fat deposition. Jacquot and Calet(35) presumed that antibiotics exerted a direct effect upon lipogenesis. The important question of antibiotic action in stimulating growth rate cannot be answered by simply determining differences in the weight between treated and untreated animals. Since the favourable influence of antibiotics on runts has been demonstrated and it has also been shown that chickens receiving antibiotics start laying earlier (both effects cannot be explained either by water retention or by slightly increased fat deposition) we ourselves define the growth effect from oral administration of antibiotics as a real formation of "structural substances." By feeding antibiotic diets to animals reared under ideal environmental conditions the biological optimum which can be attained by every animal according to its inherent genetic capabilities is not increased; but animals reared in an unfavourable environment are enabled to reach their optimum when fed antibiotic supplemented diets. Antibiotics have, therefore, no influence on genetically affected runts. However, the more unfavourable the environmental conditions of an animal (feeding included) the better is its response to an antibiotic. The action of antibiotics in promoting growth might be explained by the inhibition or restraint of those environmental factors which impair the development of the growing animal.

All animals have an intestinal flora which is highly complicated in composition(42). The biological functions of some of the component microorganisms have been elucidated. The physiological influence of the intestinal microflora on the organisms of man and animal is still obscure. The intestinal flora appears to be vital; it can almost be considered as an (exogenously supplied) *organ*. According to experience in human medicine it is of importance whether this "organ" works well or badly.

There is some reason to consider the antibiotic action on the intestinal flora as

* I want to thank Dr. R. Bonicke, Dr. H. Wolter, and Mrs. Rosenfeld for their collaboration.

the decisive factor in the growth effect. However, the following points should be considered: (1) There are innumerable chemotherapeutics having an inhibitory effect on the germs of the flora, but only some of them produce a growth effect. Therefore, the antibacterial effect is not sufficient in itself. Up to now Chloromycetin is not known to produce a growth effect. This fact was at one time explained by a quick resorption of the drug from the upper intestinal sections; but as shown in Table 1, Chloromycetin is not only excreted into the intestine, but is also found again in the colon. (2) The *parenteral* administration is not as dramatic in producing an effect on growth as is oral administration of these drugs. Crystalline Aureomycin was less effective when fed however, than were crude fermentation by-products from the manufacture of this drug. Since parenterally administered antibiotics are excreted into the intestine in considerable amounts through the bile (and the intestinal mucosa itself), an unrestrained growth effect ought to occur if the antibacterial influence on the flora were the decisive factor. Even substances, parenterally administered, which are badly or not at all resorbed from the intestine, will be found in the intestine in quantities sufficient to obtain a growth effect (Table 2; streptomycin).† (3) From human medicine it is known that large doses of chemotherapeutic drugs applied for long periods can cause severe intestinal distress. In an experiment on tadpoles kept during their growth towards metamorphosis under relatively high levels of Aureomycin (first group 19 mg/1, second group 20 mg/1 of aquarium water), we observed a change in the intestinal flora accompanied by a reduction of the total number of microorganisms. The sensitivity of the bacteria to Aureomycin decreased. The animals died before reaching metamorphosis. Considerable changes in the intestinal flora occurred together with severe damage to the animal. It must, however, be recognized that in this experiment Aureomycin also had the effect of a cytostatic agent; therefore the inhibition of metamorphosis cannot be defined with certainty. To be sure, this inhibition cannot be ascribed solely to the effect of the antibiotic. The low levels used in the diets of animals ordinarily do not affect the total bacterial count of their intestinal flora. Under normal feeding conditions we found optimum Aureomycin values of only 0.17 γ in the contents of the small intestine and 0.05 γ in the faeces(23). Such concentrations cause a biologically favorable influence on the animal. The growth effect of antibiotics can, therefore, not be elucidated by a reduction of the total count of the microorganisms in the intestinal flora (by reduction of vital nutrients, normally metabolized by bacteria, or by decreased production of toxins through a decrease in the number of toxin-producing microorganisms). The findings(40) have also shown that an abundant supply of toxin-forming microorganisms does not affect the growth and development of animals and that the additional supply of isolated toxins might even cause a growth promoting effect.

With respect to the foregoing, dissenting considerations of some authors should be reviewed. Very low concentrations of antibiotic agents could possibly have a stimulating effect on bacterial growth. This widespread hypothesis has not yet been

† It would be a task for human medicine to study whether or not the often surprising and quickly occurring curative effect of this substance (streptomycin) might be explained as an additional effect via intestinal flora. This should be more readily disclosed in children than in adults.

TABLE 1

CONCENTRATION OF CHLOROMYCETIN IN THE INTESTINES OF RATS*

Experimental Animal	Maximum Dose of Chloromycetin	Region of Intestine	Auxanogram (*E. coli*) Gut Wall a	Gut Wall b	Gut Wall c	Gut Contents a	Gut Contents b	Gut Contents c	Chloromycetin Concentration (γ/g) Gut Wall	Gut Contents
1	10 mg	Jejunum	0	0	0	14.0	0		< 2.0	3.2
		Ileum	13.0	13.0	13.0	15.5	16.0	16.0	2.6	7.5–9.0
		Caecum	0	0	0	0	0	0	< 2.0	< 2.0
		Rectum	0	0	0	0	0	0	< 2.0	< 2.0
2		Jejunum	0	0	0	16.5	17.0		< 2.0	10.0–12.5
		Ileum	13.0	13.0		15.0	15.0	15.0	2.6	6.5
		Caecum	0	0		0	0	0	< 2.0	< 2.0
		Rectum	0	0		0	0	0	< 2.0	< 2.0
3	5 mg	Jejunum	–	–		13.0	14.0		2.0	2.6–3.2
		Ileum	0	0	0	–	–		< 2.0	2.0
		Caecum	0	0	0	0	0	0	< 2.0	< 2.0
		Rectum	–	–	–	13.5	13.5	14.0	2.0	2.9–3.2
4		Jejunum	0	0	0	–	–	–	< 2.0	2.0
		Ileum	0	0	0	0	0	0	< 2.0	< 2.0
		Caecum	0	0	0	0	0	0	< 2.0	< 2.0
		Rectum	0	0	0	0	0	0	< 2.0	< 2.0
5	2.5 mg	Jejunum	0	0	0	–	–	–	< 2.0	2.0
		Ileum	0	0	0	0	0	0	< 2.0	< 2.0
		Caecum	0	0	0	0	0	0	< 2.0	< 2.0
		Rectum	0	0	0	0	0	0	< 2.0	< 2.0
6		Jejunum	0	0	0	0	0	0	< 2.0	< 2.0
		Ileum	0	0	0	0	0	0	< 2.0	< 2.0
		Caecum	0	0		0	0	0	< 2.0	< 2.0
		Rectum	0	0	0	0	0		< 2.0	< 2.0
9	0 Control	Jejunum	0	0	0	0	0	0	< 2.0	< 2.0
		Ileum	0	0	0	0	0	0	< 2.0	< 2.0
		Caecum	0	0	0	0	0	0	< 2.0	< 2.0
		Rectum	0	0	0	0	0	0	< 2.0	< 2.0
10		Jejunum	0	0	0	0	0	0	< 2.0	< 2.0
		Ileum	0	0	0	0	0	0	< 2.0	< 2.0
		Caecum	0	0	0	0	0	0	< 2.0	< 2.0
		Rectum	0	0	0	0	0	0	< 2.0	< 2.0

* In this experiment all rats received 15 g of a highly albuminous diet daily (5 g at 0830 hrs., 10 g at 1700 hrs.). At 0830 hrs. on each of four successive days (30/7/55–2/8/55) the experimental animals received in this ration various concentrations of Chloromycetin, as follows: two rats received 2.0 mg on the first three mornings and 10.0 mg on the fourth; two rats received 1.0 mg on the first three mornings and 5.0 mg on the fourth; two rats received 0.5 mg on the first three mornings and 2.5 mg on the fourth. The animals received no food on the afternoon of the third day, and on the fourth day they were chloroformed. The parts of the intestine named in this table were removed and separate determinations of the concentration of Chloromycetin in the walls and contents of each part were made.

TABLE 2

CONCENTRATION OF STREPTOMYCIN IN THE INTESTINES OF RATS*

Experimental Animal	Dose of Streptomycin	Region of Intestine	Auxanogram (*E. coli* Lb) Gut Wall a	Gut Wall b	Gut Wall c	Gut Contents a	Gut Contents b	Gut Contents c	Streptomycin Concentration (γ/g) Gut Wall	Gut Contents
1	22 mg	Jejunum	θ	θ	θ	?	?		< 5.0	?
		Ileum	θ	θ	θ	15.0	15.0		< 5.0	25.0
		Caecum	θ	θ		17.0	17.0	17.0	< 5.0	40.0
		Rectum	θ	θ	θ	15.0			< 5.0	25.0
2		Jejunum	θ	θ	θ				< 5.0	
		Ileum	–	–	–	17.0	17.0	17.0	5.0	40.0
		Caecum	θ	θ		16.0	16.0		< 5.0	33.0
		Rectum	θ	θ	θ	14.0			< 5.0	18.0
3	11 mg	Jejunum	θ	θ	θ	θ			< 5.0	< 5.0
		Ileum	θ	θ		16.0	16.0	16.0	< 5.0	33.0
		Caecum	θ	θ		15.5	15.0	16.0	< 5.0	28.0
		Rectum	θ	θ		14.0	14.0	13.5	< 5.0	16.0
4		Jejunum	θ	θ		θ			< 5.0	< 5.0
		Ileum	θ	θ	θ	13.0	13.5	13.0	< 5.0	12.0
		Caecum	θ	θ		14.5	14.0	14.5	< 5.0	21.0
		Rectum	θ	θ		–	–		< 5.0	5.0

* In this experiment two pairs of female rats, each rat weighing about 200 g, were injected at 0830 hrs. on each of 3 successive days (1–3/8/55). Each of pair 1 received subcutaneously 22 mg of streptomycin s.c.; each of pair 2 half as much dissolved in 0.5 ml of water. At 1100 hrs., 2.5 hrs. after the last injection, the animals were chloroformed. The parts of the intestine named in this table were removed and separate determinations of the concentration of streptomycin in the walls and contents of each part were made. Blood serum and liver were tested in the same way (auxanogram). The medium was Hottinger-Agar, pH 7.8; the test organisms were *Escherichia coli* Lb and its variant Lb Sr resistant to streptomycin.

established. In our laboratories we have not observed a stimulating effect on *E. coli* under culture conditions with Aureomycin at very low concentrations (fig. 1); some especially typical performances of the metabolic activity of *E. coli* are not affected by the presence of Aureomycin (Table 3). The growth effect, therefore, cannot be ascribed to an increase in the total number of microorganisms in a subsequent production of larger amounts of nutrients, which are important for the host animal. The possibility, however, is not remote that an increased number of decomposing microorganisms resulting from a more rapid multiplication might liberate absorbable essential nutrients which subsequently take an active part in the over-all growth rate of the host animal. It is known for example that decomposed bacterial cells especially those of certain strains of *E. coli* are used therapeutically in human medicine. Even though antibiotics do not either greatly increase or decrease the total bacterial count, they might perhaps produce certain changes in the numbers within one or several genera and species. Using streptomycin and folic acid in combination (50) a decrease in the number of coliform organisms and an increase in the number of lacto bacilli was observed. However, this finding cannot be substantiated using commonly accepted levels of the drug. Considerable temporary variations within component genera and species of the flora have already been observed even

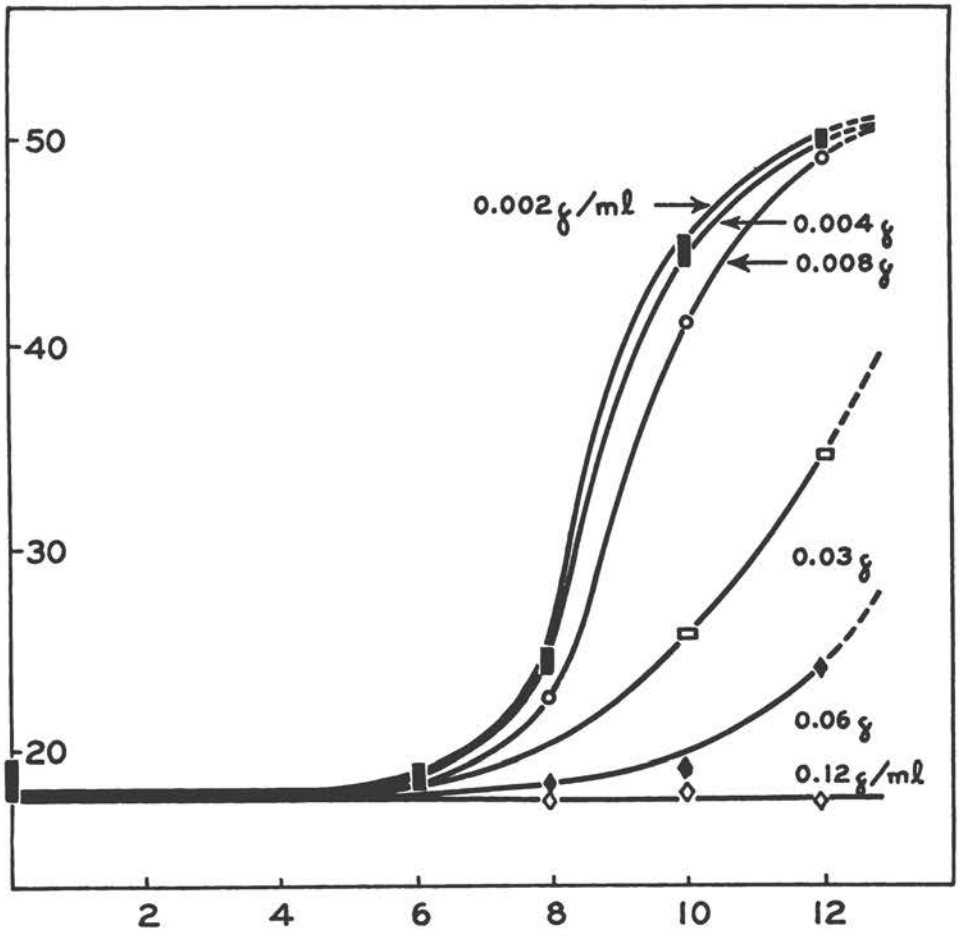


FIG. 1.—Growth of *E. coli* in the Presence of Aureomycin.*

* The X-axis numbers represent culture period in hours; those on the Y-axis a nephelometer scale. The curve for 0.002γ/ml was the same as that for the control culture without Aureomycin.

where no antibiotics were administered. Certain changes can also be produced experimentally simply by changing the diet(27) (28). The assumption that induced variations with antibiotics in the individual components of the intestinal flora, influence the growth effect can only be maintained by additional hypotheses. There would, for instance, have to exist within the flora an extremely small number of a particular bacterial species which is highly sensitive to Aureomycin (less so to other antibiotics) and highly detrimental to the well being of the host. Theoretically this could, of course, also be a hitherto unidentified mutant of the known intestinal population. In this case the antibiotic action could be related only to these particular and still unidentified toxin-producing flora components. Up to now such strains are unknown; there are, however, findings indicating that this hypothesis might not be entirely erroneous(61).

Furthermore there might exist known or unidentified components of the flora

TABLE 3

ACTION OF SUB-BACTERIOSTATIC CONCENTRATIONS OF AUREOMYCIN ON SEVERAL PHYSIOLOGICAL PERFORMANCES OF *E. coli*.*

Period of action (No. of transfers)	Concentration of Aureomycin in medium, γ/ml.	Glucose	Lactose	Citrate	KCN	Indole	Gelatin	Urea
10	0	+	+	−	−	+	−	−
	0.1	+	+	−	−	+	−	−
	0.05	+	+	−	−	+	−	−
	0.025	+	+	−	−	+	−	−
	0.013	+	+	−	−	+	−	−
	0.006	+	+	−	−	+	−	−
20	0	+	+	−	−	+	−	−
	0.1	+	+	−	−	+	−	−
	0.05	+	+	−	−	+	−	−
	0.025	+	+	−	−	+	−	−
	0.013	+	+	−	−	+	−	−
	0.006	+	+	−	−	+	−	−
40	0	+	+	−	−	+	−	−
	0.1	+	+	−	−	+	−	−
	0.05	+	+	−	−	+	−	−
	0.025	+	+	−	−	+	−	−
	0.013	+	+	−	−	+	−	−
	0.006	+	+	−	−	+	−	−
61	0	+	+	−	−	+	−	−
	0.1	+	+	−	−	+	−	−
	0.05	+	+	−	−	+	−	−
	0.025	+	+	−	−	+	−	−
	0.013	+	+	−	−	+	−	−
	0.006	+	+	−	−	+	−	−

* Sub-bacteriostatic concentrations of Aureomycin in growing cultures of *Escherichia coli* (Kvm. 1) had no influence on the fermentation performances examined; i.e., decomposition of glucose, lactose and citrate; KCN-resistance of respiratory system; production of indole; liquefaction of gelatin; decomposition of urea. In this experiment the medium was Hottinger-bouillon, pH 7.2; 1 drop of a 24-hour culture was innoculated every 24 hours.

which are of biological value for the host and which are favoured by small amounts of antibiotics. Romoser *et al.*(55) for instance, observed that *Aerobacter aerogenes* increases in number in the caeca of chicks when diets containing penicillin are fed. Chicks showed a slight increase in growth rate when fed large numbers of these organisms which were isolated from the caecal contents of 4 week old chicks which were grown on a ration containing penicillin. This effect with dietary micro-organisms was observed even when penicillin was omitted from the basal ration. Anderson *et al.*(2) fed an "atypical" strain of *E. coli* obtained from cultures under similar conditions. They also observed a slight growth effect. It is, however, of smaller magnitude than that obtained when penicillin is fed(40).

Numerous attempts have been made(43), (67), (37), (61), (2), (50), (33), (55), (58), (53), (19), (65) to measure directly the effect of antibiotic agents on the intestinal flora with reference to a "new distribution" of microorganisms, but

the results are so contradictory that they will not be discussed in detail. In regard to the methodical difficulties involved in such investigations and the great physiological variations in the composition of the intestinal flora, it must be concluded from available data that the influence of antibiotics on the total microflora cannot be very great. According to Jukes only the decrease of anaerobia can be reproduced. He fed experimental animals with toxin-producing Clostridia and large amounts of pure toxins produced by this genus of organisms. The organisms not only failed to inhibit growth, but the toxins even caused a slightly stimulatory effect. It is thus indicated that it is untenable to assume that a shift of the individual components of the microflora completely explain the mode of action of antibiotics in promoting growth.

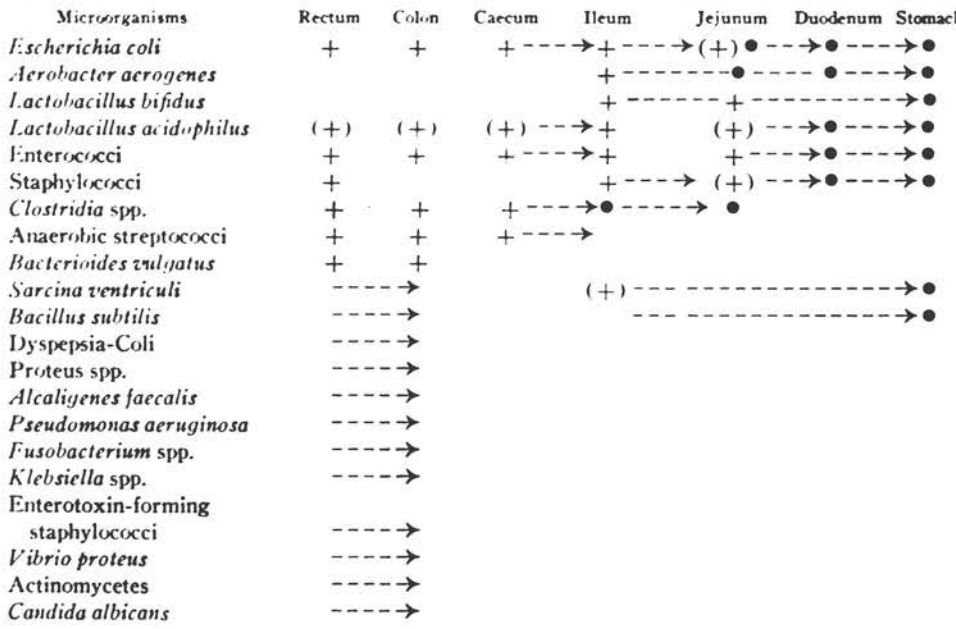
There may exist certain pathogenic microorganisms, not necessarily endogenous to the flora, but introduced because of unfavorable environmental conditions which produce a "sub-clinical" infection. This hypothesis was suggested as a result of experiments conducted with chicks reared in germfree environments. The growth rate of chicks reared under such conditions could not be improved by antibiotics in their rations(40), (41), (68). Baby pigs and chicks kept in entirely new quarters showed the same rate of growth as those kept in old quarters and fed an antibiotic supplement(12), (13), (14), (15), (16), (40). Since animals kept in sanitary quarters and those kept in old unclean ones both possess physiological intestinal flora, the effect observed cannot be ascribed to an antibiotic influence on this flora. The possibility exists, however, that certain pathogenic microorganisms absent from the intestinal tract which have been introduced into the flora from the external environment are inhibited by dietary antibiotics. Thus growth stimulation would be directly related to the so-called "disease level"; animals kept under ideal sanitary conditions would not show an antibiotic growth effect. This point of view is mainly held by Catron, based on a large number of experiments with pigs. This opinion is in accordance with our present knowledge of the importance of certain components of the intestinal flora.

Under normal conditions there are organisms in the intestinal flora which normally comprise a small part of the ecological sphere of the total flora, but which increase considerably if the physiological balance of the flora is disturbed. This explains the beneficial effect of Aurofac on enterotoxemia of lambs (Jordan) and on diarrhea of pigs.

Obviously the growth effect of diets containing antibiotics cannot be explained in terms of the *physiological* intestinal flora; on the other hand it appears justifiable to assume that there is a suppression of the exogenous pathogenic microorganisms which have entered the intestinal flora. In any case the biologically active antibiotic itself appears to be essential—especially since Dornbush *et al.* observed that inactivated Aureomycin is ineffective in promoting growth. Freerksen has suggested that it is not the physiological flora, but the pathogenic microorganisms "rising" to the upper sections of the intestine and the stomach which may play a more important part in describing the feeding effect of antibiotics. We know of such "rising" in human medicine, especially in pediatrics and under certain conditions of poor health such as pernicious anemia it is of pathognomonical importance. By this "rising" of certain bacterial species (Table 4) the normal function of the affected intestinal

TABLE 4

RISING OF MICROORGANISMS INTO UPPER REGIONS OF THE INTESTINE



sections will be disturbed thereby causing a highly disagreeable circulus vitiosus. This view is supported by the experiment of Dintzis and Hastings(17) who showed that the microbial decomposition of urea in the stomach of mice can be depressed by antibiotics. It may be assumed that microorganisms "rising" under "subclinical-pathological" conditions and thereby entering an environment which is unfavourable to them, can be inhibited by the administration of small concentrations of antibiotics. The inhibition of these organisms will contribute considerably to the improvement of the general status of health. This would also explain the growth effect of penicillin, which although after feeding is not found in the colon, is nevertheless recovered in significant concentrations in the stomach and small intestine(25). Aureomycin recovered from the stomach and small intestine is three times as high in concentration as that recovered from the colon. In the stomach and in the small intestine therefore a real antibacterial action is to be expected.

Obviously the rectal flora has a tendency to advance into the upper sections of the intestine. However, physiological factors (such as inhibitors, lysozyme, structure of the epithelium, pH-values, etc.) prohibit this advance or permit it only to a very limited degree. These endogenous "protective factors" are supported by exogenous ones, e.g. through antibacterial agents either taken in with the diet or supplied in another manner(26). Diets containing antibiotics support the process of self-purification of the upper sections of the intestine. Good health is accompanied by the absence of or at least an extreme scarcity of bacteria in the upper intestinal sections; illness or poor health is accompanied by a corresponding increase in the number of microorganisms in the upper intestinal sections. Even animals confined

to pens (for instance experimental animals) possess a greater number of organisms than animals of the same species in their natural environments (Table 5). The growth effect of antibiotic supplements is, therefore, achieved through antimicrobial action: (1) on the atypical microbial populations of the stomach and small intestine(23), (24), (25), (26); (2) on organisms in the rectal and colon flora which have been introduced under unsanitary conditions(9), (10), (11). This action, however, can only partially account for the growth promoting effect of antibiotics because not all antibiotics show a growth promoting effect. Therefore, nonspecific effects must be considered as a further factor. Theoretically these nonspecific effects may be produced through the antibiotic effect itself regardless of its effect on intestinal microorganisms or by known or unknown factors contained in the *fermentation products.*

Nonspecific Effects of Antibiotic Substances (independent of the antibacterial action).—(1) Aureomycin acts on most seeds of plants through developmental inhibition. The application of large doses prevents germination. Cuttings of chrysanthemums are inhibited in their development when exposed even for a short period to the influence of Aureomycin. A cytostatic effect can be observed in tissue culture. The levels of antibiotic required for this effect, however, are much higher than those which are used routinely for dietary purposes. Whereas the inhibitory action on cells is reproducible at any time, satisfactory reports of investigations showing a cell-stimulating action of antibiotics, especially at very low dosages, have not appeared. (2) The following pharmacological and physiological effects of Aureomycin have been described:

(*a*) liver protective effect;

(*b*) kidney protective effect (Baxter and Campe);

TABLE 5

BACTERIAL COLONIZATION OF VARIOUS PORTIONS OF THE INTESTINE OF THE RAT

Region of Intestine	Wild Rat	Experimental Rat	
		Aminopterin Intoxication	Normal Animal
Stomach	Predominately Gr. +, Cat. —, sporeless bacilli (Lactobacilli and cocci)	As in the wild rat, numerous additional yeasts	
Duodenum	Few lactobacilli	Abundant lactobacilli, additional yeasts	
Jejunum	Lactobacilli	Lactobacilli	
Ileum	Very small colonization of lactobacilli (several tests sterile)	Lactobacilli	
Caecum	As before, plus colitype bacilli	As in the wild rat, plus Proteus	As in wild rat
Colon	As in caecum	As in caecum, plus Proteus	As in caecum
Rectum	As in caecum	As in caecum, plus Proteus	As in caecum

(*c*) inhibition of frequency and amplitude of the frog's heart (Nakatsuka(51));
(*d*) influence on blood coagulation (Green(31), however denied by Parker(54));
(*e*) anti-irradiation effect (Furth);
(*f*) prolongation of survival time after poisoning (emetin) (Guggenheim(32));
(*g*) improvement of sugar resorption (Catron);
(*h*) increased accumulation of Ca in growing bones (Migikovsky(48));
(*i*) increased storage of vitamin A in the liver (Burgess *et al.*(6), Squibb *et al.*(63));
(*j*) inhibition of oxidative phosphorylation in the rat liver (Van Meter). Waisman and Cravioto(67) state that the aminopterin damage can be eliminated with Aureomycin, but not with Terramycin, Chloromycetin, streptomycin or penicillin; vitamin B_{12} alone is also not able to do so. However, these effects, all of which are not sufficiently proved, generally require higher levels of Aureomycin than are normally used for dietary purposes. The "nonspecific" actions of Aureomycin thus far elucidated cannot, therefore, significantly explain its growth promoting property.

Some of the reports regarding the action of dietary antibiotics on the thyroid should be discussed briefly (Menge and Connor(46); Calesnik, Harris and Jones(7); Mellen, Waller, and Ershoff(45)). These authors observed an inhibition of the thyroid by chlortetracycline and penicillin which however could not be confirmed by Libby and Meites(44) nor by Greul, Gardner and Taylor. Menge and Connor(46) associate the effect with a stimulation of thyrotropin secretion via the anterior pituitary lobe. Unfortunately since each experimental group of Calesnik, Harris and Jones(7) were comprised of only four animals, their findings cannot be considered significant. Since there are considerable individual variations in the adrenal cortex as well as in the thyroid gland only large numbers of experimental animals and very precise methods can be relied upon. Furthermore, morphological changes in endocrine organs only indicate that regulatory processes are in operation; these changes by no means offer significant proof of an active "participation" of these organs in the actions of Aureomycin or other antibiotics. Therefore, at the present time there is no reason to assume the existence of a relation between antibiotics and the adrenal cortex, as suggested by Schole(59). The extensive clinical and experimental knowledge of antibiotic substances neither shows their effect on the thyroid gland (i.e. hypertrophy or inactivation) nor an influence on the suprarenal gland. Though the growth effect of antibiotics, which must be imagined as being a complex one, possibly touches upon the endocrine apparatus, a true causal interrelation cannot be detected. It is more likely that the biologically favourable effects of antibiotic agents can also be expressed through regulations within the endocrine system. However, such regulations are not the "cause" of the growth effect. (It may be noted that according to Saxena *et al.*(57) penicillin favours the production of subcutaneous fat. Cortisone shows the same effect in rabbits (Freerksen), though only at high levels. Given at nutritional feeding levels, penicillin does not stimulate cortisone production; therefore, the effect of penicillin may not be considered to be an indirect cortisone effect. So the experimental evidence available at the present time regarding an antibiotic effect via the endocrine system is not sufficient to ex-

plain developmental stimulation. The nonspecific (non-antibacterial) part of the growth effect of antibiotic feeding supplements is not to be sought in the antibiotic itself, but in still unidentified factors contained in crude antibiotic fermentation residues.

Nonspecific Actions of "Crude" Fermentation Products.—Barnard and Appel(3) stated that the crude fermentation product as opposed to the crystalline antibiotic and pure B_{12} has therapeutic effects on certain haematoblastoses in man. (According to Schwartz(60) the amount of citrovorum-factor in the caecum and liver is increased by dietary Aureomycin, which would explain the "detoxifying" effect of Aureomycin on aminopterin.) Hausmann, Ludwig and Mulli(34) demonstrated the importance of the intestinal flora in the pathogenesis of megaloblastic anaemia. Therefore, a number of findings exist, which would suggest that there are interrelationships between the action of dietary antibiotics or crude fermentation products and the folic acid system.

In our laboratories we have studied the protective effect, in animals receiving aminopterin, of Aureomycin, Terramycin, Aureomycin crude fermentation products, rhepuron (a fraction derived from plant material which has no antibacterial action) and an antibacterially negative fraction G ‡ derived from Aureomycin fermentation products. None of the agents studied had a protective effect (whereby with respect to Aureomycin we substantiated the results of Sauberlich). According to these results neither Aureomycin nor Terramycin alone nor Aureomycin fermentation products are antagonistic to aminopterin (Fig. 2).

Barnard and Freeman(4) prepared a nonantibacterial fraction SGE from streptomycin-mash which they presume to possess the aminopterin protective effect. These authors suggest that this effect may be due to a precursor of a hematopoietical hormone of the anterior pituitary lobe, for EPH shows biologically quite similar effects. If they are correct the biological function of the intestinal flora would then be to provide for preliminary stages of hormone synthesis, especially those of the pituitary gland. Although this cannot yet be verified it is not beyond modern possibilities of conception, especially since Takada *et al.*(65) showed the formation of some kind of corticotropin through *Ps. fluorescens,* and since extrinsic ACTH-sources have become known (Nelson; ACTH from fish meal, mashes, etc.). This interpretation would explain why crude fermentation products are more effective than pure antibiotics.

Although the interpretation of the hormonal nature of their fraction given by Barnard and Freeman(4) is not quite convincing, we agree with them to the extent that unidentified factors may be present within the fermentation products, which at least partially produce the growth effect. It has not yet been established whether there exists relationship between these unidentified factors and the folic acid system.

The above shows that our present knowledge of the importance of "nonspecific" factors for the growth effect is still very unsatisfactory. It can be seen, however, from previous investigations with added vitamins, especially of the B-group, and minerals, trace elements, AP-factor, etc., that these nonspecific factors are of some

‡ Substances from Dr. Mulli, Hamburg.

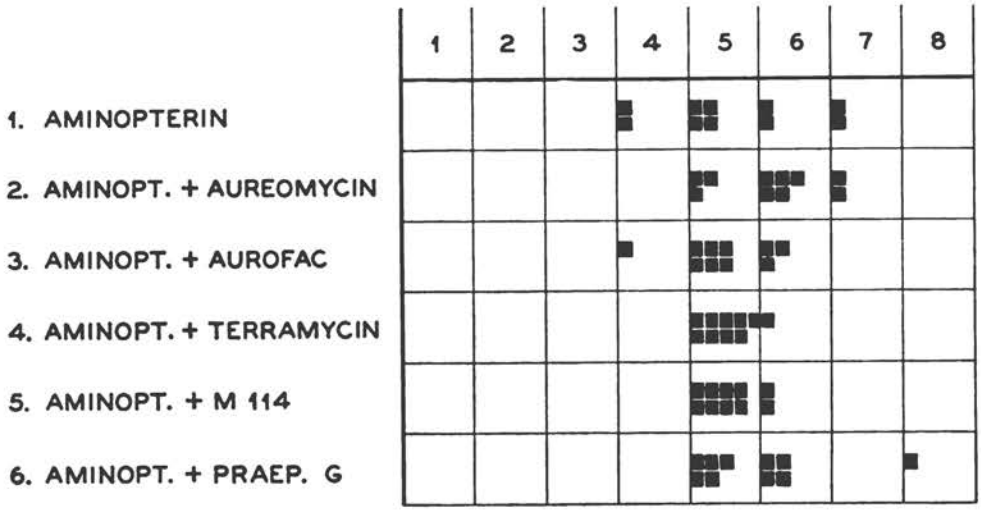


FIG. 2.—Aminopterin-intoxication in the Rat.

importance. This is also shown by more recent studies which indicate that equally beneficial effects can be obtained with "nonspecific" substances as with antibacterial agents.

We are thus facing a problem which is highly complex. Reduction of the intestinal flora, promotion of stimulation organisms which are important for the host animal, formation and assimilation of vitamins, elimination of "sub-pathogenic" and pathogenic microbes, involvement of the endocrine system—all of these items are important in the formulation of an hypothesis. None of them, it is true, have as yet been definitely established. However, when trying to survey the material accumulated in numerous widespread investigations, and taking into consideration our own studies we arrive at the following conclusions:

1. In crude fermentation products the antibiotic with its antimicrobial effect is essential for the growth effect. It is achieved mainly by antimicrobial action:

a) on organisms in the rectal and colon flora which have entered the system under unsanitary conditions (Catron);

b) on the nonphysiological bacterial populations of the stomach and small intestine (Freerksen).

2. With regard to their being kept and fed in enclosures our livestock are reared under environmental conditions (especially from the standpoints of nutrition and exposure to germs) which are not optimal for their development. The animals are not really sick, nor are they in good health. Under these conditions the microbes of the intestinal tract attempt to invade the upper intestine including the stomach. It is possible to inhibit these microbes by low concentrations of antibiotics found normally in nature, especially in plants (Freerksen). Thus traces of antibiotics which are vital substances are sufficiently available in normal diets; but like vitamins

they must be added to the diet if the animals are kept in "suboptimal environments." Therefore, it is a mistake to say that these additives are "unnatural." However, they must not be used to excess, for then they will cause damage, unless the higher level is necessary for medical reasons.

3. By supporting the self-purification process antibiotic supplementation leads to a restoration of the normal functions of the stomach and the upper intestine, normalizing perhaps, as a consequence, certain metabolic processes. These metabolic readjustments therefore, are not a direct effect of the antibiotic or the crude fermentation product. This action of antibiotics is not, of course, restricted to growing animals, but in growing animals the effect is more noticeable, first, because improvement in the general condition is shown by the easily measurable gain in weight, and second, because very young animals are more susceptible to unfavourable environmental conditions.

4. The "nonspecific" side of the growth effect of fermentation products is probably not due to "nonspecific" actions of the antibiotic itself, but rather to other factors of the fermentation products, which up to now have not been identified. They may possibly represent metabolic by-products of microorganisms or may be otherwise produced during fermentation.

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MODE OF ACTION OF ANTIBIOTICS IN POULTRY

Since antibiotics which differ widely in their chemical and physical properties stimulate growth of chicks and poults, it is not likely that their effect on metabolism can be a direct one. The only known property which these substances have in common is their antibacterial potency. It is to be expected then, that their action might be explained through their effects on bacteria. That the site of this action is primarily restricted to the intestinal tract, is further indicated by a number of observations:

(1) Antibiotics are of little or no effect in promoting growth of chicks reared in a germfree environment(56), (57).

(2) Aseptic injection of antibiotics into the chick embryo, a sterile animal, is not effective(48).

(3) Antibiotics exert relatively little or no growth promoting effects in chicks reared in clean or new environments as compared to their growth stimulating properties where chicks have been reared in used quarters(11), (19), (20), (25), (42), (71).

(4) For the most part, injection or implantation of antibiotics in the growing chick or poult has had no consistent effect on their rate of growth(13), (116). Where improved growth has been observed, a corresponding change in the numbers and types of bacteria in the intestinal tract has also been noted in some instances indicating that significant amounts of the injected antibiotic may have been excreted into the intestinal tract(32).

(5) Administration of enzymatically hydrolized penicillin is relatively ineffective(49), (117). Where autoclaved penicillin has been effective, it is possible that the antibacterial properties of the antibiotic were not completely destroyed by the treatment(33).

(6) Chloromycetin, which is rapidly absorbed from the intestinal tract, is less effective in promoting growth in poultry(14). On the other hand, bacitracin and streptomycin, which are not readily absorbed, have growth promoting properties.

(7) Although their effects are not consistent, the oral administration of antibiotics to chicks and poults appears to influence the number of various types of microorganisms found in the cecal and intestinal contents.

Effect of Bacterial Environment.—The conclusion that antibiotics exert their growth promoting effects in young animals through their action on bacteria is largely based on the lack of growth response when animals are maintained under germfree or so-called "new" environments. Luckey(56) observed no growth response from streptomycin, Chloromycetin or bacitracin in chicks or from procaine penicillin in poults maintained in sterile environments. Oxytetracycline and procaine penicillin gave variable responses in the chicks but the average of the data indicated no positive reaction.

More recently, slightly more rapid growth has been observed in certain trials(57) with birds fed oxytetracycline in germfree environments. However, the small numbers used and variability obtained make it difficult to interpret the results. Similarly, chlortetracycline, penicillin and streptomycin have been aseptically injected into chick embryos, but no growth response was obtained(48). Moreover, growth of chicks reared under germfree conditions was reported to be more rapid than that of controls reared in normal environments(77). This suggests that bacteria in general exert a growth depressing effect upon the animal.

Other investigators(11), (19), (20), (25), (42), (71) have found that orally administered antibiotics exert much less effect in promoting growth when chicks were reared in "used" quarters, while King and White(51) found no difference. Waibel *et al.*(111), also found that after using penicillin and chlortetracycline in chick rations with growth responses for approximately 21 months, growth responses were no longer obtained with the use of these antibiotics. The authors suggest that the harmful bacteria may have been largely eliminated through the long continued use of these antibiotics. Berg *et al.*(8), found that the growth promotion of a supplement containing chlortetracycline was interrupted if the supplement was removed from the ration at four and one-half weeks of age. This work suggested that the presence of the antibiotic in the feed was necessary for continued acceleration of chick growth.

Libby and Schaible(53) have summarized the results obtained from several comparisons over a four-year period during which antibiotics were fed. These workers obtained increased growth but decreased response to antibiotics with time. They interpret this to mean that the "germ load" or "disease potential" in the environment has been steadily reduced rather than that antibiotic resistant strains of organisms were becoming established, thus rendering antibiotics ineffective.

From these observations, it appears that undesirable bacterial types become prevalent in quarters used continuously for rearing poultry, and that the presence of these organisms greatly influence the growth response of chicks to antibiotics.

The bacterial infection which is established in used environments has been studied in detail by Coates(21). Chicks reared in a small isolation unit showed no response to penicillin, but when fed the contents of the intestinal tract of chicks reared under normal conditions, their growth was markedly depressed. This depression could be largely overcome by the addition of penicillin. The feeding of the intestinal contents from other chicks reared in isolation units or autoclaved material from normal chicks did not depress the growth. The inclusion of a few infected chicks among noninfected chicks also caused the noninfected chicks to respond to antibiotics. Sieburth *et al.*(86), also demonstrated that the feeding of incubated feces to poults

resulted in a growth depression. Autoclaving of the culture of feces or adding procaine penicillin overcame the growth retarding effect. These results show clearly the importance of sub-clinical infection as one of the principal agents in determining the response to antibiotics. No doubt, differences in magnitude of responses to antibiotics at various laboratories can be explained, in part at least, in the sub-clinical "disease level" at each location.

Hill and Kelly(43) found that 10 milligrams of chlortetracycline did not stimulate growth of chicks fed a practical type ration in new quarters but that a higher level, 100 milligrams per pound, did.

Several reports have appeared which demonstrate the value of antibiotic therapy to poultry infected with specific recognized diseases. For this purpose, of course, higher levels of antibiotic administration are required, and injection of the antibiotic is usually more effective. It is presumed that the antibiotic under these circumstances acts on bacterial infections in the tissues of the bird. This discussion of mode of action of antibiotics in poultry has been confined to their use at low levels in feeds as growth stimulants.

Effect of Injected Antibiotics.—Injection of antibiotics in chicks and poults might not be expected to be as effective if their action is restricted entirely to the bacteria of the intestinal tract. Whitehill *et al.*(116), failed to obtain growth responses from intravenous injection of penicillin or chlortetracycline while these antibiotics administered orally produced normal growth responses. On the other hand, Elam *et al.*(33), obtained growth responses from the injection of penicillin in chicks with no detectable effect on the fecal microflora. Simultaneously, they found that autoclaved penicillin also increased the rate of growth of chicks, yet had no detectable effect on the fecal microflora. Bacitracin also was effective when administered either orally or parenterally with no observable influence on the fecal microflora.

The subcutaneous implantation of a bacitracin pellet in the neck of the newly hatched chick had no effect on their growth or feed efficiency to six weeks of age. In other studies, however, it was observed(32) that the administration of penicillin either orally or intravenously resulted in a marked increase in the number of penicillin resistant microorganisms in the intestinal tract. DL-penicillamine was found to stimulate growth of chicks slightly when injected but not when fed(36). Jukes (49) and Williams *et al.*(117) obtained only small growth responses in chicks with an enzymatic hydrolyzate of penicillin, which was only 10 per cent of the response obtained with the unhydrolyzed product. In studies with poults, Reid *et al.*(76) have recently reported that injected penicillin and bacitracin failed to influence either the growth rate or the fecal microflora but that the parenteral administration of chlortetracycline improved growth and also produced a corresponding change in the fecal microflora. In view of these reports, it would appear that the growth responses due to the injected antibiotics may well be explained on the basis of their excretion into the gastro-intestinal tract of the animal. Since it is difficult to determine quantitative changes in fecal microflora, the failure to observe such an effect does not exclude the possibility that the antibiotic may have been excreted into the intestinal tract in significant quantities.

In comparative studies, Chloromycetin (chloramphenicol) did not promote the growth of chicks as well as certain other antibiotics(14). This is explained by

Yacowitz and Bird(119) as being due to the very low concentrations of Chloromycetin found in the small intestine and cecum due to its rapid absorption as compared to the other antibiotics. Since both bacitracin and streptomycin are effective in promoting growth in chicks and at the same time are considered to be retained almost entirely in the intestinal tract when added to the diet of chicks, it would indicate that the action of antibiotics in promoting growth of chicks is primarily located in the intestinal tract.

Effect on Bacterial Flora.—Much more remains obscure about the specific effects of various antibiotics on the types and numbers of bacteria in the intestinal and cecal contents. Sieburth *et al.*(87) have indicated that variations in apparent numbers and types of bacteria in the intestinal tract may result from the use of different basal diets, bacterial procedures, age of birds studied, and types and levels of antibiotics. Their results even showed marked differences in the cecal and fecal microflora from day to day. Stokstad *et al.*(107) obtained greater growth responses from chlortetracycline when chicks were fed sucrose than when they were fed starch, glucose or lactose.

Moore *et al.*(69) observed that the feeding of streptomycin markedly reduced the numbers of coliforms (*Escherichia coli*) and enterococci in the cecal contents of the chicks. These workers also observed a possible reduction in the number of lactobacilli. March and Biely(61) reported a reduction in the number of coliforms and lactobacilli in the feces of chicks fed high levels of chlortetracycline. Similarly, Sieburth *et al.*(88) found a general decrease in the numbers of coliforms and enterococci. On the other hand, Anderson *et al.*(2) reported increases in the coliforms and lactobacilli in the cecal contents of chicks receiving penicillin or chlortetracycline.

Similarly, Anderson *et al.*(3) noted increases in the lactobacilli counts of the cecal contents of turkey poults, accompanied by a reduction in the pH when oxytetracycline was fed. Cook and associates(28) also observed an increase in the coliforms in the intestinal tracts but not in the ceca of turkey poults. These workers, however, noted a decrease in the lactobacilli in the intestinal tract.

Romoser *et al.*(79) found an increase in the numbers of *Aerobacter aerogenes* in the ceca of chicks fed penicillin. The coliform count also was increased by feeding lactose alone or in combination with penicillin. A decrease was also observed in the numbers of lactobacilli. Increases in the numbers of coliform bacteria in the intestinal tract of chicks were found by Rhodes *et al.*(78) when chicks were fed chlortetracycline and the numbers of lactobacilli were reduced. Either bacitracin or penicillin was more effective in reducing the number of lactobacilli than was chlortetracycline.

Eisenstark and Dragsdorf(31) exposed some 200 intestinal isolates as well as many stock cultures to cecal and intestinal filtrates from birds fed antibiotics. These workers observed stimulation of bacteria accompanied by numerous enlarged and filamentous cells; they suggest that the stimulation of these bacterial cells may be related to the growth stimulation which results in animals from feeding antibiotics in low concentrations.

Kratzer *et al.*(52) reported a marked increase in the number of yeasts in the feces of poults fed streptomycin, whereas, Williams *et al.*(118) found little or no

change. However, when the yeasts were isolated and fed to other poults and chicks, there was no consistent increase in growth(52). In another study by Morimoto *et al.*(70), the counts of *yeast imperfecti* and lactobacilli in the intestinal tract were actually diminished by feeding oxytetracycline to chicks.

Effect of Feeding Bacterial Cultures.—Since it was observed by Romoser *et al.* (79), that the numbers of *Escherichia coli* and *Aerobacter aerogenes* present in the ceca of chicks were increased when penicillin was fed, pure cultures of these organisms were grown and fed to chicks. Little or no difference in chick growth was obtained in the absence of the antibiotics. However, when viable cultures of these organisms were fed in combination with penicillin, the growth of chicks was increased significantly over that obtained with the feeding of the antibiotic alone(80). Similarly, Anderson *et al.*(4), (5) fed cultures of *E. coli* to chicks and poults and obtained growth responses. A culture of micrococci depressed growth in the absence of penicillin but this inhibition was overcome when dietary penicillin was fed. Feeding of a mixed coliform culture to poults improved the weight of poults when the diet contained penicillin. A culture of atypical *E. coli* caused significant growth improvement in the weight of female poults in the absence of dietary penicillin and appeared to enhance slightly the activity of penicillin. However, neither the killed organisms nor the filtrate from this culture influenced poult weight. Cook *et al.*(29), on the other hand, were able to change the numbers of intestinal coliforms by feeding massive doses of *E. coli* to poults without improving the growth rate of poults fed diets with or without penicillin.

Feeding of penicillin or tetracycline to turkeys was found to reduce the numbers of *Clostridia perfringens*(89), suggesting the possibility that the growth promotion of antibiotics might be due to the prevention of enterotoxemia. Subsequently, Elam *et al.*(34) fed fecal Clostridia to chicks and observed a depression in growth which was overcome by feeding penicillin. This was done with chicks reared in clean quarters where the normal Clostridia population was low per gram of feces. Under these conditions, penicillin failed to stimulate growth before Clostridia cultures were fed. Furthermore, the feeding of Clostridia cultures to birds reared in old quarters where fecal Clostridia counts were already high, failed to depress growth. Penicillin produced a growth stimulatory effect only in those cases where a decrease in the Clostridia count occurred in the feces. These results suggest that antibiotics are able, under certain conditions, to stimulate growth due to reduction in the number of Clostridia in the microflora. An earlier report(105) on the other hand, revealed only slight changes in the concentration of *Clostridia perfringens* during a six-week period from feeding chlortetracycline, while the inclusion of penicillin actually resulted in a sharp rise by the fourth week. These workers did not feel that this organism has a significant role in the stimulation of chick growth by the inclusion of antibiotics in the feed.

Jukes and Williams(50) reported results of chick studies where high levels of live cells of hemolytic toxin-producing Clostridia were fed with no impairment in growth of chicks not receiving antibiotics. Cell-free toxins obtained from hemolytic Clostridia also failed to depress growth of unsupplemented chicks. Thus, it appears that the observed decrease in the number of toxin-producing Clostridia does not account for the explanation of antibiotic growth response in chicks.

Sieburth, and others(87), made some interesting observations in the intestinal tracts of chicks fed antibiotics. These workers found that the cecal and fecal microflora did not necessarily reflect the changes in the anterior segments of the intestinal tract. All types of microflora studied were consistently decreased in number in the small intestine of chicks fed a diet containing either penicillin or chlortetracycline. The rate of respiration (O_2-uptake and CO_2 production) was also reduced. The odor of the intestinal contents and feces was generally much milder when antibiotics were fed. The mesentery blood vessels along the small intestines were usually more prominent and dilated in those birds receiving the antibiotic supplemented diet. These workers suggest that a greater utilization of nutrients from the feed by the host animal may be involved in the growth promoting effects of antibiotics.

Possible Modes of Action.—Although there is good evidence to support the view that antibiotics exert their growth stimulatory action on young animals through their effects on the intestinal microflora, the exact mode of action is not clearly understood. In general, however, the following possibilities have received considerable support: (1) antibiotics may increase the bacterial synthesis of essential or growth stimulatory factors, including unidentified nutrients; (2) antibiotics may inhibit certain bacteria which compete with the host animal for nutrients; (3) antibiotics may inhibit bacteria which are in some other way harmful to the host animal, such as by the production of toxic substances, through damaging the intestinal tissues, or by establishing sub-clinical infection. Good evidence for the latter has been cited above. The first two mentioned possibilities are discussed below.

Sparing Effects on Vitamins.—It is well established that under certain conditions the oral administration of antibiotics and certain other antibacterial compounds may reduce the dietary requirement for certain B-complex vitamins. One way to demonstrate this effect of antibiotics is by using a diet deficient in an essential nutrient and show that the growth increments from the addition of antibiotics to the deficient diet is greater than that which is obtained with a complete diet. Another way is to demonstrate increased liver storage or blood levels of the vitamin in animals fed the same dietary levels.

It is somewhat difficult, however, to differentiate between an effect which might be the result of improved bacterial synthesis or better absorption on the one hand and a reduced utilization and/or destruction of a limiting nutrient on the other. In general, a reduction of fastidious organisms such as lactobacilli would be expected to reduce the loss of dietary nutrients to these organisms. Conversely, one would expect an increase in those non-fastidious types, such as coliforms, to spare the dietary requirement of the host for such nutrients through possible synthesis.

A sparing effect of orally administered antibiotics on the dietary requirement for unidentified growth factors is indicated from the results of Groschke(39), Combs *et al.*(26), Jones and Combs(47), Heuser and Norris(41), Scott and Jenson(84), Branion and Hill(15), and Matterson and Singsen(63). These workers obtained greater responses from antibiotic supplementation of all plant-protein rations than of rations containing animal proteins. The animal protein supplements used are considered to contain an unidentified growth factor, or factors. Slinger *et al.*(92), found that the addition of a grass juice concentrate to the ration of poults step-wise reduced the response to the administration of procaine penicillin. This was inter-

preted to suggest that penicillin is stimulatory to the bacterial synthesis of an unidentified factor present in the grass juice concentrate. Until all of the nutrient essentials become available for addition to feeds, it will be difficult to rule out completely the possibility that part of the effect of antibiotics may be due to an increased production of certain critical nutrient essentials, yet to be identified, by microorganisms in the intestinal tract.

Jukes and Williams(50) summarized in tabular form the effects of antibiotics on the B-vitamin requirements of chicks. A similar table (Table 1) is included.

TABLE 1

EFFECTS OF ANTIBIOTICS ON THE B-VITAMIN REQUIREMENTS OF CHICKS

Investigator	Vitamin studied	Antibiotic used	Sparing effect
Common *et al.* (27)	Riboflavin	Chlortetracycline	*
Biely & March (9)	Riboflavin	Chlortetracycline	Yes
	Niacin	Chlortetracycline	Yes
	Folacin	Chlortetracycline	Yes
Coates *et al.* (23)	Thiamin	Penicillin	No
	Riboflavin	Penicillin	No
	Pyridoxine	Penicillin	No
	Pantothenic Acid	Penicillin	No
	Biotin	Penicillin	Yes
	Folacin	Penicillin	Yes
	Niacin	Penicillin	Deficiency increased
Nelson & Scott (72)	Niacin	Chlortetracycline	No
Waibel *et al.* (112)	Pyridoxine	Chlortetracycline	No
	Thiamin	Chlortetracycline	Yes
	Thiamin	Penicillin	Yes
Morimoto *et al.* (70)	Riboflavin	Oxytetracycline	†
	Vitamin B_{12}	Oxytetracycline	†
Davis & Briggs (30)	Vitamin B_{12}	Penicillin	No
	Vitamin B_{12}	Chlortetracycline	No
	Vitamin B_{12}	Oxytetracycline	No
Stokstad & Jukes (108)	Vitamin B_{12}	Chlortetracycline	Yes
Jukes & Williams (50)	Folacin	Chlortetracycline	No
	Pantothenic Acid	Chlortetracycline	No
	Niacin	Chlortetracycline	No
	Pyridoxine	Chlortetracycline	No
	Riboflavin	Chlortetracycline	Yes
Monson *et al.* (68)	Folacin	Penicillin	†
Coates *et al.* (22)	Vitamin A	Penicillin	†
Burgess *et al.* (16)	Vitamin A	Penicillin	*
Almquist & Maurer (1)	Vitamin A	Chlortetracycline and penicillin	†
Ross & Yacowitz (81)	Vitamin D	Penicillin	No

* Blood level of nutrient increased when antibiotic was fed.
† Liver content of vitamin was increased.

Studies with vitamin B_{12} requirements(108) showed definite sparing effects of chlortetracycline in five out of eight trials. The antibiotic response was greatest at the sub-optimal levels of vitamin B_{12}. Even in those experiments where no vitamin B_{12} sparing action was evident as measured by growth, the mortality of chicks fed the deficient rations was reduced by the presence of the antibiotic in the ration. However, Davis and Briggs(30) observed no sparing action of Aureomycin, streptomycin, or penicillin on the vitamin B_{12} requirement of the chicks.

Slinger and Pepper(93) also reported a sparing effect of both penicillin and oxytetracycline on the requirement of the poult for biotin. Penicillin also reduced the poults' requirement for pantothenic acid(93) and riboflavin(46). Slinger *et al.* (94) also found that the niacin requirement of poults from hatching to four weeks of age for optimum growth and the prevention of perosis was decidedly lower when penicillin was included in the ration.

Morimoto *et al.*(70) found higher riboflavin and vitamin B_{12} contents of livers of chicks fed oxytetracycline supplemented rations. An increase in liver content may be a better criteria of measurement of sparing effects than are differences in growth rate.

Coates *et al.*(22) reported that penicillin feeding to chicks improved the liver storage of vitamin A as well as increased the efficiency of conversion of beta carotene to vitamin A in the intestinal wall(21).

Almquist and Maurer(1) also found the addition of a mixture of chlortetracycline and penicillin increased the liver vitamin A level. Since conversion of carotene to vitamin A occurred in the intestinal wall, it was suggested that the improved conversion, or absorption of carotene, or both may have resulted from the maintenance of a healthier intestinal condition. Burgess *et al.*(16), found that penicillin fed chicks had 25 to 40 per cent higher levels of vitamin A in the liver and 50 to 100 per cent increased carotenoid levels of the serum. Ross and Yacowitz(81) observed no difference in the vitamin D requirement of chicks for growth but did find increased calcification of the bones of chicks fed low levels of vitamin D when procaine penicillin was fed. No differences were observed at higher levels of vitamin D.

Monson *et al.*(68) have presented good evidence to indicate the sparing effect of antibiotics on the folacin requirement of chicks fed synthetic diets. The increased growth on folacin-low diets resulting from the addition of antibiotics was accompanied by an appearance of coliform organisms in the small intestine which produced increased amounts of folacin. This change was apparent in the ileum after only two days in four-week-old chicks. It was also observed that the increased folacin production was correlated with increased liver folacin content, although no change in the concentration of intestinal folacin occurred.

A recent report of Luckey *et al.*(58) revealed that chicks reared in a germfree environment and fed diets low in certain B-vitamins excreted appreciable quantities of these vitamins in their feces. The concentration of thiamin, for instance, in the rectal contents was about twenty times that found in the liver. The presence of these vitamins in the excreta cannot be attributed to microbial synthesis in germfree birds. It may well be that the vitamin content of the feces of chicks reared in usual environments may not be as largely influenced by the presence of bacteria in the intestinal tract as one might expect. This observation raises serious questions as to

why germfree birds excrete such relatively high levels of vitamins under conditions of dietary deficiencies.

In addition to growth studies with chicks and poults, antibiotics have been used in breeding rations to determine their effect on hatchability. In many instances, rations containing deficiencies, primarily of vitamin B_{12} or unidentified hatchability factors, have been used. Mariakulandai *et al.*(62) found that the addition of oxytetracycline to the hen ration improved the hatchability of fertile eggs when the ration contained a low level of vitamin B_{12}. The improvement obtained from the addition of the antibiotic in the absence of vitamin B_{12}, however, was not as great as that obtained in the presence of vitamin B_{12}. The feeding of penicillin to laying hens by Waibel *et al.*(113) was found to increase the biotin and folacin content of the eggs by 36 and 30 per cent, respectively. This increase presumably reflected changes in the intestinal synthesis of these vitamins.

The feeding of chlortetracycline to hens was observed by Squibb *et al.*(106) to increase the serum carotenoid level but had no effect on the vitamin A, riboflavin, or ascorbic acid levels. Carlson *et al.*(17) and Elam *et al.*(35) also obtained improvements in hatchability of fertile eggs from feeding antibiotics. Since the rations used by Carlson *et al.* were considered to be deficient in an unidentified hatchability factor, the improvement in hatchability was attributed to a sparing effect of penicillin and streptomycin on this limiting nutrient. Egg production and hatchability were also improved by prolonged feeding of antibiotics to hens fed a vitamin B_{12} deficient ration(35), but further improvement was observed when both vitamin B_{12} and the antibiotic were added. The parenteral administration of penicillin or bacitracin in water failed to increase the hatchability of eggs or decrease the number of embryos exhibiting typical vitamin B_{12} deficiencies. However, the parenteral administration of penicillin-in-oil or inactivated penicillin-in-water increased egg production and hatchability. The explanation for the latter observation is difficult to find, unless the autoclaved treatment did not completely destroy the antibacterial properties of the penicillin. Other workers including Lillie and Bird(54), Slinger *et al.*(95), Peterson *et al.*(75), Halick and Couch(40), and Bentley and Hershberger(7) have failed to obtain improvement in hatchability by feeding antibiotics. Halick and Couch(40) found that hens fed purified diets designed to be low in unidentified factors required for hatchability not only failed to respond to chlortetracycline or penicillin in the feed but these antibiotics apparently assisted in depleting the birds of vitamin B_{12} and possible unidentified factors. On the other hand, the feeding of antibiotics to breeding chickens or turkeys has been reported by Bentley and Hershberger(7), Slinger *et al.*(96), (97), and Carlson *et al.*(18) to influence the subsequent growth of the progeny. For example, Slinger *et al.*(96) reported that progeny of hens fed penicillin grew more rapidly than did those of hens not receiving the antibiotic when the maternal diet contained no animal protein supplement. They postulated that the antibiotic may have exerted by synthesis in the intestinal tract a sparing effect on nutrients normally supplied by animal protein supplements. This suggested that the antibiotic feeding improved the carry-over of some factor, presumably vitamin B_{12}, through the egg to the poult. Bentley and Hershberger(7) also reported that bacitracin in the maternal diet appeared to exert a significant carry-over effect on the growth of progeny when the antibiotic was

added to a vitamin B_{12} deficient maternal ration and the chicks were fed a vitamin B_{12} deficient ration. Little or no effect on the growth of progeny was observed, however, when antibiotics were added to the vitamin B_{12} supplemented hen ration.

Sizemore and others(90) have reported that when a vitamin B_{12} antibiotic feed supplement was fed to chicks during the growing period, the subsequent performance of the pullets was affected. When they were fed a vitamin B_{12} deficient ration, the hatchability of fertile eggs, produced by the pullets reared on the rations containing the antibiotic, was greater than that for the control groups. In some cases, the hatchability differences persisted throughout the second laying year(91). The addition of crystalline chlortetracycline to the breeder ration, however, further increased hatchability during the second year of production in three out of four comparisons. A definite relationship between the growing and breeding rations and embryonic mortality during the latter two weeks of incubation was noted. In every case, the eggs of hens, whose growing ration contained an antibiotic, showed a lower embryonic mortality than did those of hens whose growing diet contained no antibiotic. The addition of crystalline chlortetracycline to the breeder diet further decreased the early embryonic mortality.

These reports support, in general, the concept that increased amounts of certain critical nutrients may be transmitted from the hen through the egg to the chick when antibiotics are included in the ration. Whether this results from increased synthesis of the critical nutrient or a reduced requirement for it by fastidious organisms in the intestinal tract or from improved absorption, cannot be determined.

Protein Sparing Effect.—Reports have varied as to whether antibiotic feeding enhances the efficiency of protein utilization, thus sparing the protein requirement. Machlin *et al.*(59), Weakley *et al.*(114), and West and Hill(115) concluded that the protein requirement of young chicks appeared to be decreased slightly by the addition of antibiotics to the ration. The latter group reported that rations containing chlortetracycline, oxytetracycline, bacitracin or penicillin required only 18 per cent protein whereas 20 per cent protein was required for optimum growth and feed efficiency when the antibiotics were not fed. McGinnis(64) reported that poults fed 24 per cent protein rations plus penicillin grew at the same rate as those containing a higher level of protein. However, growth was stimulated at all levels of protein by the addition of the antibiotics. In contrast, Slinger *et al.*(98), (99) did not find penicillin or chlortetracycline (with broilers) or penicillin (with poults) to alter the protein requirements. In fact, in the studies involving poults, it appeared that slightly higher protein levels in the starting ration were effective when penicillin was supplied in the feed. These authors point out that when the protein level is increased in practical rations, it is possible to obtain suboptimal energy levels which may tend to limit growth. Studies by Biely *et al.*(10) also failed to reveal any effect on the protein requirement of growing chicks due to the addition of chlortetracycline. In further studies with poults, Slinger *et al.*(100) observed that increasing the levels of penicillin or chlortetracycline in rations of growing poults increased the incidence of feather depigmentation, normally associated with a lysine deficiency. The authors suggest that the increased growth and improved feed efficiency which was obtained with the higher levels of antibiotics tended to increase the requirements for lysine. Jones and Combs(47) indicated that the dietary requirement of the chick for lysine

and methionine was not spared by the addition of antibiotics but that the dietary requirement for tryptophane was spared when antibiotics were included in the ration.

Other reports by Saxena *et al.*(83) and Morimoto *et al.*(70) show greater nitrogen retention in chicks fed antibiotics. However, the difference obtained by the first group(83) was not statistically significant. Thayer and Heller(110) found penicillin and chlortetracycline to increase the amount of nitrogen absorbed from the intestinal tract of four-week-old cockerels. An increase in the nitrogen retention and a decrease in the urinary nitrogen excretion was observed.

Slinger *et al.*(101) found that the addition of 10 per cent corn oil to a turkey poult starting ration decreased the rate of growth. This growth depression was considerably relieved by supplementing the diet with penicillin. Since an increase in the energy content of the ration is comparable to a decrease in the protein content of the ration, one might interpret these results as suggesting that the antibiotic tended to spare the protein requirement.

Moreover, Baldini *et al.*(6) found that the bobwhite quail grew as rapidly on a ration containing 20 per cent protein with chlortetracycline as it did on a 28 per cent protein diet without the antibiotic. The feed and protein intake were less on the 20 per cent protein diet than on a 24 per cent diet and yet equal gains in weight were observed. On the other hand, Scott *et al.*(85) observed that the feed and protein intake increased when an increase in chick growth was obtained by the addition of chlortetracycline. The efficiency of utilization of feed and protein were equal in all rations and no protein sparing effect was evident.

It would appear that under certain conditions, lower protein levels are satisfactory when antibiotics are fed but the factors which may influence this effect must not be overlooked.

Effect on Feed Intake.—A recent report by Hohls(45) indicates that the effect of antibiotic feed supplements on gain and body weight is greatly affected by the rate of feed consumption. A report by Slinger and Pepper(102) also concludes that the mode of action of penicillin in stimulating the growth of turkey poults may be explained largely, though not entirely, on the basis of the increased feed consumption per unit of body weight during the first weeks of life. These authors show that a supplement of antibiotics increases the feed intake of poults and suggest an effect on intermediary metabolism. Slinger *et al.*(103) have also observed that the feeding of penicillin to chicks having free access to feed resulted in an increase in feed consumption per unit of body weight during the first 16 days.

Nevertheless, restriction of the feed intake of poults receiving the antibiotic did not eliminate this growth promoting effect(86), hence the growth promoting action of antibiotics is not due entirely to an effect on feed intake. Hohls(45) has interpreted these results as meaning that the feed intake is influenced by the basal metabolism and that the basal metabolism perhaps is affected by antibiotics. However, unpublished results obtained by these workers show that there is no appreciable difference in the basal metabolism under fasting conditions of animals fed rations with or without antibiotic supplements.

Effect on Absorption.—There is good evidence that the absorption of certain minerals is improved when antibiotics are fed to poultry. Hillerman *et al.*(44) observed that penicillin reduced the average time of food passage through turkeys and

chickens by 18 minutes. Migicovsky *et al.*(67) found that a greater percentage of an oral dose of Ca^{45} administered to two-week-old chicks appeared in the tibia after 48 hours when the chicks had received penicillin in the ration. This indicated an enhancing effect of penicillin on the absorption of calcium. Common *et al.*(27) found that the increase of serum calcium which follows the injection of estrogen was enhanced when chlortetracycline was included in the diet of pullets. Slinger *et al.* (104) found that the increase in perosis produced by feeding high levels of sodium chloride was prevented by penicillin. Similarly, Pepper *et al.*(73) obtained a greater growth response to chlortetracycline with diets deficient in manganese than with adequate rations and, in addition, noted that the chlortetracycline fed chicks had a lower incidence of perosis when the diet was low in manganese. These workers observed, however, a reduction in the percentage of bone ash when chlortetracycline was included in a diet which contained the NRC recommended allowance of calcium and phosphorus, suggesting that these levels of calcium and phosphorus were not adequate for maximum calcification when rapid growth was obtained by chlortetracycline feeding. In a later paper, Pepper *et al.*(74) confirmed their previous finding that chlortetracycline enhanced manganese utilization by the chick but further observed that the antibiotic did not appear to reduce the dietary requirement for the mineral. In studies with chicks, Ross and Yacowitz(82) reported that dietary penicillin significantly increased the bone ash. However, penicillin did not stimulate the growth of chicks fed a vitamin D deficient, low phosphorus ration. No effect was observed on the vitamin D requirement for growth from feeding penicillin. Lindblad *et al.*(55) failed to note an effect of chlortetracycline on the requirement of calcium and phosphorus of female chicks but obtained results which suggested that the antibiotic increased the requirement of phosphorus in male chicks. These workers observed the greatest increase in weights of chicks and poults from the addition of antibiotics in rations containing the lowest levels of calcium and phosphorus.

Gabuten and Shaffner(37) and Bogdonoff and Shaffner(12) fed several antibiotics to yearling hens and observed slight increases in the specific gravity and breaking strength of eggs. No consistent effect on the plasma calcium level of the hens was noted(12); but male chicks fed penicillin, bacitracin, chlortetracycline, or oxytetracycline, had slightly higher plasma calcium levels. However, the feeding of penicillin to chicks receiving various levels of calcium showed no consistent effect on the bone ash or plasma phosphorus at any calcium level. Sturkie and Polin(109) injected penicillin intramuscularly into laying hens during egg formation and found no effect on the blood calcium levels. This would suggest that any effect of the antibiotic on mobilization and utilization of calcium as reported by others requires a longer period of time. If the enhancement of these antibiotics on the utilization of minerals by poultry is explained on the basis of more efficient absorption, the negative results of Sturkie and Polin would be expected.

Other Effects.—Other changes, which have been described in germfree animals and in animals fed antibiotics, tend to support the view that absorption of nutrients might be improved. Gordon(38) has reported that the small intestine and cecum of the germfree chicks are one-half to one-third smaller than in controls. He also reported that the ileocecal lymph node was also smaller in the germfree animal. When

penicillin or oxytetracycline were added to the diets of conventional chicks, the small intestine, the ileocecal lymph node and, in some cases, the ceca were decreased in weight. Coates *et al.*(22), (24) and Pepper *et al.*(74) also observed a decrease in the weight of the intestinal tract. Coates *et al.* found that the total weight of the intestinal tract of chicks fed penicillin was decreased by 17 per cent with no change in length while the weight of the chicks was increased by 9 per cent. This reflects a decrease in the thickness of the intestinal wall. Since numerous investigators have reported increases in the blood levels of various nutrients, it would appear that absorption is enhanced because of a thinner intestinal wall. The increased feed consumption which has also been reported to follow antibiotic supplementation of rations might well be due to an increased rate of absorption. The decrease in thickness of the intestinal wall might result from a reduced bacterial damage, particularly since the intestinal walls also are comparatively thin in germfree animals.

Maghrabi and Turner(60) observed a slight decrease in thyroid size in young chicks from feeding chlortetracycline, while Mellen and Waller(65) and Menge and Connor(66) have reported that chlortetracycline supplementation of chick diets increased the size of the thyroid gland significantly. The latter workers found that the antibiotic dosage up to 100 milligrams per kilo of diet was correlated with an increase in the thyroid size. The maximum increase in the thyroid size, however, was not as great as that obtained when thiouracil was fed and chick growth was not depressed. The significance of these findings is not known.

Summary

The oral administration of the commonly used antibiotics at low levels to poultry appears to exert a growth promoting effect primarily through their antibacterial action on certain microorganisms in the intestinal tract of the host. This effect has been shown to be greatly influenced by differences in the bacterial contamination of the environment, adequacy and composition of the ration, and other factors.

The observations on changes in the types and numbers of bacteria in the intestinal tract of chicks and poults are variable and inconclusive. However, definite changes have been observed by most workers, even though the extent and nature of the changes are inconsistent. Since many factors may influence the response to antibiotics, lack of agreement among workers is to be expected.

Many investigators have noted a "sparing action" of antibiotics on the dietary requirements for various nutrients including minerals, protein, and vitamins as measured by improved growth rate, blood levels, liver storage, hatchability, or by a reduction of specific deficiency symptoms in some instances.

There is good evidence which shows that antibiotic feeding increases the absorption of nutrients, particularly certain minerals. This appears to be due to the thinner intestinal wall which is found in antibiotic-fed or germfree chicks.

The exact way in which antibiotics enhance growth and, in some cases, reproduction of poultry is not known. Presumably, these effects occur primarily through their antibacterial action on the bacterial population of the intestinal tract, which in turn influence the health and nutritional status of the host animal.

The nutritional effects of antibiotics are most likely a result of (1) increased bacterial synthesis by non-fastidious organisms of essential nutrients, including those

not yet identified; (2) decreased bacterial competition with the host for essential nutrients by fastidious organisms; and (3) improved absorption due to reduced bacterial irritation and destruction of the intestinal wall.

The most important effect of antibiotics, from the standpoint of practical use in poultry rations, appears to be through a reduction or elimination of harmful bacteria which are associated with environments previously used for rearing poultry. These organisms may be harmful to the host animal by producing toxic substances, mechanically damaging the intestinal tissue, or by establishing a sub-clinical infection. A decrease in numbers of these organisms may not only be desirable for the growth of the host but also may permit certain desirable bacteria to grow more rapidly.

Antibiotic supplementation of poultry rations appears to reduce the numbers of harmful bacteria and improve the nutritional status of the host.

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MODE OF ACTION OF ANTIBIOTICS ON ANIMAL GROWTH *

Rats, Swine, and Ruminants

While poultry are especially amenable to the growth enhancing property of antibiotics(10), (41), many other animals may be affected likewise. The bulk of this paper will consider some of the work conducted upon rats, swine and ruminants (particularly calves); few data useful for formulating any modes of action have come from studies on animals other than these and poultry. The voluminous literature on this topic has had several excellent reviews(3), (4), (33), (36), (46), (56), (64), which have been of considerable use in the following discussion.

Rats.—The rat has been particularly useful in studying the mode of action of antibiotics on animal growth. It appears, however, that the growth of this species is enhanced by antibiotics only when a dietary imbalance exists, since a number of investigators find the administration of antibiotics to rats fed a nutritionally adequate practical or purified diet to have no significant effect on growth(2), (31), (61), (65). Rats fed rations deficient or insufficient in one of the B-vitamins(3), (31), (33), (46), (48), (64), in certain amino acids(17), (27), (31), (50), (61) or in protein(2), (50), (61) are generally benefited by the incorporation of any one of a number of antibiotics in the ration.

Swine.—Reports of the action of antibiotics on the growth of swine are numerous(3), (4), (33), (64). Many workers find slight, if any, growth improvement in pigs fed antibiotics if a good dietary and sanitary environment exists. The better growth responses, sometimes exceeding 100 per cent, have been obtained with animals suspected of, or known to be, suffering from gastroenteritis (scours) and/or with pigs fed low quality rations such as those high in content of vegetable proteins. The more striking effects of antibiotics on weight gains have occurred in fast growing weanling pigs, or with suckling pigs fed antibiotic supplements. The addition of antibiotics to artificial sow's milk has been rather successful. Also, runt pigs respond to antibiotic supplementation.

Ruminants.—The reaction of ruminants to antibiotics has been reviewed by Knodt(36) and Reid, *et al.*(56). Although the value of feeding antibiotics to lambs is questionable, calves fed an antibiotic often will grow more rapidly during the first three to six months of life than the control animals receiving no antibiotic.

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Chlortetracycline and oxytetracycline appear to be the best growth promotants while penicillin usually has no effect. Since calves, like swine, are highly susceptible to scours, the growth-promoting action of antibiotics on calves may be a result of subduing or preventing enteritis. Oftentimes, the best growth responses to antibiotics are obtained when the control animals evidence subnormal growth, such as will occur with scours.

Discussion

The nature of the growth stimulus exerted by antibiotics and other antimicrobial agents is poorly understood and subject to considerable speculation. On the basis of present knowledge, very little of which is objective, one is justified in concluding that antibiotics promote growth via alterations of the intestinal or ruminal microflora. The evidence in support of this contention is persuasively presented by Jukes and Williams(33). Because of the intimacy of the intestinal microflora with the biochemistry of the host, almost any antibiotic-induced effect, from elevated tissue stores of certain nutrilites to the prevention of tissue damage, can be attributed to effects on the internal microflora. It is not sufficient, however, to demonstrate quantitative changes in the intestinal microflora; rather, the significance of such effects on the biochemical balance of the animal must be determined before a mode of action can be recognized. Even under the "normal" state, without the complexity of antibiotic effects, the role of the flora of the alimentary canal is obscure(32), although it is certain that ruminants are dependent on their gastric flora(1).

Effects of Antibiotics on the Population of Different Members of the Intestinal and Ruminal Microflora.—It is now clear that the composition of the intestinal microflora is affected by antibiotics, whether the route of administration is parenteral or oral(3), (16), (22), (31), (33), (48), (49), (52), (54), (62), (64). The population of a number of genera and the "total number" of intestinal microorganisms may be altered by these drugs. Furthermore, the type of dietary carbohydrate (48) and protein(62) influences the response of the intestinal flora, as well as the host, to an antibiotic. Quinn *et al.*(52), (54) noted that intestinal protozoa disappear and that the numbers of glucose-fermenting bacteria decrease in the feces of pigs fed chlortetracycline. An effort has been made to relate the latter observation to the decreased glucose tolerance of swine receiving antibiotic-supplemented rations(7). Much stress has been placed on the intestinal population of toxin-producing species of Clostridium, although it is not known whether such substances are produced *in situ*. Moreover, Larson and Carpenter(38) found the number of *C. perfringens* in the feces of pigs fed antibiotics to fluctuate so widely that there was no correlation between the growth response and the fecal population of this bacterium. Daily variation in the population of specific types of intestinal microorganisms is a common observation.

One report of increased numbers of filamentous fungi in the feces of swine fed chlortetracycline is of interest because dietary supplements of cultures of the most frequently appearing species, *Aspergillus flavus,* stimulated growth to an extent comparable to that engendered by the antibiotic alone(53). Since an unidentified factor stimulatory to bacteria was found in cultures of this fungus, it was concluded that the antibiotic caused the synthesis of a new growth factor by creating conditions within the intestine which permitted filamentous fungi to thrive.

Several early studies indicated that only slight changes of the ruminal microflora accompany the administration of antibiotics(56). Mann *et al.*(39) found chlortetracycline to have no significant effects on the microbial population of the rumen of calves, while Horn, *et al.*(30) reported both chlortetracycline and penicillin to affect the ruminal flora of yearling steers.

One wonders how many changes occur in the intestinal microflora which cannot be detected because of limitations in methodology. Undoubtedly the combined metabolic activities of the intestinal microflora are modified by antibiotics through population changes. Furthermore, the establishment of a drug-fast intestinal microflora(22), (31), (48) may be significant because of an altered metabolism which is often seen to accompany increased antibiotic resistance in bacteria(20), (55).

Effects of Antibiotics on Frank Infection or Toxemia.—Scours in swine and cattle apparently can be controlled by antibiotics(3), (56), (58). It has been suggested also that enterotoxemia of sheep is alleviated by antibiotics(64). Naturally, the control of infection or toxemia should have a beneficial effect on growth, thriftiness, etc. The control of recognized disease by antibiotics is not the answer to the problem since the growth of animals in apparent good health will be stimulated by antibiotics. The rat is particularly resistant to infectious disease and rarely exhibits, under usual conditions of maintenance, frank disease. Contrariwise, the guinea pig, with its unique intestinal microflora composed almost exclusively of Gram-positive bacteria(11), suffers from the administration of antibiotics through the resultant establishment of a pathogenic internal flora(5), (57), (59). It is surprising that few difficulties of this sort have been encountered in other animals.

Effects of Antibiotics on Subclinical Disease.—The existence of subclinical disease evidently is more common than once realized; indeed, it may be the "normal" state of health in many animal species(13). It is logical to believe that young animals may reveal delayed growth and lowered food efficiencies as the only noticeable symptoms of subclinical disease. These symptoms, which would vary in their severity with the "disease level," may be reversed by the administration of antibiotics, particularly if the etiologic agent is a bacterium. Since some workers feel that scours in swine(4) and cattle(56) is often subclinical, one explanation for the mode of action of antibiotics on growth, viz., suppression of subclinical infection, is obvious and is gaining wide favor. The occasional absence of undiagnosed disease may explain why a number of workers report no effect of antibiotics on growth. Perhaps the reason why penicillin has so little effect on the growth of calves is because it is rapidly inactivated in the rumen(28), (34), (39) and does not reach the intestine in sufficient concentration to impede the scours-producing microflora. Furthermore, the bacteria believed to cause scours are Gram-negative and hence are relatively resistant to penicillin.

Another type of disease which may persist in many animal species in a subclinical form is intestinal autotoxemia. Other than the classical endo- and exotoxins, certain products of microbial growth in the rumen or intestine may produce a chronic toxicity of very low degree, which in turn would be manifested in delayed growth. Products such as amines, ammonia, indole, skatole, hydrogen sulfide, and undoubtedly others, may be elaborated in toxic amounts within the intestinal lumen (42). While the body is normally possessed of excellent detoxifying mechanisms, certain toxic substances which are normally encountered may be metabolized at a

rate too slow to permit the young animal to grow at its full genetic potential. An excellent example of this is the late development of amine oxidase activity in the tissues of animals(66). Recently, chlortetracycline has been reported to suppress the intestinal flora's formation of amines as demonstrated by a reduction in the number of amines in rat feces as well as a lowered amino acid decarboxylase activity of mixed in vitro fecal cultures grown in the presence of a small amount of the antibiotic(42). If antibiotics interfere with the formation of amines, the young animal, which is less able than the adult to oxidize amines, will be benefited. Young rats are known to respond to tyramine poisoning which can be induced by a diet containing a high level of tyrosine(19), (40). However, unpublished data from this laboratory show that oxytetracycline and chlortetracycline can reverse the toxicity of a high tyrosine ration. Furthermore, if the level of tyrosine is not too high the weanling rats will recover within a few weeks without the assistance of antibiotics. These studies suggest that certain amines formed in the intestine may be toxic, particularly for the young animal. The toxicity may be mild and recognized only by the growth stimulus exerted by antibiotics. Even the rumen, according to one preliminary study(51), may support a microflora capable of producing sympatheticomimetic amines.

Ammonia is another substance which may be elaborated by the intestinal flora in toxic amounts. Dintzis and Hastings(12) reported antibiotics to inhibit the urease activity of the intestinal microflora of mice. In fact, the ureolytic activity of the gastric and intestinal mucosa was found to be due to adhering microbial cells which could not be removed by flushing. These observations have been confirmed by others (37). Enzymatic deamination of amino acids is another ammonia-producing system of the intestinal microflora. Michel and Francois(44) found bacterial cells recovered from the contents of the small intestines, ceca and colons of pigs to have a lower citrulline, ornithine and glutamic acid deaminase activity than comparable preparations from the control animals. Also, the addition of chlortetracycline or penicillin to fresh suspensions of cecal bacteria depressed several amino acid deaminases. In a second report, they(45) found an inverse relationship between the arginine and citrulline deaminase activity of the cecal cells and the degree to which an antibiotic stimulated growth.

Apparently antibiotics interfere with the production of amines and of ammonia from amino acids or urea through effects on the alimentary microflora. In the case of deamination the drugs may interfere directly with enzymic action; this does not appear to be true for urease or the amino acid decarboxylases. Perhaps Metchnikoff's(43) theory of intestinal autointoxication will have to be reconsidered in the light of these recent investigations.

Effects of Antibiotics on Nutritional Economy.—The so-called vitamin-sparing action of antibiotics has been studied extensively in the rat. The concept has been extended to include other nutrilites, e.g., methionine and protein. No useful purpose would be served by discussing the many reports which relate to this phenomenon because few are enlightening as to the cause of the sparing effect.

If antibiotics stimulate the microbial synthesis of a nutrilite in the intestinal canal or reduce the absorption of a nutrilite by the intestinal flora, the growth factor

should be available in greater amounts for absorption through the intestinal wall. With this thought in mind, investigations of the effect of antibiotics on levels of vitamins in intestinal and ruminal contents and tissues have been made(8), (9), (21), (24), (29), (35), (47), (48), (63). The results were variable, depending on the species of animal, the type of antibiotic, the composition of the ration, the vitamin under study and the manner of conducting the assay. It has been shown that chlortetracycline enhances the absorption of cystine, leucine, lysine and methionine from the small intestine, but not from the large intestine, of rats(6). Also noteworthy is the finding that the nitrogen content of fecal bacterial cells is lower in swine fed antibiotics than in swine ingesting an antibiotic-free ration(60). The possibility that antibiotics affect the permeability of the intestinal wall is supported by the report that both penicillin and chlortetracycline increased the absorption of casein hydrolysate *in situ* from an isolated portion of the ileum of the rat(15).

One should not overlook the possibility that antibiotics may induce a nutritional deficiency. An example of this is the induction of a biotin deficiency in the rat by administering streptomycin(14). Moreover, streptomycin, oxytetracycline and chlortetracycline have been reported to lower the biotin content of rats' intestinal ingesta(9), (24), (25).

One provoking action of several antibiotics is the partial prevention of hepatic injury induced in the rat by a deficient ration containing yeast as the sole source of protein(23). Antibiotics delay but do not prevent the onset of hepatic necrosis and cirrhosis. Prevention is accomplished by dietary supplements of vitamin E or the sulfur-containing amino acids, cystine or methionine. György(23) has concluded that antibiotics prolong the time of disappearance of stores of nutritional factors concerned in normal maintenance of the liver. While alterations of the intestinal microflora by antibiotics may delay the depletion of these unknown nutritional factors in animals on the "necrogenic diet," György believes some other mode of action is operative.

Because of the importance of the rumen to the nutritional economy of cattle and sheep, some effort has been made to determine what effects antibiotics have on the biochemistry of this important organ. In most cases, antibiotics have little effect on the efficiency of utilization of feed by ruminants(36), (56). Nevertheless, antibiotics appear to interfere with the digestion of cellulose by ruminal microorganisms, in vivo as well as in vitro(36), (56), although penicillin has been reported to stimulate cellulolytic activity(18), (56). Evidence from studies on an artificial rumen indicates that the suppression of cellulolytic action by antibiotics is temporary, lasting but a few days(26). Chlortetracycline and, to a lesser degree, penicillin have been reported to depress the ruminal flora of yearling steers concomitant with an impairment of nitrogen retention and the digestibility of crude fiber(30). Chance *et al.*(8) noted chlortetracycline to lower the concentration of 10 amino acids, riboflavin and niacin in rumen ingesta. On the other hand, Kelser(35) could find no effect of oxytetracycline on the concentration of either thiamine or riboflavin in rumen fluid. The relationship between the effects of antibiotics on activities of the ruminal flora and growth is obscure.

Conclusions

It is impossible to determine, from existent data, a mode(s) by which antibiotics enhance growth. While we should not ignore the suggestion that extra-intestinal sites are operative in this phenomenon(23), (49), the participation of the alimentary microflora seems to offer the more plausible basis for further investigation. It is becoming increasingly apparent that under certain conditions the intestinal microflora may cause unrecognized (subclinical) disease, e.g., gastroenteritis or toxemia, thereby affecting the animal's growth, efficiency of utilization of dietary components, etc. Even the sparing action of antibiotics on certain growth factors may be attributed to subclinical disease since an animal may be prone to infection under the stress of a nutritional imbalance. On the other hand, some evidence implies that the sparing action is a consequence either of an interference with the intestinal flora's absorption of growth factors, or an enhancement of the capacity of the animal to absorb nutrilites from the intestinal lumen. Because both a marked reapportionment of the intestinal flora and a rise of drug-fast strains accompany the administration of antibiotics, biosynthetic processes of the gut's microflora are probably affected.

The classical microbiologic methods, e.g., plate counts, pure culture studies, etc., are inadequate to obtain the needed precise knowledge of the *in situ* biochemical and pathological activities of the intestinal microflora. Such information will not be easy to gather because of the dynamic state of the flora. Unfortunately, in vitro studies with intestinal microorganisms cannot duplicate in vivo conditions, although work with mixed cultures under various in vitro conditions has been helpful(42), (44), (45).

Obviously, more research is necessary. Certain areas in particular have been neglected. For example: (1) What are the effects of antibiotics on mammalian tissue cultures? (2) What products of the intestinal microflora are toxic to animals and how do antibiotics affect their elaboration? (3) How do the metabolic activities of microbial cells freshly isolated from intestinal ingesta of animals fed antibiotics differ from those cells from animals on an antibiotic-free regimen? (4) What effect does drug-resistance have on the capacity of intestinal bacteria to synthesize and absorb growth factors? (5) What are the etiologic agents, if any, of subclinical disease? These questions and many others need to be answered.

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MODE OF ACTION OF ANTIBIOTICS—EVIDENCE FROM GERMFREE BIRDS *

Nutritionists have found sulfa drugs to be most useful in precipitating increased requirements for vitamins in several species. Some have assumed this effect is caused directly or indirectly by changes in the intestinal flora and/or fauna. Discussions of these phenomena usually ended with statements regarding the usefulness of germfree animals to help solve these questions. With the help of many people in the Biochemistry and the Agricultural Bacteriology Department of the University of Wisconsin,† I worked from 1943 to 1946 in different ways to raise germfree chicks in order to attack such problems directly.

When we received some streptomycin from Dr. J. M. McGuire of Lilly and Co., we fed it to chicks alone and in combination with sulfasuxadine in an attempt to "completely inactivate all bacteria in the intestinal tract" in order "to be provided with an essentially sterile animal. . . ."(9). As you know, the drugs "failed to sterilize the intestinal tracts of the chicks and no significant decreases in the total count of intestinal bacteria were observed." An observation more pertinent to this audience was seen in this experiment: Streptomycin fed with an adequate amount of folic acid produced increased growth rates rather than the expected growth depression. We suggested (in 1946) that this new phenomenon might be due to "the inhibition of intestinal bacteria that are producing toxic material or are rendering certain dietary vitamins unavailable to the animal, an effect different than is usually encountered with sulfonamides." "However" we stated, "the possibility that these agents are acting systemically cannot be overlooked."

Resolution of the mechanism of this growth stimulation rests upon the broad and basic question of the presumed contribution of the intestinal microorganisms to the nutrition of the non-ruminant host. Since we cannot approach such a broad problem this afternoon, we may restate the question: Do the antibiotics stimulate growth via the intestinal organisms, do they act directly upon the tissues or systems of the host,—or do they act upon *both* the microorganisms and the host? Evidence obtained by feeding antibiotics to germfree birds should be most useful in answering this question. All data available reproduced here are in detail. It is interesting if

* Ed. Note.—See also recently published article by E. G. Hill and N. L. Larsen. Effect of chlortetracycline supplementation on growth and feed utilization of the unsuckled baby pigs obtained by hysterectomy. *J. of Animal Sci.* Vol. 14, p. 1116, November 1955.

† These include Barbara Lackey, A. Lepp, P. Moore, Drs. E. McCoy, P. Wilson, C. A. Elvehjem, and E. B. Hart.

not ironical that germfree animals must be used to answer the question posed by an attempt to obtain chicks with sterile intestinal tracts.

Methods

The methods used in rearing germfree chicks have improved greatly since the first abortive attempts of Nuttal and Thierfelder(11). Schottelius(15) obtained germfree chicks and maintained them using skilled aseptic techniques, but they did not grow. Cohendy(3) by using more complete diets, grew germfree chicks for several weeks in his cylinders and Balzam(1) executed experiments related to the "intestinal synthesis of vitamin B" with germfree chicks. My Wisconsin work (unpublished) was done with simple apparatus such as a bell jar, or a gallon cooky or churn jar (Plate 1). Reyniers used both simple and elaborate apparatus to rear germfree chicks. The work reviewed herein was done inside a series No. 20 or series No. 200 germfree rearing unit (Plate 2) according to the method given by Reyniers, Trexler, Ervin, Wagner, Luckey, and Gordon(12). The turkey poults were reared in the large germfree units as described by Luckey, Wagner, Gordon and Reyniers(5).

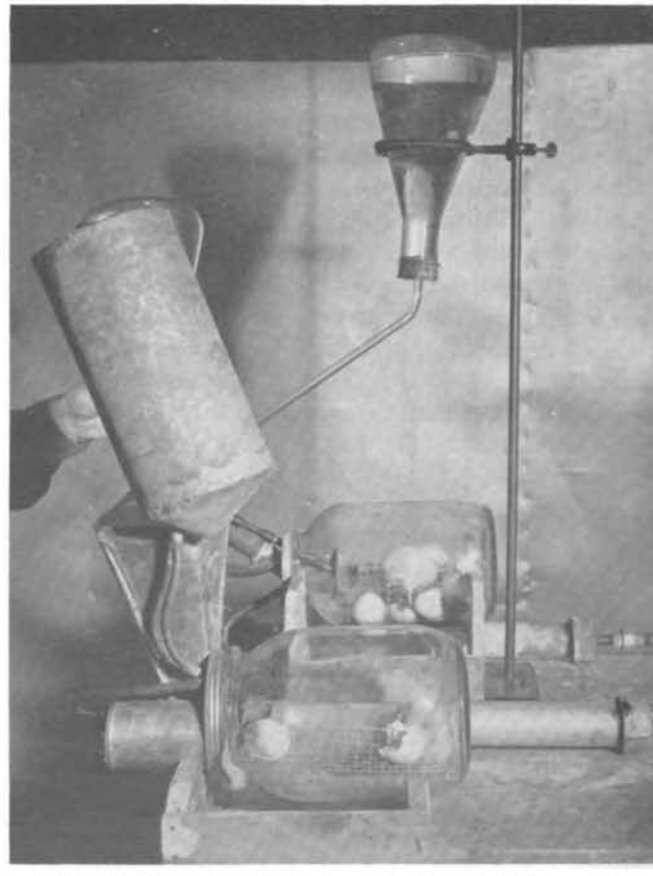

Plate 1.—Germfree Chicks in a Jar System (Wisconsin).

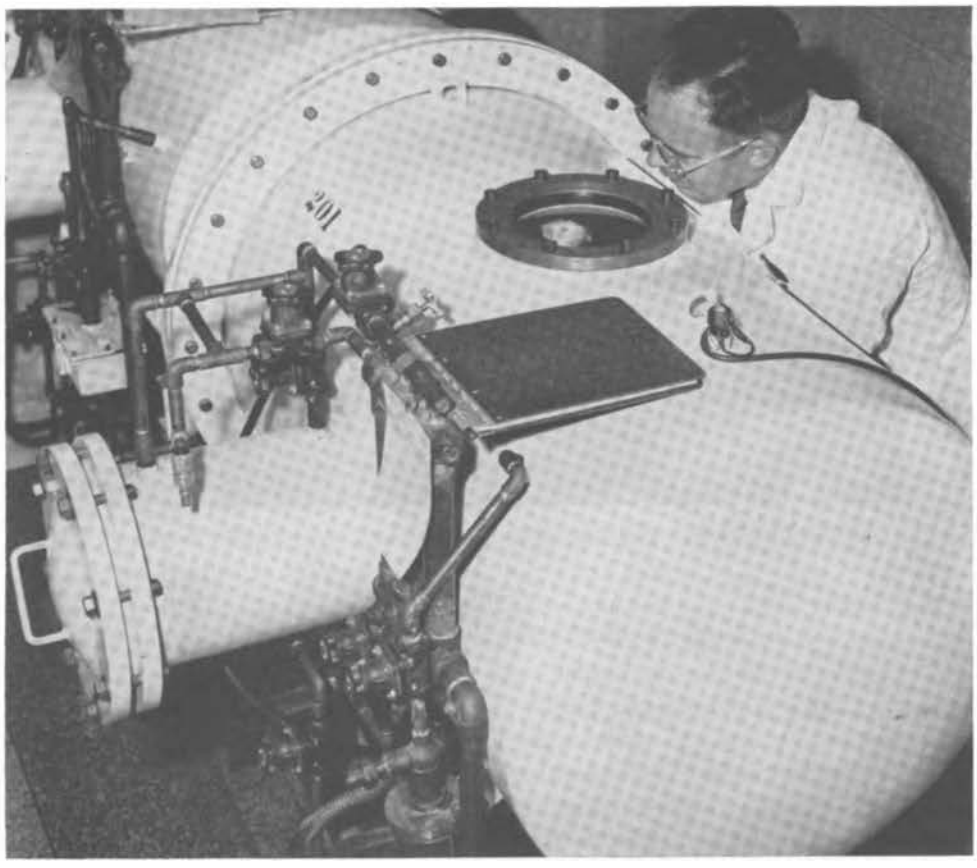

PLATE 2.—Reyniers type Germfree Unit, Series No. 200.

Clean embryonated eggs (1–2 days prior to hatch) were dipped into 2 per cent Hg Cl_2 for 5 minutes and passed into the steam-sterilized chamber. In some of the early work the two groups were maintained in separate chambers but in most of the work they were placed in two wire cages in the same unit (Plate 3). No living microorganisms were detected in any of the birds using accepted visual and cultural techniques. After hatching, the birds were divided into two groups and fed the sterile diet with and without antibiotic. Sterile food and water were available ad libitum. The diets used were Pfizer broiler ration fortified to allow for destruction of vitamins and proteins during 25 minute steam sterilization at 17 psi. (Table 1). The antibiotics (except Chloromycetin which was steam sterilized and streptomycin which was filtered) in starch or lactose diluent were sealed in triple polyethylene bags and sterilized with cathode rays (1.5 million rep). The inner two bags were introduced into the germfree cage in the manner of the eggs, i.e. through a germicidal trap. This procedure gave a minimum of destruction of the antibiotics.

Conventional (non-germfree) birds fed sterilized diet with antibiotics consistently gave increased growth rates (they averaged 6 per cent heavier than birds fed no antibiotics at 4 weeks) during the four years the germfree work was in progress.

PLATE 3.—Germfree White Leghorn Cockerel (wgt. at 4 weeks—292 gm).

TABLE 1

COMPOSITION OF DIETS*

Constituent	Chicks gm	Poults gm
Casein, technical	7.0	7.0
Corn, cracked	49.0	34.2
Soya meal (s)	23.2	23.2
Alfalfa leaf meal	2.8	3.7
Wheat mids	9.3	4.7
Fish meal	2.3	8.4
Oats, ground	—	2.8
Meat scrap powder	2.3	4.7
Corn gluten meal	—	3.7
Yeast, dried	—	4.7
Fish solubles	—	1.9
Salt (iodized)	0.5	0.4
$CaCO_3$	1.7	0.7
Ca_2HPO_4	1.5	0.7
$MnSO_4 \cdot H_2O$	0.024	0.026
Viadex (4000 A, 1000 D)	0.23	0.16
	mg	mg
Thiamin Cl	0.19	5.25
Riboflavin	0.33	2.43
Ca Pantothenate	1.12	11.49
Nicotinamide	1.64	12.18
Choline Cl	63	385
Pyridoxine Cl	0.33	2.43
Inositol	—	100
Biotin	—	0.05
Folic Acid	—	2.0
Cobaltamine	—	0.002

* A synthetic type diet was used in the experiments where streptomycin was fed.

The Growth Index (GI) was obtained from the following formula:

$$GI = \frac{\text{No. } ♂ \dfrac{\text{Av. Wgt. } ♂ \text{ fed drugs}}{\text{Av. Wgt. } ♂ \text{ fed no drugs}} + \text{No. } ♀ \dfrac{\text{Av. Wgt. } ♀ \text{ fed drugs}}{\text{Av. Wgt. } ♀ \text{ fed no drugs}}}{\text{Total No. of birds}} \times 100$$

Statistical calculations were made from relative weights using the average weight = 100 for the birds fed no antibiotic for each sex in each experiment. Thus all birds are on a common basis irrespective of breed, species, sex or environmental conditions. The data in the two presentations being reviewed are not strictly comparable because in one, weights are given at the end of a four week experiment (the birds were up to five weeks old) and in the other the weights were given for the birds four weeks of age.

Results

Growth.—When a variety of antibiotics in relatively high levels were fed to germfree chicks, growth was not stimulated (Table 2) (6). The apparent consistent growth depression was never statistically significant. Since the levels fed were 5–20 times higher than was usually being fed at this time (1952) and the only experiment wherein a positive growth response was obtained was one in which 25 mg of Terramycin/kg had been fed, it was assumed that the level fed was too high.

The results obtained when lower levels were fed give an entirely different picture; a positive growth index was obtained in each experiment (Table 3) (7). The experiment numbered 1–4 was one of the early experiments and is given in this table because the lower level of Terramycin was used. The results with streptomycin fed at 70 mg/kg are given only for completeness. All birds in this experiment appeared to be sick and grew very little. In three of these experiments the increase in weight at four weeks was statistically significant. The numbers of birds in each experiment is small but the pattern of growth stimulation by antibiotics is consistent.

From these two series of experiments it appears that germfree chicks react differently to antibiotics at the levels of 25 mg and 50 mg/kg diet. This point is not reached in conventional chicks until levels of 1000–5000 mg/kg are fed.

The results with germfree Beltsville White turkey poults are summarized in Table 4(7). The general pattern of response indicates an increased growth rate when antibiotics were fed. When all germfree turkey experiments were combined using a relative weight basis, the difference in weight between the 14 poults fed procaine penicillin and the 13 poults fed no promotant is statistically significant ($p = 0.003$). Figure 1 shows the action of antibiotics over a long period of time.

While these data are for a small number of animals in each group, you should be aware that consistency of results obtained in repetition of this delicate experiment becomes a most convincing factor not to be overlooked. You have both the raw and the processed data from which you can form your own interpretations or conclusions. Although many experiments were discarded due to contamination, in obtaining these data no birds have been omitted which were germfree for four weeks with no slipups.

TABLE 2

WEIGHT OF GERMFREE CHICKS FED ANTIBIOTICS FOR 4 WEEKS

Expt.	Supplement*	No. ♂	gm	No. ♀	gm	Av. gm
1	None	2	227 (213,241)	2	228 (186,269)	227
	Streptomycin 500 mg/kg	–	—	4	221 (209,241)	221
5	None	2	414 (401,427)	1	365	398
	Chloromycetin 23 mg/kg	2	451 (451,451)	2	325 (315,335)	388
9	None	4	501 (486,518)	2	480 (428,424)	493
	Procaine Penicillin 46 mg/kg	2	512 (504,519)	4	497 (484,527)	502
14	None	2	301 (300,301)	–	—	301
	Procaine Penicillin 46 mg/kg	1	270		278	275
10	None	4	344 (324,362)	2	332 (331,332)	338
	Bacitracin 35 mg/kg	3	343 (320,357)	3	306 (257,359)	324
7†	None	1	674	1	564	619
	Terramycin 50 mg/kg	2	448 (426,470)	1	483	460
8†	None	1	628	2	584 (568,579)	598
	Terramycin 50 mg/kg	1	619	2	496 (440,552)	537
13	None	–	—	3	248 (227,271)	248
	Terramycin 50 mg/kg	3	282 (262,301)	–	—	282
15	None	2	328 (326,330)	1	332	329
	Terramycin 50 mg/kg	1	223	2	296 (248,343)	271

* Diet L-289 was used throughout excepting diet L-284 was used in Expt. 1. The supplements were added at a level of 50 mg/kg diet.
† These chicks were New Hampshire Red. All others were White Leghorn.

RESULTS

Germfree Chick Characteristics.—The question of the suitability of the germfree chicks for experimental work may be considered. Germfree chicks and conventional chicks are very similar from the viewpoint of gross morphology, nutritional requirements and chemical composition. In the comparison we may point to the differences but let this not detract from the general similarity. "Chicks reared for short periods of time (4 to 8 weeks) on experimental diets in the complete absence of other demonstrable living forms grew and developed as well as control chicks fed the same diets" (13) (Fig. 2). Bantam chicks reared in the germfree state laid eggs

TABLE 3

WEIGHT OF GERMFREE CHICKS FED WITH AND WITHOUT ANTIBIOTICS

Expt. No.	Antibiotic: Kind	Antibiotic: mg/kg diet	Weight (gms. at 4 wks.): Av. ♂	Weight (gms. at 4 wks.): Av. ♀	GI	P
1–4*	None	0	420 (409,430)	381	—	—
	Terramycin	25	472 (466,467,482)	374	108	0.001
1–20	None	0	287 (265,280,293,310)	201	—	—
	Terramycin	25	292 (266,317)	239 (221,225,271)	109	0.011
1–22	None	0	240 (213,222,284)	122	—	—
	Terramycin	25	214 (192,235)	233 (185,212,301)	134	0.27
1–24	None	0	357 (338,361,370)	369	—	—
	Procaine Penicillin	11	379 (370,382,384)	338	103	0.026
1–2	None	0	140	134 (126,142)	—	—
	Streptomycin	70	194	143 (118,168)	117	0.310

* New Hampshire Red Chicks used in this experiment; White Leghorn Chicks used in all others.

TABLE 4

WEIGHT OF GERMFREE POULTS FED WITH AND WITHOUT ANTIBIOTICS

Expt. No.	Antibiotic: Kind	Antibiotic: mg/kg diet	Weight (gms. at 4 wks.): Av. ♂	Weight (gms. at 4 wks.): Av. ♀	GI	P
2–8	None	0	500	378 (354,370,411)	—	—
	Terramycin	25	386 (339,376,451)	373	88	0.22
2–1	None	0	438 (428,448)	422 (412,412,443)	—	—
	Procaine Penicillin	46	511 (505,509,518)	458 (451,465)	113	0.001
2–3	None	0	446 (415,422,501)	390	—	—
	Procaine Penicillin	46	456	387 (383,384,393)	101	0.126
2–4	None	0	340 (322,357)	—	—	—
	Procaine Penicillin	46	363 (335,357,398)	—	107	—
2–13	None	0	392	373	—	—
	Procaine Penicillin	11	430	385	107	—

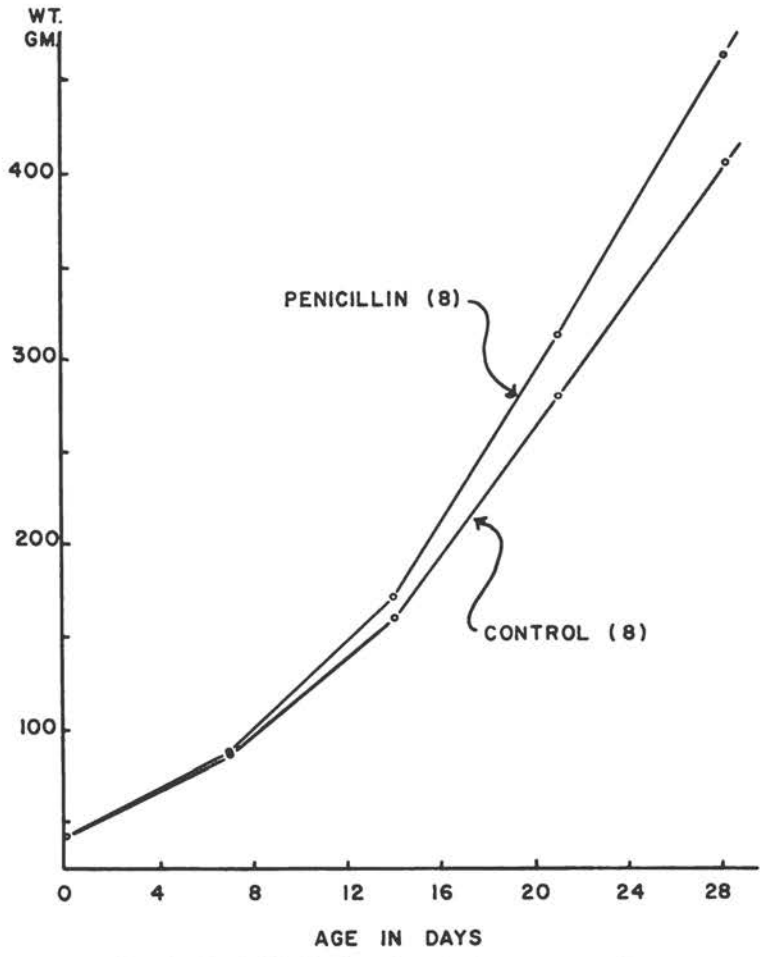

FIG. 1.—Penicillin in Germfree Turkey Poults (♂).

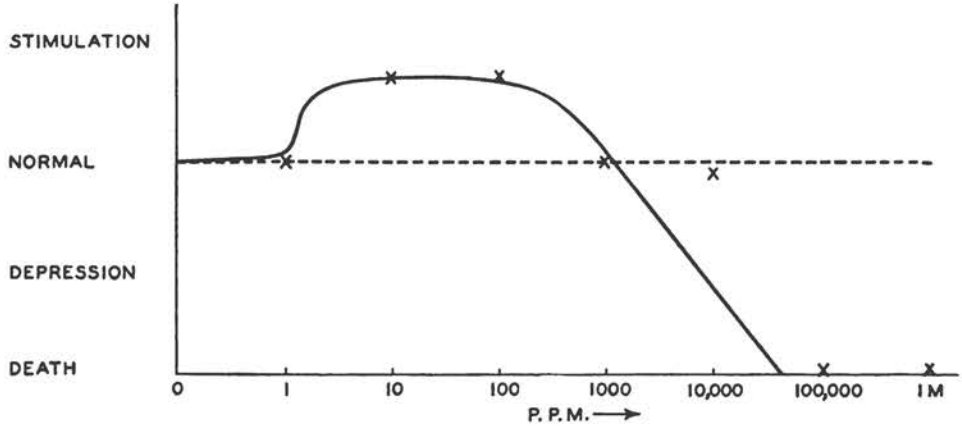

FIG. 2.—Complete Activity Spectrum of Terramycin Compared to a Hormoligant.

from which one chick hatched to complete the life cycle within a germfree chamber. Autopsy indicated the adult Bantam chicken to be "normal" macroscopically and microscopically with respect to skin, plumage, comb, general activity, appendages, bones, muscles, fat deposition, nervous system, circulatory system, respiratory system, (except slightly less peribronchial lymphoid tissue), *bursa Fabrichii,* liver, pancreas, spleen, kidneys, testes, sense organs and thyroid glands. Differences found in this germfree bird were smaller proventriculus, intestines, ceca and adrenal glands, less lymphatic tissue (particularly in the lower part of the small intestine) and the thymus was larger (containing more thymocytes) than in control chicks. Low antibody titers against bacterial antigens were found in older birds(14). When the morphological picture of the germfree chick was examined more fully by Gordon(4) this picture remained unchanged. The data for germfree chicks were taken from those fed no antibiotics in these experiments.

The vitamin requirements of germfree Leghorn chicks were qualitatively similar to those of conventional birds and the quantities of B-vitamins found in the liver and cecal contents were generally of the same order of magnitude, even in deficient chicks (Table 5)(8). The picture of the vitamins in cecal contents of germfree and conventional chicks would be similar if the expressions had been made on a dry weight basis.

Effect of Antibiotics upon Morphology and Biochemistry.—The next pertinent question which has been partially answered is the effect of dietary antibiotics upon the morphology and tissue composition of germfree chicks. Although these data were taken from chicks fed the high levels of antibiotics and showed no growth stimulation, they are of considerable interest as an exploratory examination. No morphological changes were seen in germfree chicks treated with either procaine penicillin or Terramycin. This was in marked contrast to the changes seen when conventional chicks are fed antibiotics. Dr. Gordon has suggested that feeding penicillin tends to make the conventional animal more like the germfree animal. He has also pointed out that the only morphological difference noted between the heaviest and the lightest germfree birds fed procaine penicillin is the relatively greater size of the thymus in the heavier bird. No significant biochemical changes were seen in germfree birds fed either Terramycin or streptomycin. Marked increases were noted in the biotin, folic acid and vitamin B_{12} content of cecal contents when Terramycin was fed to conventional birds. The ash content of the liver increased. When

TABLE 5

THIAMIN CONTENT FOUND IN LIVER, CECUM, AND RECTAL CONTENTS OF BANTAM CHICKS

		Liver		Cecum		Rectal	
Diet		No. of chicks	Av. Thiamin value	No. of chicks	Av. Thiamin value	No. of chicks	Av. Thiamin value
Low Vit. B_1	Control	4	2.2	3	15.5	2	45.1
	Germfree	8	1.2	7	4.9	1	29.1
Complete Diet*	Control	2	3.0	2	18.3	1	55.5
	Germfree	2	5.6	2	7.1	–	—

*1 mg B_1 per 100 gm diet.

streptomycin was fed to conventional birds no liver changes were seen, niacin and biotin were found to be high in cecal contents and folic acid was high in the blood. These figures give relative changes observed as the average values of 3–5 chicks per group.

Discussion

The complete mechanism of action of the growth stimulation of dietary antibiotics will probably not be elucidated through any one study. The results presented from the germfree work indicate there is a direct action on the tissues. Similar results were obtained by Nickell and Finlay using sterile cultures of the duckweed plant (lemna) (10). There is equally strong evidence both for an "infectious agent" (2) and for effects upon the bacterial flora of conventional chicks (1). It must, then, be admitted that the antibiotic is affecting both systems—the intestinal microorganism and the host.

Since our chairman requested us to suggest research paths, I should like to speculate a bit to see if this information can be fitted into a more general pattern. The curves in figure 2 show the complete activity spectrum of antibiotics. The solid line gives the curve predicated, and the points indicate the actual data obtained with Terramycin. The prediction was made with the assumption that antibiotics act like many other drugs. Too much will cause death, therapeutic amounts are often somewhat harmful in some respects (actually the net effect at this level is helpful) and very small quantities are stimulatory. A proposed name for this action is hormoligosis (hormone = excite, oligo = small amounts). In this sense strychnine, Terramycin, arsenic and penicillin are hormoligants. This gives a fresh view of antibiotics which may explain effects which one worker obtains but others cannot repeat. Under different conditions the same quantity of a drug may act differently. Different tissues or systems may be affected differently as the drug quantity changes, but the observer usually reads only a part of the cumulative effect on the whole organism. Hormoligology suggests the view that any drug in the proper quantity will give a stimulating effect under any given circumstance. This is the opposite viewpoint from that taken by those who screen thousands of compounds at one or three levels looking for new miracle drugs. Prediction of the proper quantity of a drug (antibiotic) needed to give stimulation would be more sure if we knew how a cell can be stimulated to grow (or reproduce) faster by the presence of a non-metabolite. This is the basic question.

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Mode of Action

PANEL DISCUSSION

Moderator

H. D. Branion

Panel Members

Knut Breirem, Johannes Brueggemann, Gerald F. Combs, Enno Freerksen, W. S. Gordon, K. R. Johansson, S. K. Kon, T. D. Luckey

Summarizer

H. R. Bird

Dr. Branion: I am going to ask the four members of the panel each in turn to add anything they may wish in the line of general remarks. I will simply take them in their order on the program. Dr. Breirem, is there anything that you would care to add?

Dr. Breirem: I will base my comments mostly on Scandinavian experiences. As to responses by feeding antibiotics to farm animals we can divide our experiments into two groups:

1. When under good sanitary conditions we feed adequate or balanced diets with liberal amounts of animal protein in restricted quantities according to the paired method of feeding, the responses in growth rate and feed utilization are very small, usually ranging from zero to 3 to 4 per cent.

2. When feeding ad libitum, good responses are obtained even if they vary with the type of diet and sanitary conditions.

It does seem to me that under our conditions the great responses by using antibiotics are obtained only when the feed intake is increased. This is what could be expected from a nutritional point of view. As the maintenance quota of energy should be nearly constant, an increased feed intake will increase fat deposition and live weight gain. It remains, however, to explain why the antibiotics increase the feed intake. It is known that the feed intake is depressed when poor rations are fed. If the antibiotics promote the synthesis of essential nutrients or if the antibiotics improve the utilization of nutrients, this may be expected to raise the feed intake. This is possibly what is happening when inadequate diets are fed. The feed intake will certainly also be increased if the antibiotics eliminate harmful effects by bacteria in the intestines. I am very much in favor of this hypothesis because it seems to give a good explanation of the variability of the responses we have obtained by feeding antibiotics.

There may be some relationship between this hypothesis and the hypothesis which attributes the effect of antibiotics to the prevention of processes which render the intestinal wall less capable of functioning. I would strongly stress that this may concern not only the absorption but also the ability to consume food. If something is wrong in the intestines, the animal will certainly take less feed, and live weight gain will be depressed. If antibiotics restore the normal conditions, especially in

the upper part of the intestines, as stated by Dr. Freerksen, feed intake will increase and responses will be obtained. The responses may further be expected to vary with the severity of the conditions which are eliminated by the antibiotics.

In support of this opinion I would like to mention some investigations on the normal growth of pigs carried out in Denmark some years ago by Dr. Clausen, Professor Jespersen, and Dr. Norgaard Thomsen. I am surprised that this work does not seem to be known outside the Scandinavian countries. As a half-Dane I think that you ought to know this excellent work. Here I will mention only an important detail. It was demonstrated that the development of the intestines is enormously rapid under the most active growth period. Whether you will believe me or not, it was found that in pigs below 20 kilograms (45 pounds) the length of the intestines increased 17 centimeters (about 7 inches) per day. I repeat, 7 inches per day. Therefore, it is reasonable that anything which retards or disturbs this fast development should depress the feed intake and the growth rate.

When we heard the speakers today I think we were all impressed by what has been done to elucidate the mode of action by antibiotics. It is, however, a mass of details reminding one of a mosaic of glittering fragments. To get a general picture I feel we need a lot of metabolism experiments in addition to the simple growth experiments and the bacteriological and biochemical work. Classical metabolism or balance experiments with a determination of what is going in and out of the animal may give valuable information about the action of antibiotics. In the Scandinavian countries there are now in press two papers which give the results of metabolism experiments in pigs fed antibiotics. In these experiments the energy metabolism has also been determined. One series of these experiments was carried out in Sweden by Dr. Nordfeldt and Dr. Kihlén. Young pigs weighing from 15 to 20 kilograms were used. The pigs were fed an all-plant diet, without and with antibiotics, Aureomycin and Terramycin.

As no control groups were used, it is not easy to appraise the effect of the antibiotics. However, it deserves to be mentioned that there was a small increase in the digestibility of protein with a corresponding decrease in the digestibility of carbohydrates. The proportion of metabolizable energy to gross energy was not affected by the antibiotics. There was no increase in nitrogen retention which could indicate increased formation of structural elements. The most interesting result was a pronounced increase in the respiratory quotients for nitrogen-free substances and a corresponding strong increase in fat deposition. This partly could be ascribed to the increasing age and partly to the increased feed intake from giving antibiotics in addition to an all-plant diet.

The other series of experiments were carried out in Denmark at Møllgaard's laboratory by Ludvigsen and Thorbek. In these experiments a good balanced grain mixture was fed together with skim milk. The paired feeding method with restricted feed quantities was used. A control group got no antibiotics, and another group were given Aureomycin supplements. The number of animals was small, and the results of these experiments can therefore not be regarded as conclusive. There was nearly no response in growth and feed utilization determined in the usual way. I have already mentioned that this is the common picture we get in our Scandinavian ex-

periments when we feed a good balanced diet in restricted quantities under good sanitary conditions.

There was a small but significant increase in protein digestibility (2.2 units in digestibility coefficient), while the dry matter digestibility was not affected. There was no difference in nitrogen retention between the control group and the antibiotics group. Most noteworthy were the increases in the respiratory coefficients for nitrogen-free substances and the increases in fat deposition. There was accordingly a decrease in heat production in the Aureomycin group, indicating a higher efficiency of energy utilization. It is noteworthy that this has happened under conditions where only insignificant responses were obtained in growth rate from feeding antibiotics. It was indicated that the increase in fat deposition could depend on increased coenzyme A activity and that there was some relationship to the utilization of pantothenic acid. A higher fat deposition could also be obtained, however, as a result of a decrease in the basal metabolism. In energy metabolism experiments in swine and in statistical calculation of the existing energy metabolism work in cattle by myself, I have demonstrated that there may be an inverse relationship between basal metabolism and the efficiency of energy utilization.

To summarize: I would say that great responses by feeding antibiotics are obtained only when the feed intake is increased. This happens when the antibiotics eliminate harmful conditions in the intestines or when the antibiotics improve inadequate diets by synthesis of essential nutrients or by a greater utilization of the essential nutrients. Even under the best sanitary conditions and by feeding complete diets in restricted quantities according to the paired feeding method, antibiotics may have a small stimulatory effect which in this case cannot be ascribed to a higher feed intake. It may be that the endocrine system is involved here as far as stimulation is concerned, but in this I think Dr. Brueggemann has greater knowledge than I.

DR. BRANION: Thank you, Dr. Breirem. Dr. Brueggemann, do you care to contribute?

DR. BRUEGGEMANN: We had planned to present a report on our findings relating to work on the metabolism of higher animals, but it proved too voluminous for presentation today. I will therefore present my report on this work in a very concentrated form, read by Miss Albertine.

DR. BRUEGGEMANN (Through the interpreter, Miss Albertine): It is well known that antibiotics have an effect on growth. What do we mean by the term "growth"? An organism grows when anabolic processes are greater than catabolic processes. In the species of interest to agriculture, the anabolic phase is controlled in large part by the pituitary growth hormones and the catabolic phase by the thyroid and the adrenal cortex secretions. The life of an organism has in this sense three stages: youth, when anabolism overbalances catabolism; maturity, when the two phases are in balance; and old age, when catabolism is greater than anabolism.

We know that there are a number of non-caloric materials which promote growth when added to feedstuffs. For our discussion today we are interested especially in those materials whose mode of action is not well understood: antibiotics, arsenicals, very high levels of certain vitamins, high levels of copper sulfate, and even in some cases inactivated antibiotic materials.

Previous published work with the hormone thyroxin indicates that this hormone

activates sulfhydryl groups and accelerates catabolism. The sulfhydryl group has been shown to be the acceptor in catabolic systems involving both cortisone and thyroxin. We believe that the pituitary growth hormone influences the same acceptor system, but in the reverse direction. It has been shown in our laboratory that this growth hormone is capable of diminishing the number of titratable sulfhydryl groups in yeast cells. We have also demonstrated this effect of growth hormone, namely, reduction in titratable sulfhydryl groups with antibiotics and high levels of vitamin A. In work with tadpoles, antibiotics, vitamin E, vitamin D, vitamin T, and estrogens reduced the effect of thyroxin. These facts indicate to us that the antibiotics function systemically; that is, they have a direct effect on the intermediary metabolism.

At the present time, there are two hypotheses on the mode of action of antibiotics in promoting growth. Probably the most widely accepted is what may be called the "enteric theory." This relates the growth effect to direct action of the antibiotic upon the microflora of the intestinal tract. The other hypothesis we might call "parenteral," which postulates a direct effect of the antibiotic upon the intermediate metabolism of the host.

Even in considering the parenteral hypothesis we must recognize that metabolism of the host and its intestinal microflora are so closely related that it is often impossible to tell which is primarily affected by antibiotic.

Parenterally administered antibiotics are less effective than orally administered antibiotics in promoting growth in most cases. In daily parenteral injections in rats we in our laboratories have been able to obtain a promotion of growth similar to that produced by oral administration in this species.

On the other hand, the growth of chick embryos is not stimulated by antibiotics. The total nutrition of the embryo is, of course, adequately supplied by the yolk sac system, and environmental conditions obviously are optimal. Thus no room is left for growth promotion within the genetic capabilities of the organism.

We know that antibiotics so inactivated as to have lost their antibacterial activity also lose their growth-promoting effects, wholly or partly, although there are some experiments which show retention of growth effect, up to 60 per cent of original maxima, by inactivated antibiotics. Obviously, except for unique cases, one would expect, with fairly simple molecules such as antibiotics, to lose both growth-promoting activity and antibacterial activity when chemical structure is changed or degraded.

As indicated by Dr. Freerksen, the amount of growth promotion may be correlated with the amount of antibiotic activity absorbed through the gut wall. At nutritional levels, however, not only is it impossible to measure the proportional amount of antibiotic absorbed from the gut, but it seems to us that it may be entirely different from the proportion absorbed when the antibiotic is given at fairly high levels.

Many workers have reported that there is a characteristic change in the relative proportions of gut microflora in animals fed nutritional levels of antibiotic. In some cases there may be a relation between changes in the form of microorganisms and the growth of the host animal. Present-day bacteriological methods are not adequate to demonstrate exactly the alterations in the balance of the gut microflora. Un-

doubtedly some effect of nutritional antibiotics upon this balance does occur. Although we know that the metabolism of the gut microflora is intimately related to that of the host, we have no experimental evidence to date that shows to what extent the changes in the gut microflora may directly affect the growth of the host.

Even nutritional levels of antibiotic help reduce the incidence of nonspecific enteritis and diarrheas in many test animals. This is certainly an example of direct influence of the antibiotic on the gut microflora, but it could also be explained on the basis that the antibiotic augments the body's own defenses against these and other disease factors, possibly by supporting in some manner the body's stress response mechanism.

Chickens and swine grown in new quarters show little or no growth effect from nutritional levels of antibiotic. This finding, of course, supports the disease level theory. But we must keep in mind that here, as wherever environmental conditions are optimal, it is difficult to improve the rate of growth by any means.

As Dr. McGinnis mentioned yesterday, there is considerable evidence that with long-continued use of antibiotics at nutritional levels, there is a gradual reduction in the growth advantage which they bring about. An explanation offered for this is that the use of antibiotics has reduced the population of disease organisms in the environment so that even non-antibiotic fed animals benefit and show greater growth than before the introduction of antibiotics. Here again, however, it is also possible to postulate that the antibiotics act by enhancing the body's defenses to reduce the spread and number of infectious microorganisms in a whole area. The same effect is observed, of course, with arsenicals.

These points have been presented to show that, although the enteric theory for the mode of action of antibiotics in growth promotion certainly is strongly supported by experimental evidence to date, it cannot be regarded as proven. It seems to us that the very term "antibiotic," with its connotation of antibacterial activity, may influence experimenters to interpret their results in terms of antibacterial effect. Arsenicals, on the other hand, have historically been regarded as materials which act directly on metabolism and only lately have experimenters tended to regard them as bacteriostatic agents, primarily from their activity in promoting growth of animals. In any case, the relative importance and soundness of the enteric and parenteral hypotheses remain open for further testing by experiment. Certainly the germfree animal studies reported by Dr. Luckey will bear directly upon the validity of the parenteral mode of action hypothesis.

Another point which to us strongly supports the parenteral hypothesis is that nutritional levels of antibiotics improve growth in a wide range of species: mammals (both omnivores and ruminants), birds, plants, and even silk worms. Because of the widely different diets and widely different environmental conditions of the various species, it seems unlikely that the gut flora would be even qualitatively the same (and plants, naturally, have none at all), but the intermediate metabolism, as we all know, is much more similar. Again, there is known today a wide variety of effects of antibiotics upon the metabolism of the thyroid, the liver, on mineral balance, in vitamin-sparing action, and in radiation damage. It is rather difficult to account for all of these effects by a mode of action mediated solely through the gut microflora.

In conclusion, it is our view that the most probable result of future experimental work will be to show that both the changes in the gut microflora and the direct action by antibiotics and other growth-promoting materials at some stages of the metabolism of the host are operative in growth promotion.

DR. BRANION: Thank you very much, Dr. Brueggemann. The man I am about to present to you for a few general comments, if he wishes, Dr. Kon, is also the editor of a scientific journal, the British Journal of Nutrition. Dr. Kon.

DR. KON: We have had several interesting and valuable papers, but my feelings as I look back upon what we have heard are at one with the feelings of one of the pioneers in dairy science here who said a cow is a peculiar animal; sometimes she does and sometimes she doesn't and sometimes she does neither. I must be a bit cynical because editors who don't improve by their experience want mental power or character, but I do feel that we are not very much wiser now than we were when we began the discussion. The reason is not that we have not had the best specialists to sum up in a most eloquent way for us the facts, but it is because the facts are wanting.

As I was listening to Dr. Luckey these thoughts were passing through my head, and I must confess to the crime that when I went to put on my pad the name he suggested for antibiotics, instead of reading it correctly, I read it as "homolitigant." Well, Mr. Chairman, if I may be allowed to take Dr. Luckey's most able presentation with what we have heard since, there are two gaps in the recent argument he gave us for indicating that the accomplishment of antibiotics is linked or related with the bacterial population. One was that the germfree animals do not react to antibiotics. In this view we confess to a vested interest because the work we have done with, call it "vectors," fell in so nicely with these findings of Dr. Luckey and fell in so nicely with the beautiful concept of Dr. Damon Catron. We are now told by Dr. Luckey that under certain conditions the germfree animals will react to antibiotics.

Looking at the early publications and looking at the publications here, some people might say that these small numbers of animals would affect the validity. I would tell all these people, and myself included, if we are to criticize this work, let us get a few hundred germfree chicks and let us repeat the experiment. We cannot have it both ways. These experiments do not prove either way. Certainly we must have more work with germfree chicks to establish the fundamental question whether in the absence of a bacterial population the antibiotics will act or will alter fundamentally our attitude toward the various theories and possibilities of the mode of action or we must accept that we have sufficient proof now that in germfree conditions the antibiotics will stimulate the growth of animals.

The other point of the argument was the inactivity of penicillin rendered useless as an antibacterial. We have read recently in Nature—and the author of the paper is sitting on my right here, and the evidence of the paper, as far as one can see, is not controverted experimentally—that pigs will react 60 per cent to penicillin inactivated in such a way that it does not exert any antibacterial effects. It would be impertinent of me to say more about this work in Dr. Gordon's presence.

So to add to the confusion I would say that in experiments which we have carried out on chicks, where also the numbers were reasonably satisfactory and the evidence

fell into the level of statistical significance, we had no improvement whatsoever from the addition of penicillin deactivated in three ways. I do not for a moment suggest this as controverting Dr. Gordon's experiments because his experiments were done with pigs and ours were with chicks, but simply as illustrating the enormous complexity of the problem which we had the temerity to tackle.

We have heard that hormonal effects do not come into consideration, yet how do we explain the fact that, as Dr. Clausen told us, in experiments with thousands of pigs, gilts produced darker meat but not barrows, when they receive antibiotics. What peculiar agency acts there in such a selective action on different sexes?

To summarize my rather silly point of view, I would say that it is my impression that we are looking through the glass darkly at something where we have not yet a crisp perception of the action and that very possibly some of our methodological approach is at fault. For example, we have now gone back to school. We thought that we could examine the contents of the intestinal tract of chickens, ruminants and swine, and establish whether or not any changes take place under the action of antibiotics. We have come to the conclusion that we knew so little about the methodology of bacteriological work and the hour to hour variation between animals that we have gone back to school and are doing work now on the normal population, and in a few years we will know something about it and can tackle it again with antibiotics.

It seems that I am in complete agreement with Dr. Breirem. I think we have heard enough about purely growth experiments with animals and would very much like to see more work done along the beautiful lines about which we have heard today from two workers responsible for this fine work. I mean Dr. Brueggemann and Dr. Breirem. It seems to me the solution of this problem will be achieved not the easy way but the hard way by careful and intense study.

DR. BRANION: Thank you very much Dr. Kon. Dr. Gordon, would you care to make a few general remarks?

DR. GORDON: I am very grateful to you for the privilege of being allowed to speak in this discussion because, not being a nutritionist, I am not hampered by any exact information about that subject. I approach the problem of "Mode of Action" from the viewpoint of the veterinary microbiologist who believes that the active principle of an antibiotic is its antimicrobial and not its growth-promoting effect.

In Britain, extensive co-ordinated trials, using Aureomycin, and penicillin in pigs, were organized by the Agricultural Research Council, and these results confirm in general the American finding of increase in growth rate and improvement in food conversion in supplemented versus control pigs. When asked if our Research Station would participate in these co-ordinated feeding trials, I was reluctant to undertake the work because I felt the subject belonged in the province of the nutritionist and not the microbiologist, further, I was sceptical about the value of the procedure. However, in association with Dr. J. H. Taylor, who was responsible for the detailed work, and who is in the audience, we have carried out quite extensive experiments not only in pigs but on calves and thoroughbred foals. While it is not possible to refer to all that work here, in order to add to the list of species, the young of which show a growth response to a dietary supplement with antibiotic, I would like to present the results obtained with thoroughbred foals (Fig. 1). There were six

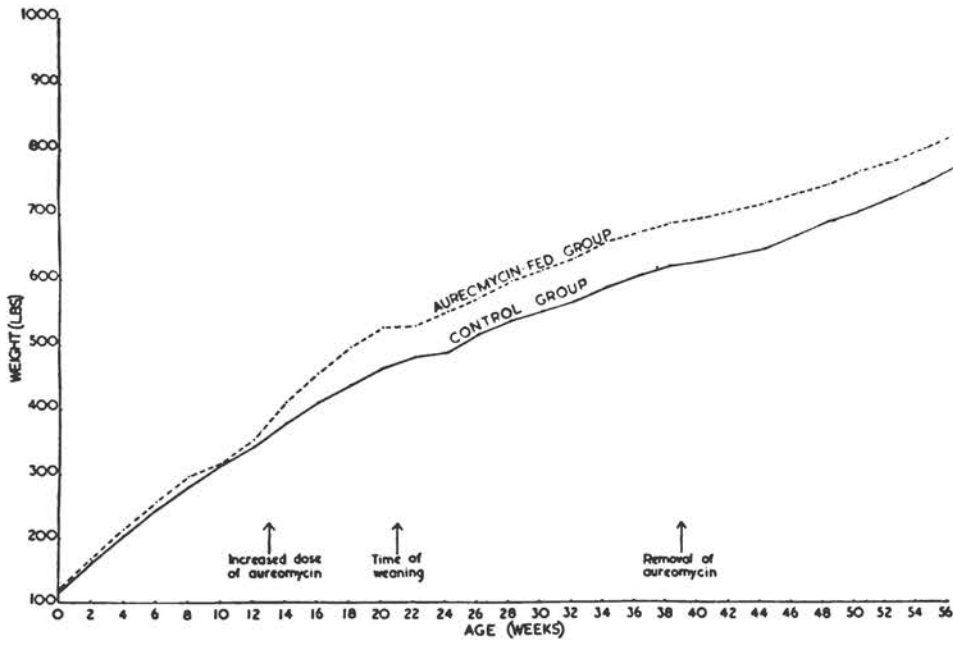

FIG. 1.—Comparison of the Mean Growth Curves of Treated and Control Groups of Foals.

foals in the supplemented group and five controls. The antibiotic was given from birth until 39 weeks of age, and the animals were kept under observation for a period of 56 weeks. Aureomycin hydrochloride was administered at the rate of 100 mg. per day from birth to 13 weeks of age and increased to 200 mg. per day from 13 weeks to 39 weeks of age. Thereafter the Aureomycin was withdrawn. The daily dose was divided into two equal portions and administered twice daily as an electuary in half an ounce of honey smeared on the back of the tongue. The increase in growth rate in the treated over the control group at 21 weeks was 14.1 per cent, at 39 weeks 10.5 per cent, and at 56 weeks 7 per cent.

As our work progressed we became more and more interested in the possible mode of action of antibiotics in improving growth rate and food conversion and we were especially attracted to the antimicrobial theory and gave special attention to following the fate of the antibiotic within the animal body. An examination of many samples of the contents of the digestive tract, of body fluids and other tissues from 480 animals (pigs, foals and calves) receiving a dietary supplement of antibiotic at varying levels has shown that both Aureomycin and penicillin can be detected in bacteriostatic concentration in the digestive tract and body fluids of these animals. To be more specific, when the level of antibiotic in the food is 16 parts per million and over, detectable bacteriostatic levels can be demonstrated in the blood and other body fluids. Further, when the equivalent of 15 parts per million of antibiotic in the food was given daily by intramuscular injection (0.5 mg. per kilo body weight) it could be shown that the concentration of antibiotic in the small intestine was approximately equal to that produced by a diet containing only 2 to 4 parts per million of antibiotic, yet the growth response to either Aureomycin or penicillin was exactly

the same by injection as by mouth. In other words, the level of antibiotic in the digestive tract following injection was not sufficient to bring about the growth response which was recorded, thus suggesting that the site of action need not necessarily be confined to the digestive tract.

Examples of detectable bacteriostatic levels in various body fluids are given in Figures 2–7. Blood levels in the pig when feeding 128 parts per million of antibiotic in the food are shown in Figure 2. Aureomycin hydrochloride is compared with procaine penicillin showing that detectable levels are still present 12 hours after feeding in the case of Aureomycin, and 7 hours after feeding in the case of penicillin. The thickness of the line on the graph indicates the range of variation in the levels detected, from which it will be seen that penicillin produced a wider variation in detectable levels as compared with Aureomycin.

The degree and duration of blood levels in the calf following regular administration of 100 mg of Aureomycin or of 100,000 international units of potassium penicillin 'G' per day are shown in Figure 3. In this case penicillin was not detectable 4 hours after feeding, whereas Aureomycin was still detectable 24 hours after feeding.

Figure 4 shows urine levels in the pig following supplementation of the diet with varying doses of Aureomycin hydrochloride. Here it will be observed that even at the level of two parts per million in the diet, detectable bacteriostatic levels of antibiotic were demonstrated in the urine. While this level of supplementation is lower than would be used in practice, it is important to bear in mind that detectable antibiotic in the urine must have been derived from the blood. Urine levels in the pig,

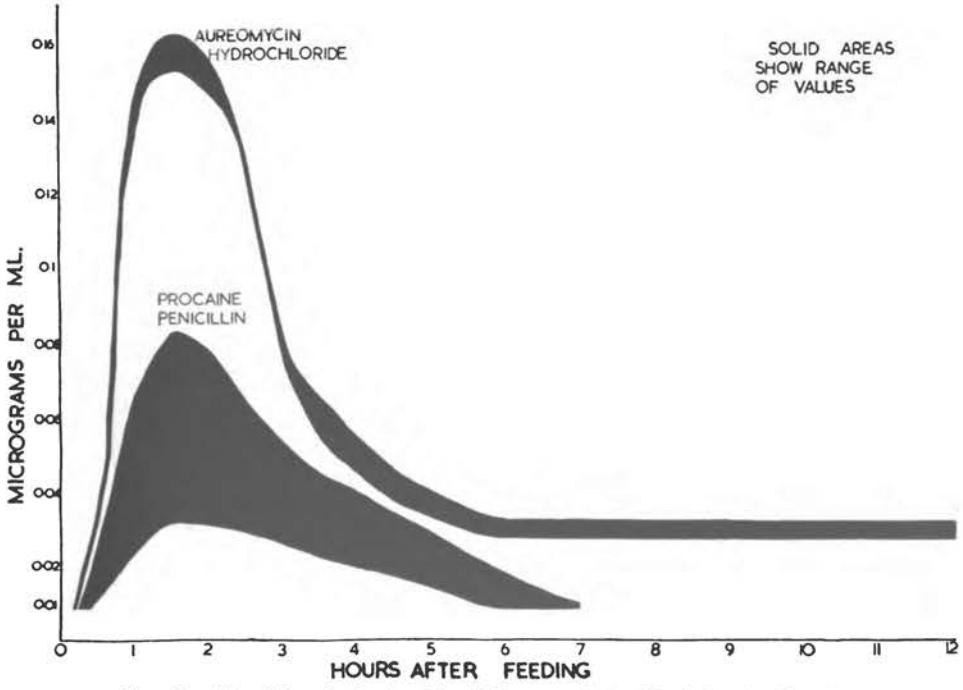

FIG. 2.—Blood Levels in the Pig (128 ppm of Antibiotic in the Food).

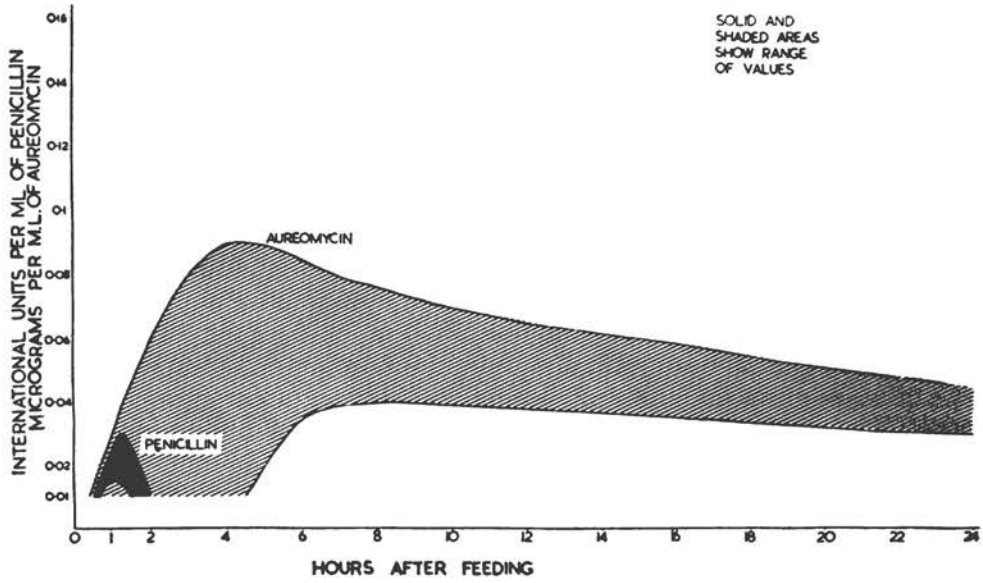

FIG. 3.—Blood Levels in the Calf. Regular administration of 100 mg. of Aureomycin and 100,000 i.u. of potassium penicillin G. Solid and shaded areas show range of values.

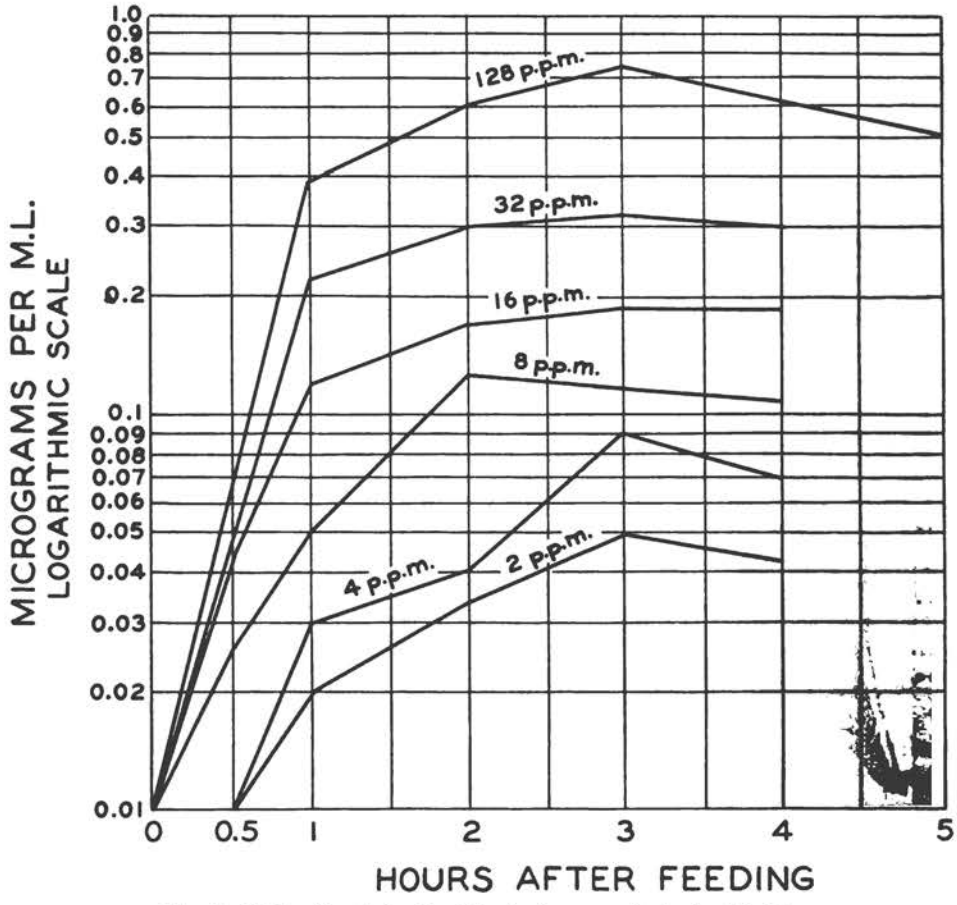

FIG 4.—Urine Levels in the Pig. I. Aureomycin hydrochloride.

using procaine penicillin are shown in Figure 5; again, even at the level of two parts per million, the antibiotic was detectable in the urine.

The effect of dose rate on the growth response and food conversion of antibiotic-fed pigs is shown in Figure 8. The increase in response with increased dosage might be due to a higher level of protection afforded by the bigger dose of antibiotic.

I venture to suggest Sir, that this work provides an important clue to help solve the problem of mode of action of antibiotics as dietary supplements, and I would like to sow the seed in your minds that we may here be dealing with the use of antibiotics in the prophylactic sense in distinct contrast to the therapeutic sense. In the therapeutic sense an antibiotic is used in massive dosage to quell an already established infection, a very heavy task, whereas in the prophylactic sense it has merely to be present in detectable bacteriostatic concentration to aid the normal defense mechanism in preventing the establishment of infections either of a sub-clinical or clinical nature. In this sense the antibiotic is not producing a growth promoting effect, but is acting as a deterrent of certain growth depressants (as yet undefined), associated with the microbial environment.

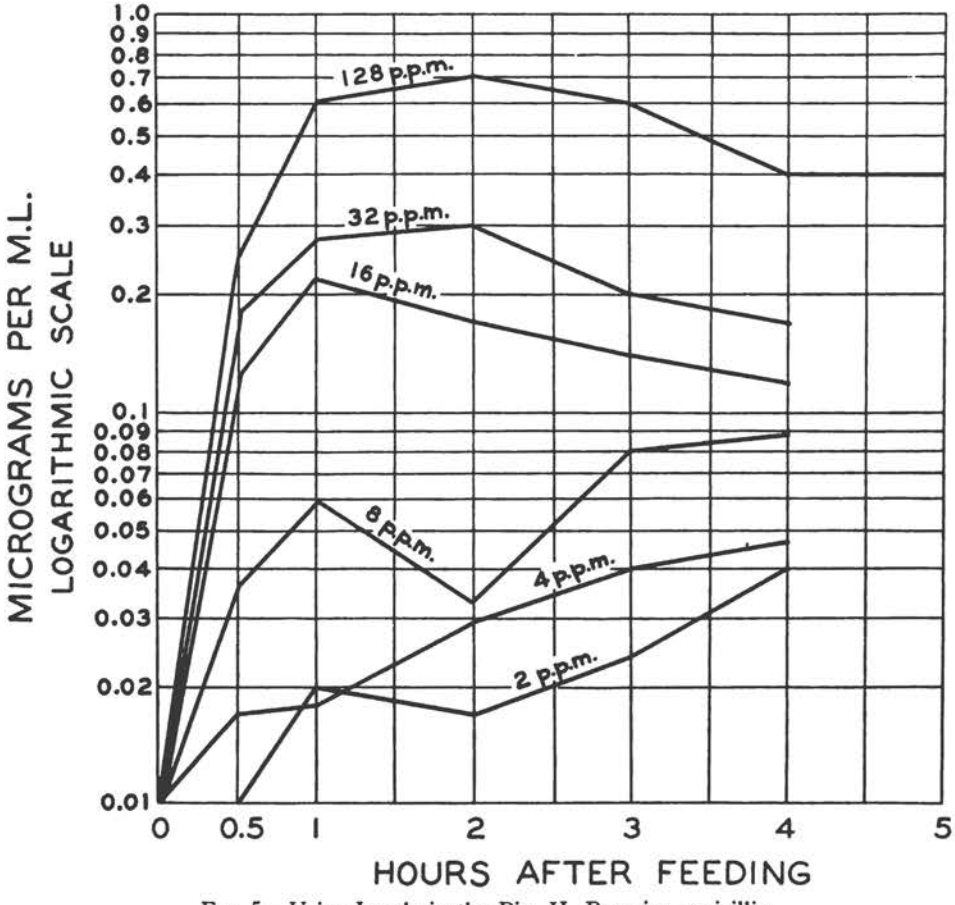

FIG. 5.—Urine Levels in the Pig. II. Procaine penicillin.

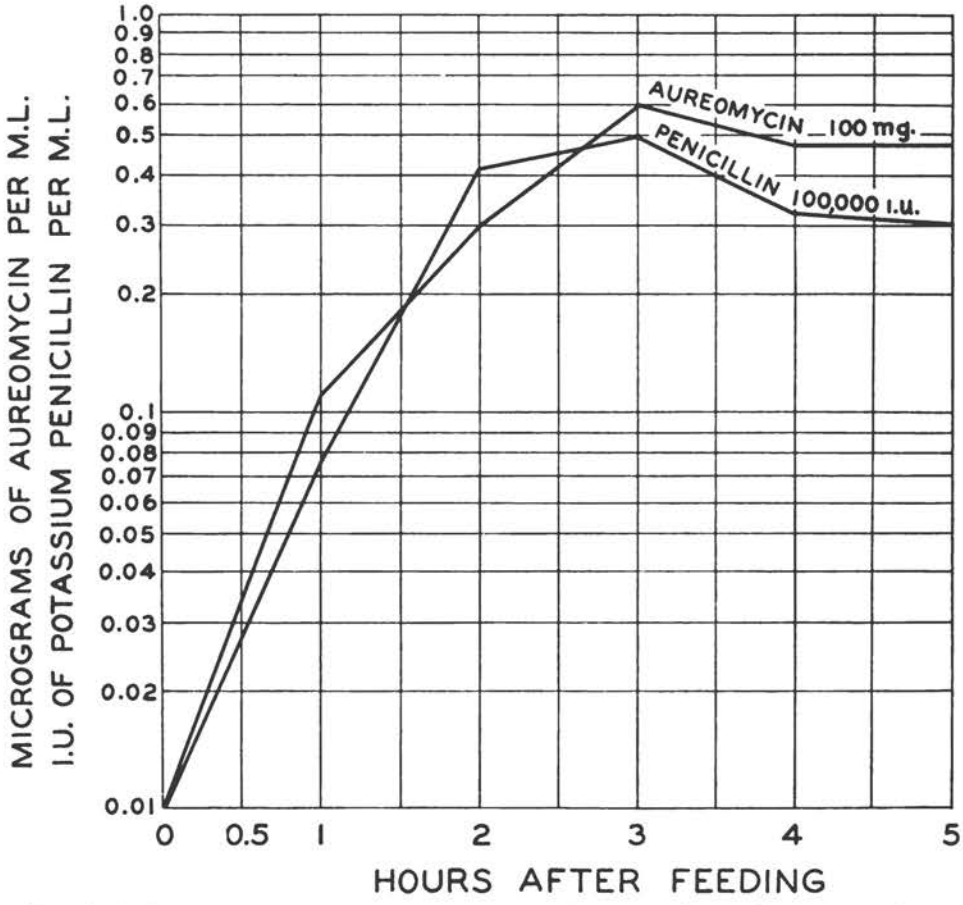

FIG. 6.—Urine Levels in the Calf, receiving 100 mg. of Aureomycin, or 100,000 international units of penicillin.

We had observed, like others, that the incidence of naturally occurring white scour in calves associated with *Bacterium coli* infection was significantly reduced by antibiotic supplements, and this prompted us to challenge a group of supplemented and control calves with an artificial infection with *B. coli*. The result is shown in Table 1.

TABLE 1

COMPARISON OF MORTALITY IN CALVES RECEIVING A DIETARY SUPPLEMENT OF AUREOMYCIN, WITH CONTROLS, WHEN EXPOSED TO AN ARTIFICIAL INFECTION WITH *BACT. COLI*.

Treatment	Number of calves	Deaths from all causes	Deaths associated with *Bact. coli* bacteraemia
Controls	25	15	14
Aureomycin-fed	25	6	4
Significance levels (Aureomycin v. controls)		P 0.02	P 0.005

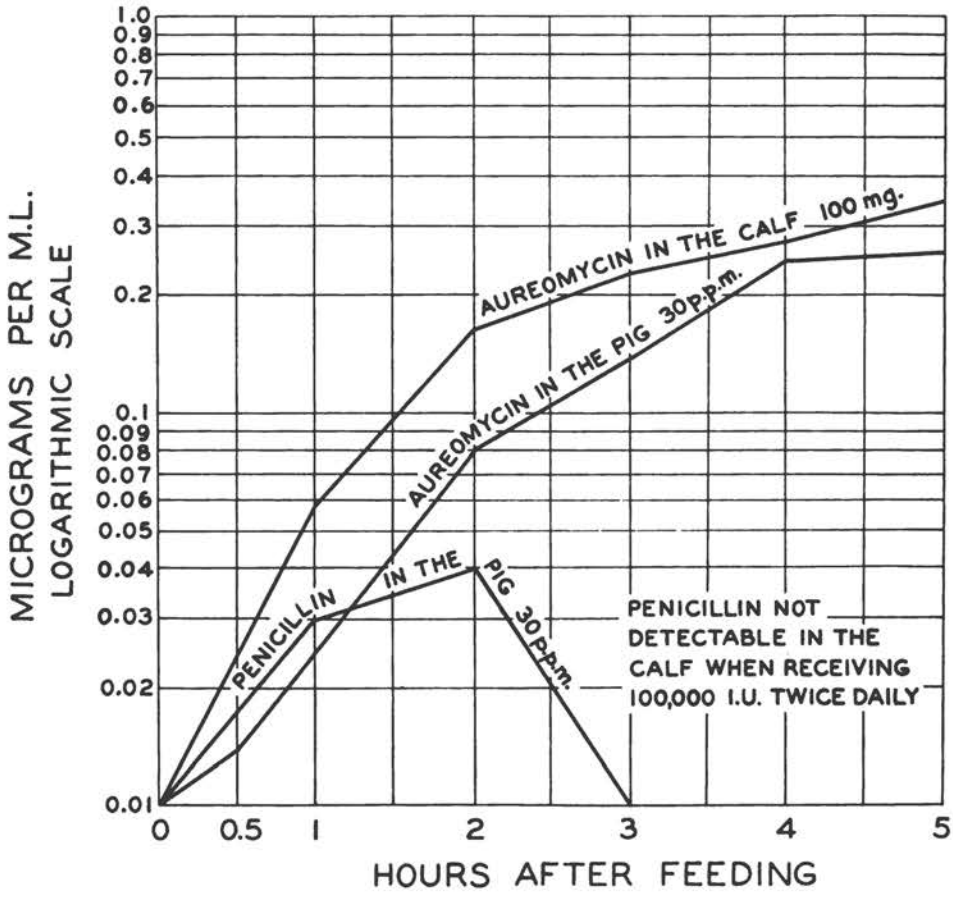

FIG. 7.—Bile Levels in the Pig and the Calf.

We used 50 calves randomised into two groups of 25 each; one group received 100 mg. of Aureomycin given as 50 mg. in the milk twice per day, and the other group acted as controls. They were kept under identical conditions, which consisted of housing them under congested conditions, feeding them irregularly and with varying quantities of milk, to which was added each week a culture of *Bacterium coli* isolated from a calf affected with white scour. It will be seen that the total mortality from all causes in the controls was 15 and in the supplemented group 6. The deaths from bacteraemia associated with *Bacterium coli* were 14 in the controls and 4 in the supplementated group. This result shows a significant difference in deaths from all causes in the two groups $p=0.02$, and a highly significant difference in the case of deaths associated with *Bacterium coli* $p=0.005$.

From this result Mr. Chairman, I submit that an important part of our future work should be directed towards challenging supplemented and control animals with a number of the different causal agents known to produce disease in young animals. I think an approach made along these lines will help towards a better understanding of the extraordinary phenomenon of the apparent growth promoting effect of anti-

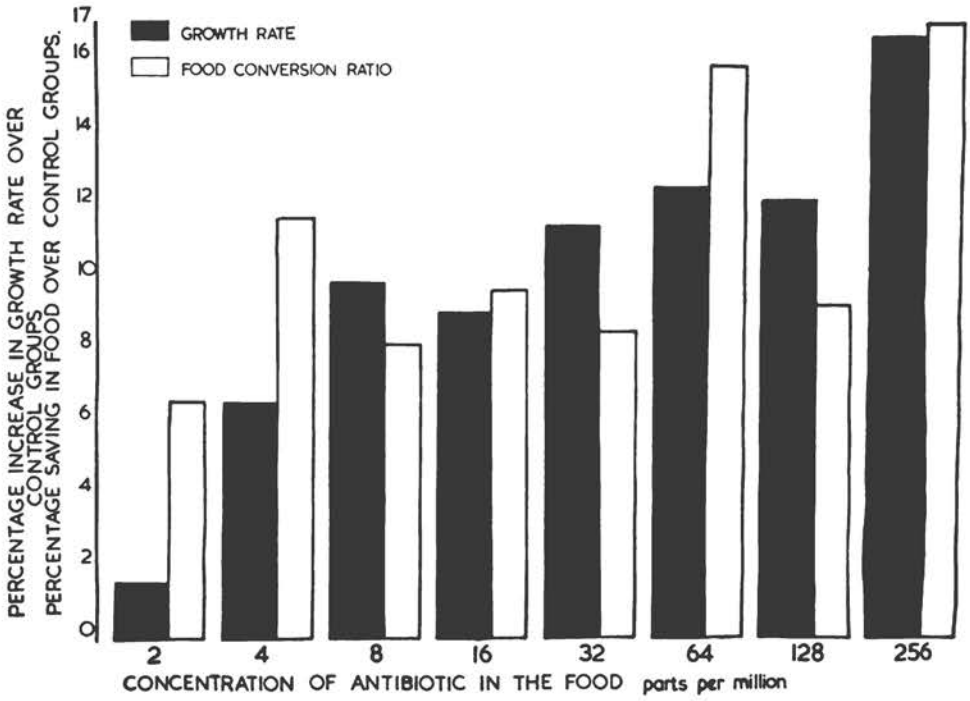

FIG. 8.—The Effect of Dose rate on the Growth and Food Consumption of Antibiotic-fed Pigs. Measurement of Mean Antibiotic Effect.

biotics. In the light of our work I much prefer the theory that the antibiotic acts as a prophylactic of growth depressants rather than as a growth-promoting substance. The mode of action of Aureomycin appears to be mainly prophylactic while with penicillin there may be in addition to the prophylactic effect, some growth-promoting factor, since, as Dr. Kon has pointed out, some growth response can be obtained with inactivated penicillin.* There may, however, be additional side effects which will yet have to be explained, such as Dr. Luckey has been discussing today. It will be interesting to repeat and extend our work before definite conclusions are reached, because the need for much replication of experiments of this nature cannot be too strongly emphasized. Meantime, however, I would like to see future experimentation developed along the lines that I have outlined today.

DR. BRANION: Thank you very much, Dr. Gordon. I am going to take just a few moments for anybody on the panel who wants to make a short comment on the first paper, given by Dr. Freerksen. Is there anyone on the panel who wants to ask any more questions of Dr. Combs? If not, the moderator does. Dr. Combs, like Dr. Kon, being a cynic, I still cannot fit in this business of loss of antibiotic potency which has occurred in Wisconsin. We have had the same thing ourselves. We did not report it because we were getting tired of confirming Wisconsin's work. Just how do you fit that into your suggestion of the disease level, and so on?

DR. COMBS: I think that fits in beautifully with the disease level concept, because

* Taylor, J. H., and W. S. Gordon. 1955. *Nature* **176**: 312.

as antibiotics are used continuously in any environment one might expect to have organisms eliminated, certain types to some degree, so that the type of flora then would become a modified flora which would be less undesirable than the original flora. Therefore, one would expect there to be less need for the antibiotic and the antibiotic to have less opportunity to improve growth.

DR. BRANION: Is there anything from the panel on the third paper, by Dr. Johansson? On the fourth paper, by Dr. Luckey, are there questions from the panel? Then we will proceed to questions from the floor. Dr. Combs?

DR. COMBS: "Have any studies been carried out on total respiratory activity of the intestinal microflora in relation to antibiotics?"

Yes, studies have been done. I think Eisenstark and Dragsdorf have done such studies where they found that the total oxygen uptake was reduced after antibiotics were administered.

DR. BRANION: Thank you, Dr. Combs. It looks as if Dr. Luckey has five or six questions. He has about ten seconds apiece to answer them.

DR. LUCKEY: The first question is "How can one completely sterilize a basal diet and retain its nutrient adequacy?"

If you get 90 per cent of thiamine destroyed during sterilization as we do, we simply put in ten times more than we need. Actually it is better to put in twenty times, and then you have a safety factor.

"How do you explain each of the differences in growth between the germfree and conventional turkeys, since turkeys respond to antibiotics to a greater extent than chicks?"

The main difference that we saw in turkeys and chickens in the germfree state was that the turkeys did respond positively to both the 25 and the 50 milligrams per kilogram level. I assume that answers that question briefly.

"What are the effects, if any, of microbial toxins and antibiotics on the permeability of gut tissues of experimental animals?"

I know nothing about this.

"Do the germfree birds that show growth response to antibiotics have thinner intestinal walls than the control germfree birds?"

The germfree bird has a thinner wall than the control bird to start with. When you add antibiotics to the germfree bird no difference is seen, as far as Dr. Gordon has reported to me personally.

"Does Dr. Luckey think that it would be useful for an experiment to be carried out simultaneously on the two levels of antibiotics which he used in these experiments to verify the toxicity or stimulation?"

I think it would be interesting. I do not think it would be essential. It would be very hard to do. I still think you would want the third control of no antibiotics and in germfree cages at the same time. I emphasize these experiments are very time-consuming, and we did roughly four years' work on three slides.

"Please define 'hormoligant.'"

The word is derived from the Greek "hormone" meaning to excite, and "oligo," small amount. A hormoligant is a compound which will excite or stimulate in small quantities. In this particular case where it was in growth, we read the stimulation

and action as a growth response. In the case of strychnine, a very toxic compound, the physician is interested in heart stimulation. To me that is a hormoligant also.

"Would it be correct to say that Dr. Luckey infers that tissue cells react in a manner essentially similar to that of bacterial cells?"

I think yes, basically. I think there are biological laws which chickens, man, and bacteria follow. In this case the law would be that living tissue responds to a stimulus. Any drug in small quantity might be a stimulus to excite the cell or the animal to prepare it for a stress. In this case the stress did not come, because we fed only a small amount of the antibiotic. So we have an over-compensation. In other words, the cell builds up a bunch of enzymes. The stress indicated did not appear, so the cell merely grows faster. Something along that line is what I am thinking.

DR. BRANION: While we are on that point I believe Dr. Jukes would like to comment further.

DR. T. H. JUKES: Dr. Luckey's theoretical structure is superficially very attractive if it were statistically or otherwise acceptable. Unfortunately, it is difficult to substantiate. In the first place, the weights and figures which he showed us appear to be statistically insignificant for a number of reasons. First, the small number of birds in each group, in many cases as low as one bird to a group. Second, the great number of variations in the experimental conditions with regard to the antibiotic used and the level used and the breed and type of animal he used. Third, there is a great variation in the weight within the groups themselves.

His second point is that Terramycin in a germfree bird at a level of 25 parts per million is stimulatory and at a level of 50 parts per million is inhibitory. This is very difficult to accept in view of the fact that, as he himself showed, in the conventional bird a level of 10,000 parts per million is not inhibitory.

Third, even if we accept the first two premises, we find on examining individual experiments that, for example, on the high level of penicillin, four experiments showed increased weight and three showed decreased weight. With Terramycin at a low level, three experiments showed increased weight; one showed decreased weight. With Terramycin at a high level, one experiment showed an increased weight, and three showed a decreased weight. So the experiment is not consistent from that standpoint, either. We are not able to have access to the sterile chambers at Notre Dame University, and I agree with Dr. Luckey that it would be very difficult to test his theory.

DR. LUCKEY: I am afraid any reply would be simply arguing further the point I presented.

DR. BRANION: Dr. Johansson, I believe you have a question from the floor.

DR. JOHANSSON: "Have you any more information on the influence of antibiotics on intestinal protozoa of different animals?"

I am afraid I have not. I think some work on this subject is in progress at Washington State College.

DR. BRANION: Dr. Gordon, I passed you a question.

DR GORDON: The question is: "How did Dr. Gordon assay Aureomycin at levels below 0.05 micrograms per milliliter?"

The answer is that Dr. Gordon did not do any of these assays, but Dr. Taylor did. He is here, and I think the questioner would very much like to hear from Dr. Taylor.

DR. JOHN HAROLD TAYLOR: I should mention that when we first started this work we could not assay Aureomycin quantitatively at these very low concentrations. Towards the end of our work, however, we were capable of detecting 0.01 micrograms of Aureomycin per ml and upon occasions concentrations down to 0.008. (Plate I.) On Plate I you will see dilutions of Aureomycin in buffer from 1.0 micrograms per ml right down to 0.007. Aureomycin is easily detectable at 1.0, 0.5, 0.25, 0.12, 0.06, and 0.03. You can see on this plate the zone of inhibition at 0.015. It is possible in the laboratory to detect the presence or absence of Aureomycin below 0.01 micrograms per ml when compared to the control. At that dilution it is obviously impossible to get an accurate assay, the question is rather whether it is detectable or not detectable.

With regard to the methods that we used, we spent sometime modifying the technique to adjust variables to give maximum sensitivity. Obviously one cannot

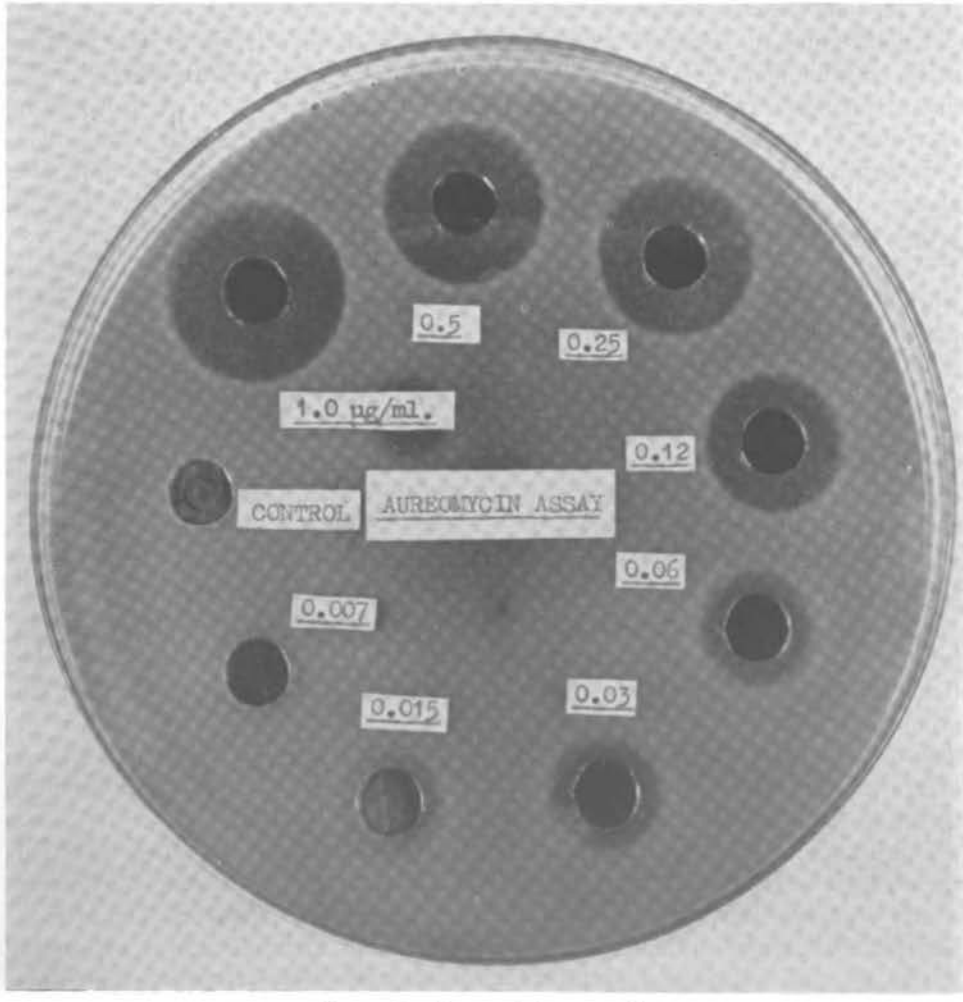

PLATE 1.—Assay of Aureomycin.

relate details here. I would mention, however, that we used a very thin layer of soft agar. I think it is of importance that after holding the plates at 4°C for two hours we incubated at 27°C instead of 37. Under the conditions I have described, we were able consistently to detect levels of 0.01 micrograms per ml and sometimes below. If there are any further specific details required, I will do my best to answer them.

DR. BRANION: There are just a few more questions that I think are worth spending a few moments on. Dr. Gordon?

DR. GORDON: I am afraid this is going to be difficult for me. The question is: "Since no bacterium grown in or on artificial media has been found responsible for poor growth of chicks, has any attempt been made to cultivate the supposed 'agent' in developing chick eggs? In other words, has an effort been made to incriminate an organism like"—something I can't read, "which is known to be susceptible to Aureomycin and Terramycin, and may occur, for instance, in the intestine of the calf for long periods?"

I don't know of any evidence in this connection at all, sir. Up to date we have made no attempt to isolate any queer organisms. We were working purely on the line of taking an organism *Bacterium coli* which had already been regarded as responsible for producing clinical disease. We think the use of known pathogens as test organisms for challenging animals receiving an antibiotic supplement in the diet along with controls is a useful procedure to obtain more evidence of the possible prophylactic effect.

With regard to infections in eggs the only person I know of who has done any work with antibiotic supplements in the egg I think is Dr. Jukes. One wonders whether he introduced the antibiotic daily while the egg was incubating and that the developing chick was continuously getting an antibiotic each day of its growth and that it finally hatched. It occurs to me there is room for experimentation there. If eggs which are known to harbor an infection or which are deliberately infected are divided into two groups, one half receiving antibiotic and the other acting as controls it would be interesting to compare the level of infection in the two groups at hatching.

I think the question is Dr. Coles', and he can tell us the organism that he was referring to here. I am sorry I cannot be more specific in my reply.

DR. COLES: I referred to Miyagawanella which is the generic name of the organisms of the ornithosis-psittacosis group. That work was done at Cornell by Dr. Baker. We know and mentioned early yesterday and again this morning that this group is highly susceptible to antibiotics. I personally feel that this group of organisms and perhaps similar organisms could well be responsible for the retardation of growth. The only animal so far in which we know definitely that the organism occurs in the intestinal tract is the calf.

I also asked what was the smallest pore through which the so-called infectious agent might be filtered, because the antibiotics, as far as one can judge, do not act on any organisms below about 70 millimicrons. I think that is correct. The Miyagawanellas and similar organisms are all approximately 70 millimicrons to roughly 150 to 200 and constitute a group which I think deserves a little more attention where the antibiotics are concerned.

DR. BRANION: Dr. Brueggemann?

DR. BRUEGGEMANN: "Can Dr. Brueggemann give us an experimental example where antibiotic directly affected titratable sulfhydryl groups in living tissue?"

Living tissue has a high content of glutathione in the form of sulfhydryl groups. If to a culture of living yeast, antibiotics are added, the content of sulfhydryl is decreased.

DR. BRANION: Does anyone in the audience wish to make a comment?

DR. R. BRAUDE: I personally have a whole theory involving the bacterial flora on which there is no time to comment now. The answer of Dr. Brueggemann has reminded me of a very recent report of Professor H. Møllgaard. With your permission, Mr. Chairman, I would like to read from it a very short paragraph.

"These experiments"—to which he refers—"seem to bring considerable confirmation to the idea, that the metabolic effect of penicillin is not due to any influence on the microflora of the intestines but to a direct effect on the metabolism common to sulfhydryl compounds. Under these conditions it is reasonable to suggest, that the basical process is the entering of the mercaptans into the coenzyme-A system of the cells, causing an increase in concentration of these systems and thereby an increase in the 'turnover' of active acetyl, which may further all the synthetic processes belonging to the cycle of Krebs."

DR. BRIGGS: Dr. Jukes would like to comment on the question Dr. Gordon asked about embryonic growth.

DR. T. H. JUKES: I believe that Dr. Gordon's question is about the rate at which we injected antibiotics in some of our work with chick embryos. Dr. Gordon, we injected the antibiotics on the seventh, eighth, and ninth days of incubation. We were careful to assay the embryos at the end of the experimental period to make sure the antibiotic was still present in the content of the egg. So we were able to show that it definitely stayed in the egg through the experimental period.

There is of course other work in that field, work with tissue cultures by Lepine and co-workers who reported no effects with low levels of antibiotics and inhibitory effects with higher levels of antibiotics on the proliferation of cells.

DR. BRANION: Does anybody in the audience want to comment on the question of permeability of the intestinal tract with and without antibiotics? That is the question Dr. Luckey said he knew nothing about. Dr. Baumann, did you mention permeability with xylose this morning?

DR. C. A. BAUMANN: I did.

DR. BRANION: Do you want to comment on this?

DR. BAUMANN: I have nothing to add to what I said this morning, namely, that preliminary experiments on the diffusion of xylose through chick intestines shows that the diffusion was somewhat greater in the intestines of chicks fed penicillin or Aureomycin. I also was careful to show the ranges of these values. At this point I am not prepared to defend them beyond the data as they were shown.

DR. BRANION: Thank you very much. Dr. Johnson?

DR. B. CONNOR JOHNSON: In our work on the mechanism of antibiotic function we have given radiothiamine labeled with C^{14} in the 2 position of the thiazole ring to rats. Antibiotic (e.g. penicillin) gives increased growth on thiamine low diets and this increased growth is brought about by more thiamine being available to

the animal as shown by increased thiamine in the carcass and an increased thiamine excretion in animals fed the antibiotic. Because of these findings we next fed radiothiamine as the low thiamine source in the diet in order to study the relative effects of the antibiotic on intestinal synthesis and on absorption of thiamine. When this was done we found an increase in the radiothiamine in the tissues of the rats fed antibiotic over those not fed antibiotic, and this was true whether the animals were pair fed or ad libitum fed. While there was also some decrease in specific activity of the tissue radiothiamine in the rats fed antibiotic this was not large compared to the total radioactivity and that data definitely indicate an increased absorption of dietary thiamine when penicillin is fed on a low thiamine diet.

DR. BRANION: We have here another question on the effect of antibiotics on fur quality. Mr. Reynolds?

MR. WARREN M. REYNOLDS: I don't know if I can give too much as to improvement in quality of fur except that it was significant to note a complete absence of "wet belly" and possibly "yellow fat" in the Terramycin-fed animals, whereas the control group had approximately a 10 per cent incidence of the above conditions. The elimination of these skin stains by the use of antibiotics suggests further investigation is in order.

We can give some information regarding increased fur yield as a result of increased animal size at pelting time with the use of Terramycin and a combination of Terramycin and vitamin B_{12}. Increased growth rates, improved feed efficiency, and reduction in mortality were obtained using the antibiotic Terramycin at the rate of 18–20 grams per ton of feed on a wet basis which was equivalent to approximately 50 grams per ton on dry basis under the condition of the trial. Apart from quality and quantity of fur, it was also observed that the pelting time for skins of animals fed Terramycin was extended.

DR. BRANION: Thank you very much. I believe we have actually covered all the questions which have come in. We have run overtime. I think the time has come for the summation, Dr. Bird.

SUMMARY OF THE THIRD SESSION

DR. BIRD: It is quite obvious that the speakers have demonstrated that this session was misnamed. Instead of mode of action, it should be modes of action. The panel members have presented evidence in support of several different ways in which antibiotics stimulate the growth of animals.

I would enumerate these methods by which antibiotics stimulate the growth of animals as follows:

1. Direct effect on the metabolism of the animal. Dr. Luckey's paper today shows that under some conditions the growth of germfree chicks was stimulated by feeding an antibiotic. This fact is of very great theoretical significance. However, neither the magnitude nor the consistency of the response was great enough to explain the effect of antibiotics on the growth of animals in agricultural practice. Furthermore, the well-known environmental effects make it clear that we have to look to the intestinal flora for at least a large part of the explanation of the antibiotic effect.

2. There is an antibiotic-mediated increase in bacterial synthesis of essential or

growth-promoting factors. This effect was discussed both by Dr. Combs and Dr. Johansson. Perhaps the best example is the increased synthesis of folacin by coliform organisms after the feeding of an antibiotic to chicks. Since growth responses to antibiotics occur in swine and poultry when no known or suspected nutrient is limiting, it does not seem likely that bacterial synthesis of nutrients is a major factor in explaining these responses. The situation is quite different in the rat, as pointed out by Dr. Johansson. In this species probably the primary effect of dietary antibiotic is to spare the limiting nutrient.

3. Effect of antibiotics on frank infection or toxemia. Dr. Johansson referred to scours in swine and cattle. Since many apparently healthy animals show increased growth when given dietary antibiotic, we must look beyond frank infections for other explanations. The levels of antibiotics commonly used in frank infections are higher than the so-called nutritional or growth-promoting levels. However, there is probably no clear line of demarcation between this effect and the next one.

4. The effect on subclinical disease. Under this heading we might include the effects on the nonphysiological flora of the upper intestinal tract, mentioned by Dr. Freerksen, and we might mention also that this effect may be largely prophylactic rather than therapeutic, as mentioned by Dr. Gordon, which perhaps accounts for the effectiveness of low levels of antibiotics. The environmental studies at Reading and elsewhere which have established subclinical disease as the primary basis of the growth-promoting effects of antibiotics in chicks have been reviewed. The definition of subclinical disease can easily be stretched to cover bacterial competition for required nutrients as well as bacterial toxin production and damage to tissue, and I would like to define it so and then divide this between two sub-headings, the first being the possibility that antibiotics may inhibit competitive organisms and exert their effect in that way. That would be one heading. It is difficult to explain the over-all growth-promoting effects of antibiotics on this basis because in most cases these effects are not eliminated, at least in chickens or in swine, or even very much reduced by feeding excesses of required nutrients.

The second heading under subclinical disease would be bacterial toxin production or damage to tissue. This of course might include the thickening of the intestinal wall, which has been mentioned repeatedly here today. If we accept the evidence for the existence of subclinical disease and reject competition for required nutrients as a major factor, we arrive at bacterial toxin or tissue damage by a process of elimination. The direct evidence for this explanation is scanty and equivocal. It has been reviewed by several of the panel members. We may say that at least the existing evidence does not exclude the possibility that subclinical toxin and tissue damage are important factors and perhaps may be the most important factors in mediating the antibiotic response, at least as we see it under field conditions.

So I have here four different effects, one of which has two subheadings, so you may say there are five. Of course, we cannot overlook the possibility, especially in the field, that these may occur in any possible combination.

It must be clear to all of us that our knowledge of the modes of action of antibiotics in stimulating growth is in a very unsatisfactory state. We can echo Dr. Freerksen's statement. After enumerating some of these possibilities, he said "All these items are important as operating hypotheses. None of them can be proved for

certain." The circumstantial evidence and process of elimination by which we reach the tentative conclusion that we are dealing with subclinical bacterial toxin production or tissue damage I should think would be especially unsatisfactory to the bacteriologist. We can only hope that it will prove sufficiently unsatisfactory to induce more of them to study these problems. Perhaps they can isolate organisms from the digestive tracts of animals which are healthy but capable of being stimulated by an antibiotic, culture the organism on an artificial medium, and then by inoculation make other animals capable of responding to antibiotics in an otherwise clean environment.

It may be that we are dealing here not with a straight line relationship between the host and a single subclinical pathogen, but rather with a mutual relationship involving the host and several different microbial species. We may all wonder with Dr. Kon how many changes occur in the intestinal microflora which we cannot detect because of limitations in methodology and how these changes may affect the biochemical balance of the animal.

I think we must refer also to the need for more experiments on the effect of environment on the antibiotic response. All of us who have worked with this subject are well aware of the perversity of the environment and how difficult it is to maintain either a clean environment or a contaminated one. You would think you could maintain one or the other, but they seem to have a capacity of shifting one to the other.

I think we need to consider that we are dealing with a relationship between host on the one hand and bacterial species on the other, and that the antibiotic has the capacity for influencing both bacterial species and apparently also directly influencing the host so that we have a number of different possible interrelationships which may enter into these effects.

The data presented by Dr. Luckey also emphasize the need for additional work to determine the basis for the response to an antibiotic under germfree conditions. Was there an effect on absorption or metabolism of some nutrient? Do antibiotics have a direct effect on gastrointestinal motility and secretion or on the function of the endocrine glands or on the circulation? In short, what is the pharmacology of antibiotics in the germfree state? It may be that these questions can be answered only in a germfree installation of the type which exists at Notre Dame, but perhaps answers could be obtained to some of these questions if someone took another look at the relatively germfree interior of the incubating egg. Dr. Johansson's suggestion of experiments with mammalian tissue culture is also very appropriate.

Let me summarize my summary in the following manner: We have discussed modes of action by which antibiotics stimulate the growth of animals. Our discussion inevitably turned out to be not so much a review of work done but rather a suggested outline for further work.

DR. BRANION: Thank you, Dr. Bird.

That is the end of the program.

. . . The meeting adjourned at five forty-five o'clock. . . .

FOURTH SESSION

A. CROP USAGE

October 21, 1955

JAMES G. HORSFALL, *Presiding*

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IMPROVING PLANT HEALTH WITH ANTIBIOTICS

Antibiosis is not a new phenomenon to plant pathologists, but the use of antibiotics as protectants against or as eradicants of plant pathogens is still a relatively new method of plant disease control. In the near future, however, antibiotics may possibly be used as widely for this purpose as they now are in control of human diseases.

Even before the discovery of penicillin, gliotoxin, the first of our present-day antibiotics, was isolated and purified by a plant pathologist(65). Because of its antifungal properties, it was studied as early as 1933 as a protectant against certain plant pathogens. Shortly after penicillin and streptomycin were released for medicinal use, several plant pathologists investigated them for control of phytopathogenic bacteria. Only limited experiments were conducted at that time because the supply was short and the cost extremely high.

Recently widespread and keen interest has developed in this type of research. Pharmaceutical firms that produce antibiotics realize the importance of plant diseases and the opportunities for marketing improved compounds having bactericidal and fungicidal properties.

Before 1951, the major investigations consisted of screening plant pathogens in vitro to determine their sensitivity to antibiotics. Since then, in addition to expanding such studies, many plant pathologists have been testing antibiotic materials in the greenhouse and field for the control of various plant diseases caused by fungi and bacteria. Although screening tests in vitro have demonstrated the effectiveness of a number of antibiotics against many pathogens, particularly bacteria, in vivo tests have usually failed to corroborate the laboratory results. Thus far, only a few antibiotics have given satisfactory disease control. At present, streptomycin appears to be the most promising. Originally thought to be solely an antibacterial agent, it has recently been shown to have anti-fungal properties under in vivo conditions.

Partially purified streptomycin is on the market under four trade names: Agrimycin 100, Agristrep, Phytomycin and Acco Streptomycin. These products are recommended for the control of fireblight of pears and apples, walnut blight, bacterial spot of tomatoes and peppers, wildfire and blue mold of tobacco, soft rot and black leg of potatoes, and bacterial wilt of chrysanthemums. Other diseases such as halo blight of beans, bacterial blight of sesame, and downy mildew of cucumbers have been controlled in the field and downy mildew of lima beans and

late blight of tomatoes have been effectively controlled in the greenhouse. The formulations tested are water-soluble and relatively stable, and at the recommended concentrations they are nontoxic to the plants tested. Agri-mycin contains 15 per cent streptomycin in the sulfate form and 1.5 per cent oxytetracycline (Terramycin) as its active ingredients. In vitro and in vivo tests have shown that including Terramycin in the formulation prevents or greatly retards the development of streptomycin-resistant strains of bacteria(25). This combination has been reported to be more effective than streptomycin alone(27a), (62a). Agristrep is similar to Agri-mycin but contains only commercial streptomycin (37 per cent) in the sulfate form. It has been reported that it lost less than 5 per cent in potency when stored for 10 weeks at 60°C.(26). Phytomycin, a liquid preparation, contains 20 per cent commercial streptomycin in the nitrate form. It remains stable for at least 18 months if stored below 70°C., but it should not be frozen. Acco streptomycin contains 45 per cent streptomycin. It is stable at room temperatures for at least two years.

The fifth antibiotic formulation on the market is Acti-dione (cycloheximide) which is antifungal only in its action. This is made by *Streptomyces griseus,* the organism that produces streptomycin. It is slightly soluble in water and stable in neutral or acid solutions, but rather phytotoxic at relatively low concentrations.

This paper, in which the more recent work on commonly known available antibiotics is reviewed, deals primarily with the control of diseases of crop plants under greenhouse and field conditions. Certain compounds on which only minor studies have been made are not discussed. Only minor attention is given to tests in vitro and to certain physiological aspects of plant responses to antibiotics. It is possible that among the large number of papers currently being published in this field in many countries, some important ones have inadvertently been overlooked.

Plant Disease Control

Fruit and Nut Diseases

Fire Blight.—Fire blight of apples and pears, caused by *Erwinia amylovora,* was the first bacterial disease of an orchard crop to be effectively controlled with an antibiotic under commercial conditions. Very promising results have been obtained with various streptomycin formulation sprays by Goodman(28), (29), (30) in Missouri, Young and Winter(66) in Ohio, Heuberger(41) in Delaware, and Ark (4), (6), (7) and Dunegan *et al.*(23) in California, Kienholz(43) in Oregon, Clayton(12) in North Carolina, Kirby(44) in Pennsylvania, and Mills(50) in New York. These sprays have drastically reduced blight infections on pears and apples wherever they were used. In 1953 Goodman(28), (29), in Missouri obtained 100 per cent control of fire blight of apples by using streptomycin, either in combination with Terramycin or alone, at concentrations ranging from 100 to 500 p.p.m. Complete control resulted where 6 applications of the antibiotic sprays were made at 4-day intervals, beginning at 30 to 50 per cent of full bloom. When the spray was applied at early petal fall or at calyx stage some blossom blight developed. In 1954 Goodman(30) using Agri-mycin at concentrations of 50 and 100 p.p.m., found little difference in blight control between a 4- and a 3-spray schedule. A 4-spray schedule with Agri-mycin at a concentration of 25 p.p.m. also gave a high degree of protec-

tion. Even though secondary blight was severe in several of the plots, trees receiving 4 or 3 applications at 100 p.p.m. or 50 p.p.m. remained relatively free of infection for as long as 30 days after the first primary infections were observed. Eradicant antibiotic sprays, applied 2 to 5 days after primary blight was observed, failed to control the disease. Young and Winter(66), in 1953, obtained 97 per cent control of blossom blight and 100 per cent control of twig blight of apples with 3 applications of streptomycin at 120 p.p.m. Terramycin sprays of the same concentration gave 97 per cent control of blossom blight and 99 per cent control of twig blight.

Ark(4), in 1953, using crude streptomycin as a dust to control pear blight, found that it was less effective than copper-lime dust, but that it did not cause the fruit russeting produced by the latter. In 1955 he (6) reported that dusts containing 500 and 1000 p.p.m. of streptomycin blended with pyrophyllite gave good control of fire blight of pear, comparable to that given by the copper-lime dusts. He noted that in these formulations streptomycin appeared to remain unchanged for a period of from 12 to 15 months. Streptomycin is readily eluted by water from pyrophyllite, thus becoming available to the plant. Other carriers such as bentonite and fuller's earth bind streptomycin in an insoluble and unavailable form. Using streptomycin formulation sprays, Ark and Scott(7) in 1954 obtained results that agreed with those of other investigators.

Dunegan *et al.*(23) reported that in tests conducted in California in 1954 pear blight was satisfactorily controlled with spray dosages of Agri-mycin as low as 30 p.p.m. applied 5 times during the blossoming period. When concentrations of 60 and 100 p.p.m. respectively, were used, the disease was almost eliminated. Dunegan also reported that 5 applications at 30 p.p.m. gave as good results as 3 at 100 p.p.m. A 7-day interval between applications was the most satisfactory.

Walnut Blight.—Ark(5) reported the control of walnut blight, caused by *Xanthomonas juglandis,* with 2 sprays of streptomycin sulfate at 10 p.p.m. The applications were made at pre- and post-bloom stages, and the control was about equal to that obtained with copper sprays. Dye and Dye(24) showed that under greenhouse conditions seedling peach trees could be protected from the blast disease of stone fruits, caused by *Pseudomonas syringae,* with streptomycin sulfate and dihydrostreptomycin sulfate sprays at concentrations of 100 p.p.m.

Cherry Leaf Spot.—Acti-dione, also, is being used in fruit disease control. Cherry leaf spot, caused by *Coccomyces hiemalis,* can be prevented by the application of this antibiotic at various periods during the growing season. Hamilton and Szkolnik (36) reported that Acti-dione, sprayed on foliage at the rate of 2 p.p.m. after an infection period of 96 hours, controlled cherry leaf spot. Cation(11) found that leaf spot was controlled by applying this antibiotic at a concentration of 1 p.p.m. twice before harvest and at 2 p.p.m. once after harvest. At present Acti-dione is being used before the formation of the fruits and after the cherries have been picked. Recent residue studies have indicated that it can probably be applied to the tree during fruit formation without leaving toxic residues. Gilmer(27) showed that a mixture of Acti-dione (2 p.p.m.) and sulfur (2 to 5 lbs./100 gals. of water) afforded excellent control of both cherry leafspot and powdery mildew of cherries when applied 4 times at 14-day intervals. Acti-dione alone at the same concentration gave excellent control of leafspot but not of powdery mildew.

Prescott, Emerson and Ford(57) showed that when cherries had been given a preharvest treatment with Acti-dione and macerated, *S. pastorianus* bioassays of the extracts detected as little as 0.04 p.p.m. of the antibiotic in the fruit. When the ripe fruit on the tree was sprayed with Acti-dione the residues were found to inactivate rapidly. The rate of inactivation was found to be much greater than that in aqueous solutions of the same pH and temperature. The investigators suggested that an enzyme system of the fruit may have been responsible for the inactivation.

VEGETABLE DISEASES

Bean Blights.—Halo blight of beans, caused by *Pseudomonas phaseolicola,* was the first bacterial disease of plants to be effectively controlled with an antibiotic under field conditions. In 1952 Mitchell, Zaumeyer, and Anderson(51) demonstrated that under greenhouse conditions bean seedlings could be protected from halo blight by the application of a minute amount of streptomycin sulfate to the stems of the plants before inoculation with the causal bacterium. The stems absorbed the antibiotic and translocated it upward into the primary leaves in sufficient amounts to protect the plants from infection.

Field studies(68) showed that when bean plants that had been artificially inoculated with the halo blight organism were sprayed 3 times at weekly intervals with 1000 p.p.m. of streptomycin they were completely protected from infection. In the untreated control, 93 per cent of the plants became infected. Later tests showed that control was equally good when the concentration of streptomycin was only 250 p.p.m.

Field tests in 1954(67) demonstrated that the halo blight organism was largely eradicated by an Agri-mycin spray. In plots treated 3 times at weekly intervals with a spray containing 200 p.p.m. of streptomycin, beginning when the first signs of halo blight appeared, 0.8 per cent of the plants were infected, as compared with 19 per cent in the untreated plots. Plots treated in a similar manner with an Agri-mycin spray containing 500 p.p.m. of streptomycin, beginning when the symptoms were more advanced, showed no active infections. In the untreated plots, 26.5 per cent of the plants were infected.

Although streptomycin protected beans from infection by the common bean blight organism (*Xanthomonas phaseoli*) in the greenhouse(51), field results have not yet confirmed these findings. Field plots that were thoroughly sprayed 2 or 3 times with streptomycin showed no common blight infection early in the season, but diseased plants were observed later. It has been demonstrated that when streptomycin is applied to beans after *X. phaseoli* has gained entrance into the plants and before symptom production, the antibiotic will not control the disease.

Marlatt(48) was unable to control common blight on Pinto beans in New Mexico with 4 applications of streptomycin sulfate or Agri-mycin spray at strengths up to 1000 p.p.m. of streptomycin. Infected seed treated for 15 minutes in a 1 : 500 aqueous solution of streptomycin sulfate produced plants with as high an incidence of infection as the controls.

Gray(32) reported that the addition of glycerin to streptomycin sprays caused marked increase in effectiveness against *Xanthomonas phaseoli* in greenhouse tests.

A spray containing 50 p.p.m. of streptomycin sulfate and 1 per cent glycerin was more effective than one containing 200 p.p.m. of streptomycin alone. One application of a solution containing 200 p.p.m. of streptomycin and 1 per cent glycerol gave almost complete control of the disease. This high degree of control was still evident 2 weeks after the spray was applied. The antibiotic was applied 3 days before inoculation and was washed off with water before inoculation. The increase in effectiveness against the disease caused by glycerol was correlated with an increase in absorption of streptomycin by the leaves.

Downy Mildew of Lima Beans.—In recent greenhouse tests at Beltsville, Md., excellent control of downy mildew of lima beans, caused by *Phytophthora phaseoli,* was obtained with the commercial streptomycin formulations(70). Spraying seedling lima beans with any of these formulations at a concentration of 100 p.p.m. gave almost complete protection to the plants later inoculated with a spore suspension of the downy mildew fungus. In repeated tests pure streptomycin sulfate sprays at concentrations of 50 and 100 p.p.m. were not so effective in controlling the disease as were the crude formulations at the same concentrations. In fact, in a few tests the crude formulations at a concentration of 50 p.p.m. gave better control than pure streptomycin at 100 p.p.m. It is possible that some minor fraction having fungicidal properties is present in the crude materials but not in pure streptomycin. In limited tests one of the commercial crude streptomycin formulations at a concentration of 50 p.p.m. of streptomycin was somewhat more effective in its control of the downy mildew fungus than were the others at the same concentration, but no apparent differences were noted at 25 and 100 p.p.m.

Streptomycin sprays containing 50 and 100 p.p.m. of streptomycin plus 1 per cent glycerin were no more effective in controlling the disease than those containing streptomycin alone or streptomycin and Terramycin in combination.

An Agri-mycin spray containing 25 p.p.m. of streptomycin and the same proportion of a neutral copper gave considerably better protection against downy mildew infection than copper alone at a concentration of 50 p.p.m. and slightly better than Agri-mycin alone at the same concentration of streptomycin(70). In numerous tests, Agri-mycin sprays at a concentration of 25 p.p.m. of streptomycin gave no protection against the fungus.

A streptomycin formulated dust containing 1000 p.p.m. of streptomycin afforded the same protection against the disease as the streptomycin sprays at 100 p.p.m. A dust containing 500 p.p.m. was ineffective(70).

Bacterial Spot of Tomato and Pepper.—Bacterial spot, caused by *Xanthomonas vesicatoria,* frequently causes considerable damage to tomatoes in the Middle Atlantic, South Atlantic and Central States. Peppers also are subject to the disease and aften are seriously damaged.

Conover(15), in 1954, reported good control of the bacterial spot in tomato plant beds with 5 applications of an Agri-mycin spray containing 200 p.p.m. of streptomycin. Of the treated seedlings, 74 per cent were disease-free and only 0.4 per cent were severely infected. In the untreated beds 12.8 per cent of the plants were disease-free and 34.8 per cent were severely diseased. In the treated beds, 95 per cent of the plants were suitable for transplanting, as compared with only 27 per cent in the untreated.

Coe(13), in 1955, reported control of the disease in Florida after it was generally established in the field and the plants were setting fruit. Five applications of Agri-mycin at 400 p.p.m. of streptomycin and 3 at 200 p.p.m. were made at approximately 4- to 5-day intervals. The treated plots showed a 42 per cent greater yield, a 28 per cent greater average fruit size and an 11 per cent greater number of fruits than the untreated plots.

Conover(16), also, reported very satisfactory field control of the disease with 7 applications of Agri-mycin at 200 p.p.m. of streptomycin. The spray applications of the antibiotic were started when the plants were about 4 inches high and were made at intervals of from 3 to 7 days. In one test there was 67 per cent less fruit infection, a 12 per cent greater average fruit size, a 50 per cent greater yield of disease-free fruit and a 25 per cent greater total yield on the treated plots than on the check plots. In a second test the sprays caused 85 per cent and 72 per cent reduction in fruit infection in areas of the field set with healthy and disease-infected transplants, respectively. A 40 per cent reduction in cracking and scarring of fruit was also noted.

Conover's(15) results in controlling bacterial spot on pepper were similar to those on tomato seedlings. Five applications of Agri-mycin at 200 p.p.m. of streptomycin effectively controlled the disease. Cox *et al.*(17) reported that 3 applications of streptomycin at a concentration of 500 p.p.m. eradicated the disease in severely infected field-grown peppers. Crossan and Krupka(20) found that Agri-mycin reduces infection of pepper leaves when used at frequent intervals at concentrations of 250 p.p.m. or higher but does not eradicate the causal organism from leaf lesions when used at concentrations as high as 500 p.p.m.

In tests during the 1954–55 season in Florida, Cox(19) found that weekly applications of streptomycin formulations gave commercial control of the disease and that the effectiveness increased up to 400 p.p.m. Yield was as much as 50 per cent more than that of the checks. Neutral coppers also effectively controlled the disease. They were compatible with Agri-mycin, and when they were used in combination with it there appeared to be a considerable additive effect. For the combined spray, the copper was dissolved in water at the rate of 2 to 4 pounds per 100 gallons and streptomycin was used at concentrations of 100 to 200 p.p.m.

Bacterial Blight of Celery.—Bacterial blight of celery, caused by *Pseudomonas apii,* is a very important disease in celery seedbeds in the Everglades section of Florida. Cox(18) reported that 5 applications either of Agri-mycin at 300 and 600 p.p.m. of streptomycin or of copper A, at 4 pounds per 100 gallons gave high degrees of control of the disease, but that the former appeared to be more effective. When copper was used, the disease gradually built up, whereas when Agri-mycin was used, the severity of the disease diminished. When these two materials were combined and applied as a spray, the mixture appeared to be more effective than either material alone, but it was not significantly better than Agri-mycin alone. No indication appeared of a true synergistic action but there may have been one of a supplementary nature.

Potato Seed Piece Decay.—Seed piece decays, caused by *Erwinia atroseptica* and *Pseudomonas fluorescens,* are frequently responsible for heavy losses of stands in

potato fields and of potatoes in storage. *Erwinia atroseptica* also causes the disease known as blackleg.

Bonde(9), in greenhouse tests, found that seed piece decays could frequently be controlled by immersing infected freshly cut potato seed pieces in a water solution of streptomycin sulfate or in a combination of this antibiotic and terramycin hydrochloride. Treatment for 10 to 30 minutes in a solution containing 25 p.p.m. of either of these preparations practically eliminated seed piece decay. Of the untreated infected seed pieces, 80 to 100 per cent decayed. Terramycin hydrochloride alone did not control the diseases. No blackleg-diseased plants were produced from seed pieces treated with streptomycin sulfate or the combination of the two antibiotics. Two to 5 per cent of the plants from the infected untreated seed pieces were infected with blackleg.

In field experimentation on commercial farms, Bonde(10) found that treating potato seed pieces in Agri-mycin solutions containing 100 p.p.m. of streptomycin produced similar results. He noted that an instant dip was as effective as a 30-minute soak. Treatment increased the emergence of the potatoes, reduced the percentage with blackleg, improved the color, size, and vigor of the plants and increased the yield.

When cut seed pieces were treated in Phytomycin solutions containing 50 and 100 p.p.m. of streptomycin nitrate, seed piece decay was reduced and plant height, number of stems per hill, number of flower clusters per plant and yield rate were increased. Webb(64), in tests conducted in Maryland in 1955, found that in the absence of seed-piece decay treating seed pieces with Agri-mycin had negligible effects on plant vigor and yield. Phytomycin-treated tubers, in contrast, produced more vigorous plants than untreated tubers, and the yield was approximately 15 per cent more.

Black Rot of Rutabaga.—Black rot of crucifers, caused by *Xanthomonas campestris,* is a seed-borne disease, and its control is difficult. Sutton and Bell(60) reported in 1954 that treating rutabaga seeds in aqueous solutions of Aureomycin provided effective control of seed-borne infection of the black rot organism. When Aureomycin-treated seeds were placed on agar plates, no colonies of the pathogen were observed; in the untreated checks, 88 per cent of the seeds were infected. Greenhouse soil tests showed that seed treatment increased germination. At 1 to 2500 and 1 to 1000 dilutions the organism was eliminated; in the untreated check, 76 per cent of the seedlings were infected. Hot water and chlorine (2 per cent) gave comparable control but lower germination of seeds than Aureomycin.

Similar results were observed in field trials. Mature roots of plants from Aureomycin-treated seed showed no evidence of infection, while 30 per cent of those from untreated seed showed infection.

Angular Leaf Spot of Cucumber.—Doolittle and Beecher(22) found that Agrimycin and Agristrep at 400 p.p.m. of streptomycin protected field-grown cucumber plants from infection with *Pseudomonas lachrymans.* When streptomycin was applied 1, 2, or 3 days after artificial inoculation, the reduction in infection was approximately the same as when it was applied 1 day before inoculation. With a very heavy primary infection of the organism, secondary infection was reduced on an average to less than one infected leaf per plant when the plants were sprayed 5,

10 or 15 days after inoculation with 400 p.p.m. of streptomycin. The use of this high concentration does not seem to be warranted in some commercial cucumber plantings.

Downy Mildew of Cucurbits.—Coe(14) reported control of downy mildew, caused by *Pseudoperonospora cubensis,* with 7 applications at 4- or 5-day intervals of Agri-mycin at 200 p.p.m. of streptomycin and 100 p.p.m. of streptomycin plus 2 and 4 pounds of tribasic copper per 100 gallons of water, respectively. There appeared to be an additive effect of the copper in control of the disease, as previously reported by Cox(18), (19).

Downy Mildew of Onion.—Nelson(55) reported significant yield increases in onions infected with mildew, caused by *Peronospora destructor,* when they were sprayed with a 2 per cent Acti-dione-sulfur mixture. This mixture was not, however, so effective as Dithane Z 78-sulfur dust. The antibiotic dust was superior to the Dithane mixture in controlling mint rust(54).

TOBACCO DISEASES

Wildfire.—Heggested and Clayton(37) reported that streptomycin sulfate gave excellent control of wildfire, caused by *Pseudomonas tabaci,* in tobacco plant beds that had been artificially inoculated. Sprays at the rate of 10 gallons per 100 square yards were more effective than drenches or dusts. The first treatments were applied when the plants were in the two-leaf stage, and 3 additional sprays were applied at weekly intervals.

The most efficient control resulted from Agri-mycin and Agristrep at the rates of 200 p.p.m. In the plant beds treated with these antibiotics no plants were killed by wildfire, whereas 7 per cent of the plants treated with bordeaux and 20 per cent of those treated with tribasic copper died from it. In the untreated checks, 30 per cent of the plants died. In the plots treated with Agristrep and Agri-mycin, leaf infection of the surviving plants was practically negligible; in those treated with the copper compounds, incidence of infection was considerable.

In addition to protecting tobacco plants from wildfire infection, streptomycin sulfate sprays eradicated the disease. Seriously infected plants in growers' beds were sprayed 3 times at weekly intervals with streptomycin sulfate at a concentration of 200 p.p.m. When the treatments were terminated, one-third more of the plants were surviving in the treated areas than in the untreated ones. Only 5 per cent of the leaves from untreated areas that were examined showed no wildfire infection, while 79 per cent of those from the sprayed plots were free. Plants treated with streptomycin sulfate sprays at concentrations of 200 and 400 p.p.m. had larger and more fibrous roots than plants that survived in the untreated plots and in those sprayed with fixed copper.

Beach and Engle(8), in 1955, reported excellent control of wildfire with Phytomycin in Pennsylvania. Tobacco seedlings were sprayed 4 times at intervals of 8 to 10 days with a solution containing 100 p.p.m. of streptomycin. Kirby(45), also in Pennsylvania, showed that Agri-mycin at a concentration of 100 p.p.m. of streptomycin plus ferbam, 3 pounds per 100 gallons of water, applied 4 times at weekly intervals prevented the development of wildfire in the seedbed and reduced the

amount of infection developing in the field. When 4 weekly sprays at 200 p.p.m. were applied to infected plants, the spread of the wildfire organism was checked and thus the plants were enabled to grow normally. In the area of heaviest infection, wildfire was checked by the spray and a field crop of tobacco plants free of wildfire was obtained.

Blue Mold.—In greenhouse tests, Grosso(35) controlled blue mold, caused by *Peronospora tabacina,* with streptomycin sulfate. This was the first report of control of a fungus disease with this antibiotic. Young tobacco plants were sprayed with aqueous solutions of 10 antibiotics at 100 p.p.m. and with Dithane. Four applications were made at 3- or 4-day intervals. The day after the second application, the plants were inoculated with spores of the blue mold fungus. Streptomycin reduced leaf damage due to the blue mold fungus to 1.8 per cent as compared with 30 per cent in the untreated check. Spraying with other antibiotics was not effective. Dithane-treated plants had 1.7 per cent leaf damage due to the fungus.

In another experiment plants sprayed with streptomycin at 200 p.p.m. showed 1.1 per cent damage as compared with 50 per cent on plants in the untreated check. Plants treated with Dithane showed 12.7 per cent damage.

DISEASES OF OIL SEED CROPS

Bacterial Leaf Spot of Sesame.—Thomas(61) demonstrated in field tests in Maryland that soaking sesame seed infected with *Pseudomonas sesame* for 30 minutes in an aqueous solution of Agri-mycin containing 250 p.p.m. of streptomycin completely controlled leaf spot. Plants grown from seeds soaked in water for the same period were almost completely defoliated by the leaf spot organism. Soaking seed in solutions containing 500, 750, and 1000 p.p.m. of streptomycin for 30 minutes caused no injury to the seedlings.

DISEASES OF ORNAMENTALS

Bacterial wilt of chrysanthemum, caused by *Erwinia crysanthemi,* was shown by Robinson, Starkey, and Davidson(58) to be controlled by streptomycin or Terramycin. Chrysanthemum cuttings were soaked in water solutions of the antibiotic for 6 hours, washed, inoculated with the bacterial wilt organism, and planted in sterilized sand. A high percentage of the treated cuttings survived, whereas all the untreated cuttings died after 25 days. Streptomycin was more effective than Terramycin.

Cuttings inoculated with the wilt organism and treated later with the two antibiotics showed a high survival after 20 days, while all the untreated cuttings were killed. As in the protection experiment, streptomycin gave better control than Terramycin.

Treating sand with streptomycin eliminated established infection in cuttings planted therein. Treating the cuttings with hormone powder containing streptomycin also controlled the disease.

CEREAL CROP DISEASES

Wallen(63), using several antibiotics as sprays for the control of wheat rust, caused by *Puccinia graminis* var. *tritici,* found Acti-dione the most promising. Con-

centrations of 50 to 500 p.p.m. were phytotoxic. The degree of infection ranged from 0 to 5 per cent in the plots sprayed every 10 days with a 500-p.p.m. concentration but from 50 to 60 per cent where the concentration of the spray was 25 p.p.m. All plants in the control plot were infected. The plots sprayed before heading, after heading, and at 10-day intervals consistently outyielded the unsprayed control plots. Emergence from seed of plants sprayed with Acti-dione was over 90 per cent in all treatments.

Leben, Arny, and Keitt(47), in small-scale greenhouse and field tests, found that seed treatment with crude helixin B controlled helminthosporium blight of oats, caused by *Helminthosporium victoriae,* and of barley, caused by *H. sativum.* In limited tests, the antibiotic was effective also in controlling wheat bunt, caused by *Tilletia foetida;* oat smut, caused by *Ustilago avenae;* and covered smut of barley, caused by *U. hordei.* Evidence as to the practical applications of this antibiotic as a seed treatment is still inconclusive. Henry *et al.*(39) found that a rapid seed treatment of wheat with 0.5 and 1 per cent Acti-dione dust mixed with Dixie clay at the rate of 0.5 oz. per bushel gave almost complete control of covered smut of wheat. Seedling emergence was not greater than that of the untreated check; in fact, where 1 per cent dust was used emergence was slightly reduced. Later, Henry, Peterson, and Millar(40) demonstrated that when the organic fungicide captan, which when used alone did not give satisfactory control of the disease, was added to the mixture of Acti-dione and Dixie clay, emergence was improved and the smut was well controlled.

DISEASES OF TURF GRASS

Vaughn and Klomparens(62) found that Acti-dione gave excellent control of dollar spot of turf, caused by *Sclerotinia homoeocarpa,* and of melting out of turf, caused by Helminthosporium sp. Howard(42) reported it to be effective in controlling the fading out disease of turf, caused by *Curvularia lunata.*

VIRUS DISEASES OF PLANTS

Thus far, only limited investigations have been conducted on the inhibition of plant viruses by antibiotics. Leben and Fulton(46), in detached-leaf studies, demonstrated that streptomycin and Terramycin both prevented the production of local lesions on cowpea leaves by tobacco necrosis and tobacco ring spot viruses. Only partial inhibition of the tobacco mosaic virus was obtained. The writers suggested that inhibitory effects were due to action on the host, since the antibiotics did not influence the infectivity of the viruses when tested in vitro.

Schlegel and Rawlins(59) studied the effect of MK61 in leaf discs infected with tobacco mosaic virus that were floated on solutions of this antibiotic. Quantitative data showed that the antibiotic at 10 p.p.m. and higher produced 69 to 90 per cent inhibition of virus infectivity. The investigators assumed that it directly affected virus multiplication rather than host metabolism.

Mode of Action

Anderson and Nienow(3), in 1947, demonstrated that streptomycin was absorbed by soybean plants growing in a nutrient solution containing the antibiotic. The ex-

tracted sap contained 4 to 5 p.p.m. of the antibiotic. Mitchell, Zaumeyer, and Preston(52), (53), having applied streptomycin in a lanolin paste to the stems of bean plants, detected the antibiotic in the juice of nearby leaves. When filter-paper discs impregnated with this juice were placed on agar plates seeded with *Xanthomonas phaseoli,* inhibition zones became evident around the discs. Similarly, a streptomycin-dependent strain of *Escherichia coli* grew on agar that contained the diffusate from the juice of plants whose stems had been treated with streptomycin sulfate but did not grow on agar that contained the diffusate from the juice of untreated plants.

Streptomycin was rapidly absorbed by the stems and translocated to primary leaves, for measurable amounts of the free antibiotic were detected within 8 hours after treatment. Concentration of the free antibiotic was greatest during the third day. It was shown that as much as 67 per cent of the total streptomycin applied to the stems was translocated to the primary leaves as free streptomycin during the 5 days immediately following treatment.

Primary leaves inoculated with the halo blight organism about 10 minutes after the stems were treated with streptomycin failed to develop visible symptoms of halo blight. Streptomycin was shown to be translocated upward from the stems and from the base of the leaves to the tip. Translocation of the antibiotic from the leaves and stems into the pods was not observed.

Robinson, Starkey and Davidson(58) showed that both streptomycin and Terramycin were readily absorbed and translocated in chrysanthemum cuttings and less readily in rooted plants. In treated cuttings the antibiotics persisted in sufficient amounts to protect the newly developing plants from infection. Streptomycin remained active in plants for several weeks.

Pramer(56) and Crowdy and Pramer(21) found that some antibiotics were readily absorbed by some plant species but others were not. Distribution within the plants varied with both the plant species and the antibiotic tested. Some of the antibiotics translocated moved more readily than others in all plants tested. Some appeared to move more freely in certain plants than in others. Readily translocated antibiotics are either neutral or acidic substances (griseofulvin and penicillin). Inconclusive results are reported for the basic (neomycin and streptomycin) and amphoteric antibiotics (Aureomycin and Terramycin).

Goodman and Hemphill(31) reported that when indole-3-acetic acid was added to an antibiotic spray formulation containing 250 p.p.m. each of streptomycin and Terramycin and 1 per cent each of methyl cellosolve and Carbowax 400 fire blight control was markedly increased in apple shoots that were inoculated 24 hours after application of the spray. Later Hemphill and Goodman(38) demonstrated that ethyl-3-acetate was just as effective as indole-3-acetic acid, in concentrations as low as 50 p.p.m. They suggested that the property of increasing the efficacy of antibiotics is not restricted to these two compounds but is shared by a large group of plant growth regulators. Most of these materials demonstrated activity similar to that obtained with indole-3-acetic acid and its ethyl ester. The authors concluded that the increased disease control was not effected by action of the growth-regulating substances on the pathogen but reflects some host-plant reaction to the growth-regulating substance such as increased water uptake or increased permeability of

cell membranes, each of which might facilitate the movement of the antibiotic. Also, increase in metabolic rate resulting from application of these substances may speed maturation and reduce susceptibility of the tissues.

Gray(34) demonstrated that streptothrycin and pleocidin, which were readily absorbed by leaves of tobacco, tomato and bean plants when sprayed on the intermediate leaves, were translocated out of the leaves and not only up the stem to the young leaves, but also down to older untreated leaves and, in some cases, to the roots. Under the same conditions no translocation was detected with streptomycin, neomycin, bacitracin or actinomycin. Where one primary leaf of a bean plant was immersed in a vial containing 2000 p.p.m. of the antibiotic in an aqueous solution, movement out of the treated leaf and up to the young trifoliate leaves occurred with Chloromycetin, penicillin, streptomycin, streptothrycin, pleocidin and viomycin. Movement across the stem to the opposite primary leaf, also, occurred with all these except Chloromycetin.

Using a spray containing 500 p.p.m. of streptomycin and 1 per cent glycerin, Gray(33) found that much greater absorption of the antibiotic occurred in 3 hours after application to bean leaves than where streptomycin was used alone. After 6 hours the absorption was 5 times as great, and after 96 hours, it was 23 times as great. The absorption of Chloromycetin and streptothrycin, also was increased by the addition of glycerin, but not that of oxytetracycline and neomycin.

Alcorn and Ark(1) reported more rapid translocation of Aureomycin, Terramycin and tetracycline than of streptomycin and neomycin in Pyracantha cuttings. In carnation cuttings, also, tetracycline moved more rapidly in the stems than streptomycin. Adding 1 per cent K_2HPO_4 to streptomycin solutions in which carnation cuttings were placed increased the rapidity and the distance of movement of the antibiotic.

Research Progress and Needs

In the relatively few years that antibiotics have been studied in the field of plant therapy, rapid advances have been made. Possibly the greatest stimulus to this type of research was the discovery that certain antibiotics inhibit bacterial plant pathogens in vitro. This discovery opened up control possibilities for bacterial diseases that hitherto had not been effectively controlled by the usual organic or inorganic sprays. It was of special importance because breeding for resistance to these diseases has not been particularly effective.

Prior to 1952, research on antibiotics in relation to plant health was conducted at relatively few institutions in the United States or elsewhere. At present, at least 50 plant pathologists in State and Federal agencies and many others employed by private companies are engaged in antibiotic research for plant disease control. A number of the major producers of antibiotics in the United States are expanding their research facilities and have planned programs specifically to develop antibiotics for plant disease protection. This expansion indicates that the future in this field is bright.

The promising results obtained by the use of streptomycin in the control of many bacterial diseases are most encouraging. It is to be hoped that antibiotics yet undiscovered will effectively control many other bacterial parasites.

The control of a large number of fungus diseases with antibiotics may not be accomplished as rapidly as that of bacterial diseases, because of the diversity of the fungi causing diseases, their varied responses to antibiotics, and the different reactions of the hosts to them. Thus far no antibiotic with a wide in vivo antifungal spectrum has been reported. Just a short time ago, Acti-dione appeared to be the only antibiotic that showed promise as an antifungal agent. Recent studies have demonstrated that streptomycin may become an important fungicide in the control of certain diseases caused by some Phycomycetes, including blue mold of tobacco, downy mildew of cucumber, downy mildew of lima bean, late blight of tomato and potato, blue mold of spinach and several others. Since a large number of the Phycomycete diseases are of considerable economic importance, their control with antibiotics would greatly expand the use of these materials in agriculture.

What is needed in antibiotics for plant disease control is not difficult to describe, but to accomplish the goal may be difficult.

In general, plant bacterial parasites that invade the vascular system, such as the various wilts, have not responded to the available antibiotics as have those that primarily invade the parenchyma. An antibiotic that would be effective for this group of diseases would be very useful.

The production of more effective antibiotic eradicants is greatly to be desired. For example, streptomycin effectively eradicates some bacterial diseases but not some others such as fire blight of apples and pears.

An antibiotic or a mixture of antibiotics with a wide antifungal spectrum is desired. A mixture of crude antibiotics may furnish the desired wide spectrum more readily than would a fairly purified preparation. The finding that crude formulations of streptomycin give more efficient control of downy mildew of lima beans than the purified antibiotic, appear to open up an interesting field of investigation.

The development of an antibiotic that can be applied to the soil for control of root parasites is greatly needed. Such a chemical must be very stable under soil conditions, must not be adsorbed by soil particles, must not destroy beneficial microorganisms and must be non-phytotoxic. In addition, it should be readily absorbed by the roots and translocated in the plant.

Oligomycin, an antifungal antibiotic, may fill this need according to recent results obtained by scientists at the University of Wisconsin(49) where it was discovered. It has been demonstrated that when oligomycin is added to the soil it does not decrease soil microflora in numbers or in activity of important physiological groups tested. It is completely inactive against all bacteria. It is relatively stable in soil up to 10 days but disappears within 3 weeks. Its solubility in water is limited, but it is highly soluble in alcohol, fat solvents and fats. When an alcoholic solution is added to water a colloidal suspension results, which is stable. Plants sprayed with oligomycin preparations up to 3000 p.p.m. showed no toxicity. Plants have withstood at least 150 p.p.m. applied in daily waterings. Seeds treated with solutions containing 100 to 150 p.p.m. of the antibiotic germinated normally. Oligomycin absorbed by plants from aqueous solutions accumulates in the roots and has been detected in the stems. About 28 plant pathogens have been tested in vitro of which 19 were sensitive to <5 p.p.m. and 11 to <2 p.p.m. Tests on the control of plant diseases are still very limited, but greenhouse results showed that oligomycin, when added to the soil in

which alfalfa seed was planted and later applied to the seedlings in waterings gave complete protection against *Pythium spp.* causing damping-off.

It has been stated that species of Pythium, all together, constitute one of the most destructive groups of plant pathogens. I believe that many species of this group causing root rots and damping-off of seedlings could be controlled by streptomycin compounds if they retain their activity when applied to the soil. This belief is based on the fact that streptomycin formulations have effectively controlled two species of Phytophthora causing downy mildew of lima beans and late blight of tomato and one species of Peronospora causing blue mold of tobacco, both of which genera are closely related to the genus Pythium. Production of an antibiotic that, when applied to the soil, would control Pythium species and other soil-inhabiting organisms such as Fusarium and Rhizoctonia that are unhampered by our best present control methods is a real challenge to the industry.

Filipin, another new antifungal agent recently reported by Ammann(2) may be helpful in this regard. It has shown protection against some seed-rotting fungi, and in dust seed treatments, filipin completely protected cucumber and spinach seedlings against pre-emergence damping-off caused by *Rhizoctonia* sp. Spraying young tomato and bean plants with 1000 and 500 p.p.m. of the antibiotic respectively showed no phytotoxicity. It is nearly insoluble in water.

With the exception of the two antibiotics reported earlier by Gray(34), those that have been investigated in relation to translocation in plants have all been shown to move in an upward direction. Screening for additional antibiotics that would move downward and into the roots might open up possibilities for control of root rot parasites.

An antibiotic effective against viruses is greatly to be desired, because of the large number of destructive virus diseases. Such an antibiotic would be particularly valuable in relation to the many virus diseases of stone fruits, which cannot be controlled by any means now known.

Regardless of how efficient antibiotics may be in the control of plant diseases, their use will be restricted unless the cost of using them will allow a normal margin of profit to the grower. Antibiotics are now used on crops of high per-acre value or where control requires only small quantities of spray materials, as on seedbeds. They could be used generally on crops of low per-acre value if only a single application were necessary to prevent secondary dissemination of the pathogen. They can economically be used on crops that require low dosages or if the pathogen can be controlled with a low concentration. Effective antibiotics would be practical as seed treatments where only small quantities of the materials would be necessary. Within the past 9 months the cost of the crude streptomycin formulations has been reduced by approximately 20 per cent; and it is believed that when a large potential use of antibiotics is assured the pharmaceutical industry, with its present-day production methods, will produce and market these products at prices sufficiently low to permit the average grower to use them. Likewise, if and when effective crude antibiotics are produced specifically for plant disease control, it seems conceivable that the cost of production will be less for these than for the antibiotics now being used, which are crude forms of antibiotics produced primarily for medical purposes.

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Crop Usage

PANEL DISCUSSION

Moderator

James G. Horsfall

Panel Members

Clyde M. Christensen
E. N. Mishustin
N. A. Krasilnikov
Harry C. Young
George L. McNew
W. J. Zaumeyer

Summarizer

John C. Dunegan

Dr. Horsfall: The first panelist we will hear from today will be Dr. C. M. Christensen of the University of Minnesota.

Dr. Christensen: Most of my work deals with the deterioration of stored seeds by fungi. The fungi which operate there are operating under rather peculiar conditions. Essentially they are growing in an atmosphere with a relative humidity of 70 to 85 per cent. No free water is available. Some of those fungi practically are poisoned by freed water. Therefore, fungicides and antibiotics which depend for their activity upon being dissolved in water just have very little effect on these fungi which cause deterioration of stored seed.

We can apply good fungicides which are commonly used in seed treatment and store the seeds at a relative humidity of 70 or 75 per cent. The molds grow right through the fungicide or with a little tuft of fungicide on top, at the head of the mold. So that is one situation where it looks like some of our present fungicides and our present antibiotics just are not going to work. If we are going to get antibiotics which will operate under those conditions, it will have to be something different than we now have.

Dr. Horsfall: Thank you, Dr. Christensen.

At this point we will hear from Dr. McNew of the Boyce Thompson Institute.

Dr. McNew: I think it is perfectly obvious from the few things which were said by Dr. Zaumeyer that we know very little about the use of antibiotics in the plant field. The very fact that we know that we know nothing puts us ahead of those people who were up here yesterday. There are a few things which should be said, however. One is that it is peculiar to know that a profession which knew these things as early as 1918 and had a good grasp of them by 1933 and 1934 has waited this long to capitalize on a perfectly natural phenomenon. As a matter of fact, one of those young men by the name of McNew in 1934 published a paper describing inhibition of seedborne pathogens by the use of filtrates from a number of fungi. I think the significance of that may have missed some of you.

We have in the bacteria a group of pathogens which invade plants through natural openings or through insect transmission. Therefore, the ordinary type of plant protectants that we have developed in the chemical world simply will not work. If you put a surface protectant on, the tissues move, as the stomata do, and then you have

a natural unprotected opening for the infection site. So the great significance here is that we have moved into materials which have a limited systemic activity. So the really significant phenomena involved here in plant protection is the one of systemic movement in plants.

I think this is very significant, not only because we can improve on what we already have by the use of other antibiotics, but also because these materials that we are working with today will eventually become the models for the organic chemist to learn how to develop other synthetic materials which can be used as systemic protectants of plants. It is a great and coming field, as indicated in Dr. Zaumeyer's closing remarks.

We have been trying to use as systemic protectants those things which were developed as surface protectants. The organic molecules there have a very definite and delicate balance between certain lipophilic and hydrophilic constituents. Those molecules therefore have a tendency to be dissolved in fatty and waxy materials so they will not move in a plant the way they should.

Furthermore, as we have learned at Boyce Thompson Institute in the last two or three years, they have a great tendency to be detoxified by the normal constituents of the plant. Where in the world can we find a model of something which would not be detoxified inside the living cell, which would exist there in an active condition unreacted? The answer is perfectly obvious: It will be found in other living cells, and that means to us today the word "antibiotics."

I would like to bring to you one or two other observations. One of them pertains particularly to the question of synergism between different antibiotics and the synergism between antibiotics and certain metallic constituents, particularly copper. I was interested that in the discussion of the last two days this question of combined use of these materials has not been emphasized very much. We started out two and a half years ago working upon the idea, from certain in vitro tests, that we would add Terramycin to streptomycin to prevent the development of resistant strains, as was shown so clearly in the Pfizer Laboratories, in, for instance, fireblight bacteria. We are now confident from tests which have been made on other species of bacteria, such as the naturally resistant common blight of beans, *Xanthomonas phaseoli,* that the combination of these two materials has led to enhanced activity which makes these materials much better than they would be alone in either case.

For example, in the conditions under strenuous laboratory tests where you would get practically no control of *Xanthomonas phaseoli* by streptomycin by the use of a concentration of 100 parts per million or less, if you will add ten parts per million of Terramycin you can bring the activity of control up to around 95 per cent, and yet the Terramycin alone has relatively no effect.

About two and a half years ago we also became interested in the compatibility of this mixture of Terramycin and streptomycin with certain other materials. We noticed, much to our surprise, that not only was it compatible with certain inorganic copper compounds, but it was even more active than we had anticipated.

We have studied this very diligently and we are confident now this is a case of true synergism. By suppressing the level of the antibiotic to 25 parts to 50 parts per million where you get essentially no control, maybe as high as 10 per cent, and then by using 100 to 800 parts per million of an insoluble copper compound where

you get practically no control, you can achieve 98 or 99 per cent control of this disease.

The full significance of this is gradually becoming evident to us because it is obvious that copper is an excellent fungicide. We therefore turned our attention to the fungi two years ago, and we found if we take an organism such as *Phytophthora infestans,* the late blight of potatoes, we can very effectively control this organism by the use of this combination and we get the same synergistic effect that we have demonstrated on the bacteria.

We feel this is rather significant because it indicates that possibly the mechanism of action is somewhat similar. Unfortunately, we have not gone far enough to know the full significance of this, but it does point out that we have in our hands the means of controlling a great number of the downy mildew type of fungi, as Dr. Zaumeyer has indicated, by the use of much lower concentrations of antibiotics than originally thought, so we have a chance to lick this economic situation which is causing us so much trouble.

We are not certain what the mechanism of action of this synergism is. We have gone far enough to study the copper chelates of Terramycin, and we are sure it does not reside there. I am sorry that all we have is negative evidence at this point, but that is where we have to drop this subject.

I would like to close by saying that there is great promise in the future. We do have in our laboratories now in the very elementary stages of evaluation, antibiotics which are remarkably wonderful against the powdery mildew type of fungi. We have control of rose mildew in a fashion I never thought I would see. We also have a material which will act as a chemotherapeutant against the rust fungi, which has been particularly effective against the leaf rust of wheat and the bean rust organism. We can get control within five days after the time of inoculation.

I think all of these things portend well for the future. We have just started to get our feet wet. We think there is great opportunity ahead of us and we are going forward with a great deal of diligence in this field.

DR. HORSFALL: The last official panelist on this group is my good friend Harry Young, of the Ohio Agricultural Experiment Station.

DR. YOUNG: Yesterday I heard several persons remark when they left the meeting that we were now thoroughly confused. What I want to say, may also add to that confusion insofar as the control of plant pathogens by antibiotics and perhaps by other compounds is concerned.

Three years ago we began experiments with streptomycin and other antibiotics for the control of fireblight. In 1953 the control was perfect, as was reported by the paper this morning. In 1954 the control was again perfect, and that time we used Agri-mycin, streptomycin sulfate, and streptomycin nitrate.

In 1955 our control experiment at Wooster and those in Northern Ohio were absolutely noneffective. In other words, we got practically no control from streptomycin in any form for the control of fireblight. We had a little inkling that this might occur, and the inkling came from New York State, from Dr. Parker, who at a meeting last year stated that unless the temperatures were at a certain point Agri-mycin, or if you will, confine it to streptomycin, would be almost ineffective. That is what we found this year with ours, and we of course attributed it to tem-

peratures. He has found in his work that unless the temperature is above 70° we cannot expect too much control from the streptomycin formulation. In our own tests this year the temperatures were slightly below that figure.

I want to add at this point, too, that this temperature of 70° or below did not prevent the streptomycin from entering the leaf. We made determinations both years and we found almost as much streptomycin this year as we did last, not quite. Even though the streptomycin was in there and even though we used such compounds as glycerine with it, we still did not get control when the temperature was below 70 degrees.

I want to add just one more word to some of the things which have been said. I happen to be in charge of a national project on systemic materials supported by the Agricultural Research Institute and the National Academy of Sciences. The development and introduction of all of these antibiotics into the plant as systemic materials for plant disease control is one of the big factors in that project. As was pointed out yesterday by Dr. Christensen, not only the organism might change through the action on the antibiotic, but the susceptibility of the host plant may change because of the action of the antibiotic on the host. Therefore, I leave you this word: While we have a new tool with which to begin working on the great group of plant diseases not now controlled, namely, over 50 per cent of them, we have at our disposal this large group of antibiotic compounds plus a large group of systemic compounds, all of which must be studied not only from their effect on the organism, but also their effect on the host plant.

You will hear a great deal more, I hope, from this phase of plant disease studies and plant disease control in the future.

DR. HORSFALL: Thank you, Dr. Young.

I would like first to ask the two scientific men from the Soviet Union to stand and take a bow before their papers are presented. First, Dr. Eugene Mishustin, from the Academy of Sciences of the USSR, and Dr. N. A. Krasilnikov, also from the Academy of Sciences of the USSR.

We will now hear the abstracts of the three papers which these scientific colleagues have brought with them.

DR. E. N. MISHUSTIN (Through the Interpreter, Mr. Mikhailov): Much attention in the Soviet Union is paid to the investigation of the phenomenon of antagonism. This phenomenon is of particular significance for the utilization of bacterial fertilizers.

Azotobacterine is the name of a bacterial fertilizer containing azotobacter, a microorganism that excretes a number of substances which tend to accelerate the growth of plants and which also can assimilate molecular nitrogen. During the past 20 years azotobacterine has undergone all sorts of testing and practical utilization in the USSR, and considerable data on its effectiveness in increasing yields have been accumulated. The results show that it is not always effective. The present paper is concerned with a study of factors affecting its action in the zone of plant roots, or rhizosphere. It is pertinent to this conference on the use of antibiotics in agriculture, because we have shown that the effectiveness of azotobacterine in the rhizosphere of spring wheat is influenced by the presence of fungi of the genus Penicillium, which has an antagonistic effect on azotobacter. The removal of such

antagonistic fungi from the rhizosphere increases the multiplication of azotobacter close to the roots of plants. Various chemical, physical and biological factors influence the repression of the antagonists and the stimulation of the nitrogen-fixing bacteria. Our conclusions are as follows:

The bacterial fertilizer called azotobacterine, which finds wide application in the USSR, is not always effective. It does not survive in acidic soils. In neutral soils its acclimatization depends on the content of moisture and organic substances. That is why azotobacterine is very effective on irrigated lands and in kitchen gardens. Its efficiency increases when used with organic fertilizer, which can be applied locally and in small doses, preferably with the addition of superphosphate.

The favorable effect of organic fertilizers on the action of azotobacterine is caused both by the nutrient value of the fertilizer for this microorganism and by the inhibition of antagonistic fungi of the genus Penicillium by the fungi of the genus Trochoderma. The propagation of the latter in the rhizosphere is abruptly accelerated in the presence of organic compounds in the given soil.

DR. HORSFALL: Thank you, sir.

The other two papers are authored by Dr. Kasilnikov.

DR. N. A. KRASILNIKOV (Through the Interpreter, Mr. Mikhailov): Let me express my satisfaction and gratitude for the kind invitation on behalf of the Academy of Sciences of the United States of America to take part in this very interesting conference.

Investigations on the use of microorganisms against plant diseases have been under way in the USSR since 1947 when crude antibiotic substances of bacterial origin were studied. At present the phenomenon of bacterial antagonism is being thoroughly analyzed and tested, chiefly along two different lines; (a) sanitation of soil by means of antagonistic microorganisms and (b) treatment of diseased plants with antibiotics.

The first method is based upon the fact that numerous pathogenic, as well as saprophytic, bacteria die very quickly after being introduced into the soil, the effect depending upon the type of soil and its content of antagonistic microorganisms, bacteria, fungi, actinomycetes, and so forth, which produce antibiotic substances. The number of antagonists depends also on the kinds of plants growing in the soil.

Our investigations show that the addition of antagonists to soils decreases the incidence of plant diseases, such as fusarium wilt of flax, fir seedlings and cotton. Soil sanitation can be achieved also by growing plants during crop rotation that tend to remove phytopathogenic microorganisms from the soil.

In more recent investigations (1951–54) we studied the effects of purified preparations of penicillin, streptomycin, Aureomycin, gramicidin, subtilin, mycitin, and others. Our experiments have shown that some of these antibiotics are taken up by plants through their root system. They can be introduced into plants also through stems and leaves. We have introduced penicillin, streptomycin, grisemin, and Aureomycin into the trunks of fruit and ornamental trees and find that fruit trees take up antibiotics more readily than do the ornamentals that were tested. We find that antibiotics introduced into plants stay in the plant tissues for a longer time than in the animal organism. In our experiments antibiotics remained in the plant from one to twenty days, depending on the antibiotic and species of plant. These observations

caused us to test antibiotics for the control of diseases of woody and herbaceous plants. Positive results have been achieved against bacterial infections of tomatoes and cabbage. Bacterial wilt of peach and apricots was controlled by grisemin which was introduced through the trunk and by spraying the leaves.

Our conclusions are as follows:

1. Antagonistic microorganisms are a powerful factor in the growth and productivity of plants. They protect plants by inhibiting the growth and reproduction of pathogenic microorganisms in the soil.

2. These antagonistic microorganisms produce antibiotic substances which can be used successfully for the treatment of infected herbaceous and woody plants.

3. Some antibiotic substances inactivate toxins that are produced by bacteria and fungi in the plants.

4. Antagonistic microorganisms in the soil produce antibiotic substances which penetrate the roots of plants and make their immuno-biological properties more effective.

DR. HORSFALL: I would like at this point to introduce a gentleman who should have been on the program yesterday, Professor A. H. Poliakov, Doctor of Veterinary Medicine, and Director of the All-Union Institute of Veterinary Parasitology of the USSR.

Now we will take a few minutes for questions from the floor. Dr. McNew, you have one or two there, I think.

DR. MCNEW: There seem to be several of you who are interested in the question of growth regulation of plants by antibiotics. After I say a few words, I will ask Dr. Zaumeyer to repeat what he said about some of the evidence of growth stimulation from streptomycin.

In the course of evaluating quite a number of these compounds in the last three years we have found some evidence of plant stimulation and regulation. Only one of these materials has given us a very strong or definite formative effect on plants, not enough to be in the herbicidal class at all or possibly even among the better growth regulants. We do have one case of that.

I don't know whether Dr. Campbell of England is in the audience or not. Is he still here? He was telling me yesterday that they have a very definite growth regulation compound antibiotic.

DR. CAMPBELL: I am here.

DR. MCNEW: Would you like to tell us a little bit about that?

DR. ALEXANDER H. CAMPBELL: The subject I mentioned yesterday to Dr. McNew involves the well-known antibiotic griseofulvin. Griseofulvin has been known for a great number of years as a systemic antifungal of some promise in controlling Fusarium and a variety of root rots.

In the course of doing some trials with this antibiotic in the control of the root rot of carnations, we were able to get a very marked stimulation in the onset of flowering. There is also evidence of some physiological changes in the plants. The color was markedly different. It had changed to the blue-green color which the greenhouse man likes to see because he knows then he has a hardy plant.

I must first of all apologize. I wasn't the least bit prepared to say anything about this. But now that I am on my feet, I may take just a minute more to explain my

interest in griseofulvin. We have known it a long time. It is not toxic to man. I thought that since it is antifungal antibiotics which we need, this one really ought to be looked at. What we have done simply is to make it available so that a large number of people can try it for themselves if they so desire.

DR. HORSFALL: Thank you, Dr. Campbell.

At this point I would like to introduce the summarizer Mr. Dunegan from the United States Department of Agriculture.

SUMMARY OF THE FOURTH SESSION

CROP USAGE

MR. DUNEGAN: In the United States our research work in the control of plant diseases has been confined primarily to use of antibiotic materials—streptomycin, cycloheximide, oxytetracycline, tetracycline, and one of the other cycline compounds, Aureomycin. In research work involving large field experiments, at least 18 different kinds of crops have been treated. Additional hosts have also been treated in the smaller laboratory experiments.

It is obvious that while these antibiotic materials are predominantly effective against bacteria, there is also some promise that the Phycomycetes can be controlled. We have evidence of control of certain of the Ascomycetes, particularly Coccomyces fungus causing cherry leaf spot, and also some of the Basidiomycetes i.e. the rusts and smuts, and at least one or two members of the *Fungi Imperfecti,* namely the powdery mildews and Helminthosporium. This is an imposing list, both of the host plants and the fungi, but when you consider the number of species of plants available or the number of fungi, it is obvious that we are just beginning.

In the antibiotic work there are many curious contradictions such as eradication versus non-eradication. It is probably a very involved situation. We do not get eradication in the fruit crops. But this may be due primarily to the low dosage which we are forced to use because of the cost factor. We have such a high gallonage requirement per acre in fruit trees that perhaps we are missing entirely the eradicant effect.

On the other hand, as Dr. Zaumeyer brought out, he has obtained definite eradication of the halo blight organism with as low as 200 parts per million; but on the other hand, the common blight organism has sort of eluded his grasp, much as the *Xanthomonas pruni* has evaded all of our efforts to control it. In fact, we cannot get antibiotics into peach trees by merely spraying them. We can control the organism in the laboratory, but we cannot get the antibiotics into the peach tree unless we inject it into the trunk or branches. This is not a good procedure considering the number of peach trees in this country.

To me, one of the most interesting compounds is cycloheximide or Acti-dione. Very definitely it has worked against a fungus and not only experimentally, for it is being used commercially with very satisfactory results. It is an extremely potent compound but can cause injury to plants. Dilutions of one or two parts per million are very effective.

Then there has been, of course, a lot of attention given in this country to the

problem of growth-promoting substances, to the addition of humectants, as well as the addition of metal salts to antibiotics. All these things are under study.

The results are promising, sometimes they are confusing, and much more research is needed. For the future we certainly need materials which have a wide band spectrum, antibacterial and especially antifungal material. We need to think about the ecological phases of the problem which Dr. Young has brought out. We need especially—and here I was most interested to listen to the comments of our Russian colleagues—materials which can be used in the soil, because the Fusarium problem is a very difficult one. We need compounds which can remain stable in the soil, be absorbed by the plant, and produce a therapeutic effect.

I might mention, for instance, the Panama disease of the banana, a most devastating disease in the tropics. We need an antibiotic for it. We of course also need anti-viral materials. We have just touched on that.

I think we could sum up the situation by saying that progress has been most encouraging, but that we are just at the beginning. We don't know how far we are going or all of the possibilities, but those we can visualize are very inspiring.

FOURTH SESSION

B. FOOD PRESERVATION

October 21, 1955

J. G. HORSFALL, *Presiding*

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CONTROL OF BACTERIAL SPOILAGE OF FISH WITH ANTIBIOTICS

It is well known that fish are almost invariably much more susceptible to bacterial spoilage when stored at ordinary refrigerator temperatures than are meats obtained from warm-blooded mammals. Probably one of the most important reasons for this is that fish are usually contaminated with those types of bacteria which thrive at low temperatures, namely the psycrophilic organisms. At temperatures around 0° C. these organisms normally have an extremely high growth coefficient (Q_{10}), and it has been shown that the flesh of fresh fish will spoil about twice as rapidly at 2.5 or 3° C. as it will at –1° C.(10). Moreover, fish presents an almost ideal substrate for growth of most spoilage organisms.

Though bacterial spoilage of fish may be prevented or delayed by such physical means as chilling, freezing, or heating, the possibility of adding relatively harmless chemical substances to inhibit microbial growth has long been the subject of extensive research. A survey of the early literature indicates that, over the years, a very wide and varied selection of chemical substances has been used with fish in attempts to delay spoilage. Our own early work, initiated in 1938(16), led to the use of sodium nitrite as an inhibitor of spoilage of fresh fish, and, indeed, this substance was very widely applied in the Canadian Maritime provinces during the last war. It is still used extensively, and is now legalized for use on fish in Canada. This substance, though undoubtedly useful, is by no means an ideal preservative, and we have therefore long sought for a more desirable substance.

The possible value of antibiotics in controlling bacterial spoilage of fish was investigated at this Station about 1943 when it was shown that penicillic acid was practically valueless for this purpose(17). Later, streptomycin and penicillin were studied and were also found relatively valueless as fresh fish preservatives(18). Early in 1950 about 15 antibiotics were investigated, and it was found that of these only three, namely chlortetracycline, oxytetracycline, and Chloromycetin were effective for both preservation of fish flesh and of beef(19). Since extensive work showed that, of the tetracycline group, chlortetracycline was always the most active, all our recent work has been conducted with this antibiotic. In our experience oxytetracycline is only about one-fifth as active as chlortetracycline for flesh food preservation, and tetracycline is less active than oxytetracycline. Since this early work was published we have also tested oxamycin and erythromycin, and found them relatively ineffective for flesh food preservation.

In some ways it is unfortunate that we have so far felt it necessary to devote most of the time spent on this problem to its more practical aspects. Consequently, considerable effort has been directed to practical problems concerning the use of chlortetracycline on fish, though some of the theoretical aspects of the problem have also been considered.

As with almost any applied research problems, certain difficulties have arisen but it has usually been possible to overcome these. We have conducted, usually at the request of the fishing industry, several quite large-scale experiments on the use of chlortetracycline under practical conditions. Some of the problems which have occurred, together with some of the results which have been obtained in the various experimental trials, will be discussed.

It was obvious when this work was initiated that chlortetracycline treatment should start as soon as possible after fish have been captured. The flesh of healthy fish is normally bacteriologically sterile(4), and as soon as the bacteria which occur in the slime or visceral tracts contaminate and penetrate the flesh, it becomes increasingly difficult to combat their activities. Several possible methods of application of chlortetracycline to fish at the time of capture were investigated, and it was concluded that, from the point of view of simplicity, incorporation in ice used for icing fish offers considerable promise. Though experiments did indicate that round or eviscerated fish could be dipped briefly in, say, 50 or 100 p.p.m. solutions of chlortetracycline immediately prior to icing them, this method is probably not too practical under some of the conditions which pertain on commercial vessels.

During the last two fishing seasons we have added approximately 1 μg./ml. (p.p.m.) of chlortetracycline hydrochloride to the water used in preparing flake-type ice at two different fish plants. This method is extremely simple and yields an ice which is not only indistinguishable from ordinary ice even when 4 μg./ml. of the antibiotic are incorporated, but also possesses outstanding bacteriostatic properties. The only real difficulty which we experienced in this work was the instability of chlortetracycline in so-called "hard" water, and this, in the particular instance, could be easily overcome by the addition of 200 p.p.m. of citric acid to the water in the tank containing the chlortetracycline stock solution to adjust the pH to about 3.5. Microbiological assays showed that in the unacidified water (pH 7.2) practically all antibiotic activity of added chlortetracycline disappeared in one day at 25° C.

The addition of chlortetracycline to commercial block ice presented certain difficulties, and one of these has not yet been completely overcome. The customary commercial procedure for preparation of block ice involves slow freezing in large rectangular cans immersed in circulating calcium chloride brine. There is always a tendency, especially if aeration is used, for dissolved crystalloids and colloidal or suspended particles to migrate to a small "core" near the centre of the ice blocks. Chlortetracycline, even in 1 μg./ml. concentration, offers no exception to this rule, and the problem of obtaining even distribution arose. It was found that, of a number of hydrophilic colloids studied, commercial carrageens (Irish moss extractives) and carboxymethylcellulose retarded migration of chlortetracycline effectively during freezing(8). Various methods of incorporating these colloids in the water used for making ice were studied, and difficulties such as relative insolubility in cold water or a tendency to form lumps had to be overcome. Experience showed that heating

carrageen suspensions in order to effect "solution" was impractical for large-scale work, but it was found that prior mixing of the finely powdered material with about 3 parts of propylene glycol overcame this difficulty. However, with carboxymethylcellulose an even simpler method was discovered, for it was found that if the finely divided material (less than 100 mesh) was mixed with about 3 parts of ordinary salt, it could be thrown into ice cans during rapid addition of cold water without any danger of lumping(2). The required amount of chlortetracycline hydrochloride may be added to the dry mixture.

Though the problem of difficulty of distribution of chlortetracycline appears to have been solved, another problem has arisen which could probably be overcome quite readily. In certain localities along the British Columbia and Alaska coastline the surface water in streams and lakes may be coloured brown by dissolved organic substances. Work at this Station (Dr. D. R. Idler, unpub.) has shown that ion exchange treatment removes the coloured substances so that the means for obtaining clear water are available. It would seem highly probable that the untreated water would make an effective chlortetracycline ice, and, in fact, use of such ice on a fishing boat showed that the white ventral surfaces of halibut are not stained by it. However, it is difficult to overcome the prevalent idea, which is probably quite without foundation, that ice made from such water is of inferior quality for icing fish.

Because of work initiated at our Station several years ago, renewed interest has been taken in the possibility of storing fish on boats or on shore in refrigerated sea water, or in salt solutions of similar concentration. It must be emphasized that this idea is by no means new, for an early French patent(6) indicates that some thought was given to application of this general method many years ago, and thorough investigations at the Halifax Station of this Board which were conducted about 1927 to 1930(10), (11) definitely established the superior keeping quality of fish so stored. This method offers one of the few ways by which fish may be maintained in non-frozen condition, and yet be only slowly susceptible to bacterial spoilage. The flesh is maintained at about 30° F. (–1.1° C.) while in ordinary melting ice the temperature can never be below 32° F. (0 °C.) and under usual commercial conditions is more likely to be between 35° and 37° F. The suggestion that fish might be held in refrigerated sea water on fishing boats was made by the writer in 1947 (20), and it was then pointed out that the method is quite possible because "fish wells" are commonly used on tuna boats to freeze the fish. Initial trials were made on small trolling boats(9), (13), (14), and these proved so successful that, during the present fishing season, a privately owned fish packing vessel has been equipped with three large aluminum tanks with capacity for around 45,000 pounds of fish. At least one large scow with a capacity of around 120,000 pounds of fish has been operating in the Alaska area this season.

Though storage and transport of fish in refrigerated sea water offers many advantages, experience has shown that certain difficulties may arise. Thus, under certain circumstances, there is a risk that bacterial spoilage may develop. This has definitely been shown to be a danger in instances where a very large load of slimy fish is placed in a tank in which the ratio of fish to sea water is very high. The sea water is heavily contaminated with protein and fish blood, and also with spoilage

bacteria from the slime and visceral cavities of the fish. This creates a heavy bacterial inoculum in a favourable growth environment, and even in a week at 0° C. unpleasant odours may develop in the sea water and, although the fish may themselves appear firm and in good condition, they form a rather serious "bacteriological" risk if, for any reason, they are stored further. This effect is shown in Table I which illustrates results obtained in trials carried out on a large fish-packing vessel during the past season. Though it would be possible to change the sea water regularly and thus preserve a clean medium, this is often neither practicable nor indeed desirable. This procedure would often create a condition where large extra refrigeration loads would be required, and also there could be a very real danger that the fish might absorb unduly large amounts of salt. We have found that the inclusion of only 1 μg./ml. of chlortetracycline, or considerably less, will successfully hinder bacterial development for extensive periods, and that, even after one month in refrigerated sea water containing this antibiotic, fish may still be quite edible.

The question is often asked whether chlortetracycline, when added to ice or to refrigerated sea water, penetrates the flesh so that it can leave substantial residues. In early work we attempted to ascertain whether penetration did occur, but these

TABLE 1

EFFECT OF FOUR STORAGE TREATMENTS ON KEEPING QUALITY OF IMMATURE COHO ("BLUEBACK") SALMON

Type of storage	Bacteria (×10⁶) per gram after days*				
	2	9	16	23	30
Ordinary ice	0	14.0	342	535*	
	0	16.4	368		
	0	16.7	286		
	0	15.6	259		
		15.7	319		
Ice containing 1 μg./g. of chlortetracycline	0	1.80	6.77	124	
	0	.81	4.50	58	
	0	.85	3.97	61	
	0	.53	6.09	53	
		0.99	5.37	74	
Sea water	0	3.1	60.9	143	80†
	0	1.9	60.9	159	
	0	1.7	58.3	157	
	0	6.4	60.4	217	
		3.3	60.1	169	
Sea water plus chlortetracycline (1.4–0.7 μg./ml.)	0	0.26	2.60	47	23†
	0	0.26	4.77	47	
	0	0.21	1.59	40	
	0	0.34	2.60		
		0.27	2.89	46	

* Counts were made by a direct method after incubating steaks for 1 day at 10° C. except in those marked with a dagger where there was no incubation.

initial attempts were unsuccessful because the flesh of most of the local fish species examined were found to contain substances which gave a fictitious assay in the chlortetracycline pad plate assay procedure. This year we have reinvestigated this subject because we have found that the flesh of a local species known as the true cod (gray cod, *Gadus macrocephalus*) rarely exhibit this fictitious assay when the fish are in a fresh condition. To test the point, these true cod were iced with ordinary ice and with ice containing 1 μg./g. of chlortetracycline. At intervals fish were removed and different portions were assayed for the antibiotic, using the pad plate procedure. A simple extraction method was employed whereby the flesh was blended in the cold with 2.5 normal hydrochloric acid to pH 4.6. It was found that extracts obtained on centrifuging these blends at high speed could be placed directly on assay pads, dried in vacuum, and then applied to the inoculated medium as usual. Some results obtained in initial experiments are given in Table 2. They show that fictitious positive chlortetracycline assay values occur rather rarely with gray cod stored in ordinary ice, and that in fish iced with ice containing chlortetracycline there was a rather slight penetration after several days storage, and that this occurred most markedly in the visceral cavity samples with skin attached. Fish stored in refrigerated sea water containing chlortetracycline absorbed the antibiotic more markedly than did the iced fish (Table 3).

Studies of the rate of destruction or disappearance of chlortetracycline in heated fish flesh have occupied a certain amount of our time. Initial and early tests as shown in Table 4 indicated that destruction on heating was quite rapid. The positive

TABLE 2

PENETRATION OF CHLORTETRACYCLINE INTO GRAY COD STORED IN ICE CONTAINING 1 μg./g. OF THE ANTIBIOTIC

	Section of fish*	Days stored					
		0	1	2	4	7	10
Ordinary ice	V + S	0	0	0	0	0	0.2
		0	0	0		0	0
	V − S	0	0	0	0	0	0
		0	1.1	0		0	0
	T + S	0	0	0	0	0	0.2
		0	0	0			0
	T − S	0	0	0	0	0	0
		0	0	0		0	0
Chlortetracycline ice	V + S		0	0	0	0.4	0.9
			0	0		0.3	0.5
	V − S		0	0	0	0.5	0.7
			0	0		0.2	0.3
	T + S		0	0	0	0	0
			0			0	0.8
	T − S		0	0.6	0	0.2	0
			0	0		0	0

* V + S = Visceral cavity wall plus skin.
V − S = Visceral cavity wall without skin.
T + S = Tail end of fish plus skin.
T − S = Tail end of fish without skin.

TABLE 3

PENETRATION OF CHLORTETRACYCLINE INTO GRAY COD STORED IN SEA WATER CONTAINING 2.8 μg./ml. OF THE ANTIBIOTIC†

	Section of fish*	Chlortetracycline in μg./g. after days					
		0	1	2	4	5	6	7
Ordinary ice	V + S	0		0		0		0
	V − S	0		0		0		0
	T + S	0		0		0		0
	T − S	0		0		0		0
Chlortetracycline ice	V + S		1.5	3.0	1.5		2.16	3.0
	V − S		0	1.23	1.23		1.26	1.14
	T + S		1.2	1.23	1.5		1.26	2.55
	T − S		0	0	0		0	0

* V + S = Visceral cavity wall plus skin.
V − S = Visceral cavity wall minus skin.
T + S = Tail end of fish plus skin.
T − S = Tail end of fish minus skin.
† Steiner, G., and H. L. A. Tarr, unpublished.

values obtained after 20 and 30 minutes heating may have been due to substances which cause the fictitious chlortetracycline assay. Results obtained in more recent tests indicate that normal cooking of fish to internal temperatures of between 82 and 99° C. will destroy from about 80 to 90 per cent of added chlortetracycline (Table 5). The next step in this work will be to ascertain whether there are any detectable residues in flesh of fish iced with chlortetracycline ice after these have been subjected to normal cooking procedures.

We have carried out some exploratory experiments in attempts to ascertain what substance(s) in fish flesh occasion the fictitious chlortetracycline assay, and also in attempts to see whether quite small quantities of added chlortetracycline could be recovered. The technique used was as follows: Chlortetracycline (0.2 μg./g.) was added to lingcod flesh, which was blended at 0° C. at pH 4.6 and the bulk of precipitated protein removed by centrifuging. The liquid with the washings was further acidified and evaporated to dryness in vacuo at low temperature. The residue was thoroughly extracted with water-saturated n-butanol and this extract dried. The residue was washed with diethyl ether, dried and extracted with absolute ethanol. The two solvent extracts were evaporated to small volume and were chromatographed on paper treated with phosphate buffer pH 3.0. Bioautographs showed that small amounts of chlortetracycline were present in the ethanol extract. However, the ether extract also gave one zone of inhibition, and the ethanol extract two zones of inhibition which were rather diffuse and of lower Rf values than the chlortetra-

TABLE 4

DESTRUCTION OF CHLORTETRACYCLINE IN HEATED FISH FLESH CONTAINING 5 μg./g. OF THE ANTIBIOTIC

	CTC present after heating (μg./g.) minutes				
	0	10	15	20	30
Lingcod	5.1	2.4	1.5	0.6	0.45
Coho salmon	4.8	1.8	7.8	—	0.30

TABLE 5

INACTIVATION OF CHLORTETRACYCLINE ON HEATING FISH FLESH CONTAINING 6.5 AND 16.0 μg./g. OF THE ANTIBIOTIC*

Temperature (° C.)		Time required to attain temperature*	CTC content (μg./g.)	Time required to raise and maintain temperature 5′	CTC content (μg./g.) after holding 5′ at temp. given
0.5 μg./g. CTC	60	5′ 40″	1.4	11′ 45″	1.4
		6′ 10″	1.2	11′ 0″	1.8
	82	5′ 29″	1.4	11′ 35″	1.8
		8′ 0″	1.8	15′ 0″	1.2
	99	5′ 8″	<0.3	10′ 0″	<0.3
		4′ 50″	0.8	10′ 30″	<0.3
16.0 μg./g. CTC	60	8′ 45″	13.2	12′ 35″	2.8
		8′ 45″	11.2	12′ 35″	2.8
		8′ 45″	11.2	12′ 35″	2.8
	82	9′ 27″	6.6	16′ 0″	2.0
		9′ 27″	5.4	16′ 0″	3.0
		9′ 27″	4.8	16′ 0″	2.4
	99	14′ 0″	2.4	10′ 35″	0.98
		14′ 0″	2.4	10′ 35″	1.2
		14′ 0″	2.4	10′ 35″	0.92

* Steiner, G., and H. L. A. Tarr, unpublished.

cycline. These substances may be lipids, but as yet no attempt has been made to identify them. Attempts to recover chlortetracycline quantitatively when 20 μg. were added to 100 g. of fish were unsuccessful.

It is common knowledge that shellfish, and the cooked flesh materials obtained from them, are particularly susceptible to bacterial spoilage. There has never been a completely satisfactory explanation for this but it is probably largely because the pH of their muscle tissues is normally considerably above 7. Thus, in one of our experiments we found that cooked crabmeat had a pH of approximately 7.9. The meat of these shellfish requires considerably higher concentrations of chlortetracycline or oxytetracycline than does that of fish where the post-mortem muscle pH is usually between 6 and 7. Probably this is because chlortetracycline is quite unstable at pH values above 7.0. In one test we found about 10 times more chlortetracycline was required to preserve cooked crabmeat than was required to preserve various kinds of fish. This has also been the experience of those at the Marine Laboratory, University of Miami, who have been concerned with the preservation of fresh shrimp in either refrigerated sea water or in ice(5), (12). Thus they have reported that between 5 and 8 parts per million of chlortetracycline must be added to refrigerated sea water in order to bring about really successful preservation of shrimp. Similar concentrations of this antibiotic must be added to flake or block ice if successful preservation of shrimp against bacterial spoilage is to be obtained. Recent studies in California(7) have indicated that low concentrations of chlortetracycline and oxytetracycline which will preserve fish effectively are not successful with California shrimp.

There is, unfortunately, little published information on results of research conducted in other laboratories on fish preservation with antibiotics. In California a series of tests in which fish fillets were dipped in two parts per million solutions of various antibiotics prior to storing them at about 45° F. has been carried out(7). Using trimethylamine nitrogen, total volatile nitrogen, and the volatile reducing substances test as criteria of bacterial spoilage, it was found that the chlortetracycline dip gave the most outstanding preservative effect, and that oxytetracycline was less effective. More information on preservation of fish in different localities using antibiotics would certainly seem desirable.

In view of the success attained with relatively low concentrations of chlortetracycline, and because it is known that living fish will withstand quite high concentrations of this and certain other antibiotics in the water in which they swim, it seemed of interest to attempt to determine whether fish, if permitted to swim in chlortetracycline-containing sea water, would eventually have relatively sterile visceral tracts. Experiments of this kind are rather complicated, because it would appear from the literature that the visceral tracts of non-feeding fish are relatively sterile bacteriologically, while those of feeding fish are normally fairly heavily contaminated with bacteria. Due to very limited experimental facilities we have only been able to perform a few tests. The results (Table 6) indicate that the data we have obtained so far are rather inadequate, but they do show that when sea fish are permitted to swim for a day in sea water containing 5 p.p.m. of chlortetracycline, the antibiotic can be recovered from the mixed visceral organs, and that it is by no means certain that it causes a definite reduction in the bacterial population(3). More data with both fresh water and sea fish would be of interest. It would be desirable if possible to conduct such tests for some days or weeks, using feeding and non-feeding fish, and to perform both microbiological assays and bacterial counts on individual organs in the visceral cavity as well as on the digestive tract itself. The problem may be complicated by slow penetration of the antibiotic into organs such as the pyloric caeca and by the emergence of chlortetracycline-resistant bacterial strains.

The sensitivity of nearly all normal spoilage bacteria present in fish to chlortetracycline is very interesting. In our earlier work we found quite consistently that when fish containing 1 μg./g. of this antibiotic was stored at 0° or 4° C., nearly all bacteria were prevented from growing, but after about two weeks the flesh was richly invaded by yeasts and occasionally by moulds. We have been interested, consequently, in trying to obtain information on how this antibiotic exerts its

TABLE 6

VIABLE BACTERIA (per g.) AND CHLORTETRACYCLINE (μg./g.) IN THE VISCERA (stomach, pyloric caecae, liver, spleen, intestines, etc.) OF ROCK COD (*Sebastodes* sp.), HELD ONE DAY IN SEA WATER

	Experiment 1		Experiment 2		Experiment 3	
	Bacteria	CTC	Bacteria	CTC	Bacteria	CTC
Sea water alone	———	—	1,000,000*	—	50,000	0
Sea water + 1 μg./ml. CTC.....	1,000,000	0.22	———	—	———	—
Sea water + 5 μg./ml. CTC.....	———	—	18,000	—	48,000	1.2

* These fish died due to faulty aeration.

activity. There is far too little known regarding the mode of action of antibiotics so that any information which we might secure could be of value. There are probably many ways by which such a problem could be attacked, and we have initiated the somewhat obvious and possibly crude idea of attempting to determine what differences there may be in the metabolic pattern of a typical organism obtained from fish both before and after it has become very resistant to chlortetracycline. The work has as yet not proceeded very far, for it has taken a considerable time to obtain a very resistant organism. Thus it was found that the particular culture with which we have been working was initially susceptible to small fractions of 1 μg./ml. of chlortetracycline, and that, during the initial weeks of almost daily transfer in an ordinary medium, the sensitivity only gradually diminished. During the last few weeks of this transfer period, which will soon have extended to about four months, the resistance to the antibiotic has increased by leaps and bounds so that the organism will grow cheerfully in concentrations in excess of 1000 μg./ml.

The possibility of using chlortetracycline to retard post-mortem bacterial spoilage in whales is being explored. Whales are warm-blooded mammals which may often weigh over 50 tons, and their visceral tracts are richly contaminated with strict anaerobes of the genus Clostridium. There is also some evidence that the flesh or blood vessels of living whales may be contaminated with these organisms, though this does not appear to be entirely conclusive(15). Where whales are processed on factory ships there may be no unduly long interval between time of capture and processing, but when the nature of the fishery is such that processing is carried out at a shore plant, the whales may be towed for a few days and extensive anaerobic putrefaction may occur. Sperm whales appear to be peculiarly prone to post-mortem bacterial putrefaction since obvious spoilage may occur within 24 hours of death. In an attempt to overcome this we have been carrying out some experiments to ascertain whether the introduction of chlortetracycline into the visceral cavity of whales post-mortem will cause a transient preservation which will overcome serious bacterial spoilage until the animals are cut up. So far the method of introducing the chlortetracycline has been by sucking an aqueous solution into the air line used for inflating the mammals prior to towing them. In this work we have allowed about 55 g. of chlortetracycline per whale, which would be about 1 μg./g. calculated on the basis of a 50-ton whale, or 5 to 10 μg./g. based on visceral organ weights of between 5 and 10 tons. This work has been ably conducted by Mr. P. B. Crean, who had considerable prior experience when attached to the British factory ship "Balaena." The results of all tests made so far have been very encouraging (Table 7), and it is hoped that more extensive work may be carried out next year. In one whale a 25 g. charge of chlortetracycline hydrochloride was placed in a linen bag in the bomb head of the harpoon. The cloth material was recovered and after drying was found to contain about 2.5 g. of the antibiotic. This indicates that it might be possible to introduce the antibiotic in this manner without any serious decomposition.

Before permission may be granted to add any substance to a food intended for human consumption, such a substance must advisedly pass rigorous tests to ensure that no harm will come to the eventual consumer. The concentrations of chlortetracycline used throughout our experiments have been extremely small and amount to a single rather small normal daily oral dose (1 gram) in, for example, one ton of

TABLE 7

CONTROL OF POST-MORTEM BACTERIAL SPOILAGE OF WHALES WITH CHLORTETRACYCLINE* (55 g. per whale)

Whale	Location of sample	Bacteria ×10⁶/g. by direct count	
Untreated 44′ male sperm, 43 hr. post-mortem	Deep muscle from behind skull	48	*
	Outer muscle from back	90	
	Deep muscle near backbone	170	2.1% F.F.A. in oil
	Liver tip	1850	
	Deep liver	1700	
Treated 50′ male sperm, 43 hr. post-mortem	Deep muscle near backbone	3	
	Deep muscle behind skull	7	
	Deep muscle near tail	2	0.7% F.F.A.
	Muscle near belly	1	
	Liver tip	37	
Treated 44′ male sperm, 48 hr. post-mortem	Surface muscle close to blubber	637	
	Deep muscle near backbone	50	
	Deep muscle opposite injected side	2	0.7%
	Liver tip	5	F.F.A.
	Muscle near liver	34	in oil
	Deep liver	100	
	Muscle near CTC injection	0	
Untreated 41′ female humpback, 26 hr. post-mortem	Deep muscle near backbone	40	
	Deep muscle behind skull	353	
	Surface muscle close to blubber	450	
	Deep liver	143	
	Liver tip	0.5	
	Belly muscle	1	
Treated 33′ male humpback, 26 hr. post-mortem	Deep muscle near throat	5	
	Deep muscle near backbone	0	
	Deep muscle near tail	0	
	Deep liver	0.8	

* >2.0% Free Fatty Acids indicates oil unfit for Grade 1. Crean, P. B., and H. L. A. Tarr, unpublished. *See* also Fish. Res. Bd. Canada, Prog. Rep. Pacific **104**, 1955 (in press).

ice. As shown in our experiments, which have been reviewed above, only a fraction of this is ever likely to enter fish, and it is extremely doubtful if the average fish treated with chlortetracycline ice would have more than .2 or even .1 p.p.m. of the antibiotic in the flesh. In fact, the amount is so small that it is only rarely that any can be detected by the pad plate assay procedure, and that after fairly prolonged storage in ice. Since fish are almost invariably cooked prior to use, it is extremely doubtful whether it would ever be possible to detect any chlortetracycline in their flesh as consumed even if they had been stored for considerable periods in chlortetracycline ice.

One danger which does occasionally exist with certain food additives is that of suppression of the natural spoilage or desirable microbiological flora and promotion of growth of certain undesirable or potential toxin-forming food spoilage organisms. Thus, sorbic acid is known to favour the growth of certain Clostridia such as *C. botulinum*. To test this point we have inoculated fish flesh with spores of Type

E. *Clostridium botulinum*, some of the fish samples containing 1 μg./g. of chlortetracycline and others being free of the antibiotic. Serial dilution tests of these samples for *C. botulinum* after storing for three days at 10° C. showed that no appreciable growth of the anaerobe occurred in the samples containing the added antibiotic, while in those without the added antibiotic there was a slight increase in growth of *C. botulinum*. A rather similar experiment was carried out using a known food poisoning strain of *Staphylcoccus aurens* (∝). Bacterial counts indicated that the growth of this organism was definitely suppressed in the presence of the chlortetracycline(1). It can certainly be concluded that treatment of fish with chlortetracycline is more likely to suppress growth of these food spoilage organisms than to enhance it.

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THE USE OF ANTIBIOTICS IN THE PRESERVATION OF FOODS OTHER THAN FISH

The primary function of agriculture is to provide food for people. Some countries produce more food than is required whereas others cannot produce enough food for their population. Right now there is not enough food in the world. Even in those countries which have a plentiful supply of food large populations in cities must be fed from farms hundreds of miles away. So it is at once apparent that feeding people is not simply a task for the farmer to produce food on his farm but also a problem in getting food from the farm to the consumer in good wholesome condition. Many of the most nutritious foods such as meat, milk, poultry, eggs, fresh fruits and vegetables are also the most perishable and difficult to preserve and transport. Modern scientific agriculture is built upon what might be called controlled biology—use of fertilizers, weed killers, insecticides, plant and animal hormones, antibiotics, synthetic nutrients, medicinals in animal rations, and antibiosis of more homely variety such as the cat vs. the rat, the geese that weed the cotton fields, or mulching or cultivating to kill some plants and protect others. These and other practices can permit the production of sufficient food. To do so is to accept the principle that controlled biology may be used to produce food. But food to serve its primary purpose must be consumed. If controlled biology can help in food production then this same principle should be applied in its broadest sense to food distribution—that is, to decrease waste because every pound of food saved is another pound for the hungry.

The biological control to be discussed here will be limited to the use of certain antibiotics to delay spoilage and decrease waste in some of the most nutrious foods—meat, milk, poultry and eggs. The other panel members are much better qualified to discuss fruits and vegetables than this author. Meat, milk, poultry and eggs are very susceptible to bacterial spoilage. Yet they do not lend themselves to heat sterilization, preservation by salt or sugar without deterioration. Consequently they are consumed from the unsterilized fresh state for the most part. Different areas of the world handle this extreme perishability in two ways—harvesting at point of consumption or refrigerated distribution. The latter method has been developed in a few areas such as the United States where these perishable foods (all products of warm blooded animals) are chilled or even frozen at once on harvesting, stored, transported, retailed, and even kept in the home under continuous refrigeration until

cooked and consumed. Should any link in this refrigerated chain suddenly be broken, such as doing away with household refrigeration, this land of plenty would probably find itself a land of food shortage. Refrigeration serves only to retard the growth of bacteria which are present and in some cases to change the type of microflora which develops. This is precisely the kind of thing antibiotics can do—inhibit growth of bacteria already in the food. Neither refrigeration nor antibiotics sterilize food. Consequently both are most effective when food is handled and processed under the most sanitary conditions. There is no doubt that antibiotics and refrigeration combined can do more for preserving perishable foods than either alone. Yet in many areas of the world where refrigeration does not exist, antibiotics alone may be able to add sufficient time to the keeping quality of some foods to permit more efficient and less wasteful distribution of food. Thus the increment of benefit resulting from the use of antibiotics in food processing may be less in the United States than in other parts of the world which enjoy only limited or no refrigeration.

Before going into a few specific examples of possible food uses for antibiotics a few general remarks applicable to all foods might be in order.

First and perhaps most important is that antibiotics are most effective when used under the most sanitary conditions. Since the antibiotics only inhibit bacterial growth and may not destroy bacteria directly they are most useful when the total number of potential spoilage organisms is low. This precludes the possibility that antibiotics would be of value in trying to recover partially spoiled and highly contaminated foods. In this respect antibiotics are analogous to antioxidants in that antioxidants are most effective in fresh fats and cannot be used to make rancid fats fresh.

Second, antibiotics do not sterilize foods and consequently they can only delay spoilage and have the greatest place in perishable foods where long time preservation is not the primary goal.

Third, antibiotics must be used to benefit the consumer in a direct manner by giving him better foods and foods which he may use over a longer period of time. Antibiotics should not be used by processors and distributors to play the markets and juggle inventories and still give the consumer a marginal or unimproved product. As an example, the use of chlortetracycline or oxytetracycline on dressed poultry (22), (23), (28) or streptomycin on fresh spinach(32) can extend the shelf life of these foods. These improvements should be for the benefit of the consumer by putting better poultry and spinach on his dinner table rather than just a permit for a processor or distributor to have more latitude in playing markets. The use of antibiotics in foods will place even greater moral responsibility on the food industry itself and also on producers of antibiotics. Certainly, if not properly done, the use of antibiotics in foods could simply become a matter of academic interest only in some areas of the world. As an example of what might happen is the use of sulfites in meat in the United States. Sulfites might be the answer to some of the meat color problems in self-serve meat merchandising, yet flagrant abuse and improper uses have definitely put this possible solution to these problems into the public doghouse to the extent that use of sulfites is inviting immediate criminal action.

Fourth, the most effective use of antibiotics for food preservation will require a better understanding of the nature of bacterial spoilage in many foods. Furthermore each food use of an antibiotic will have to be worked out on an individual basis.

There are no general or easy ways through this problem and in this way antibiotics differ from refrigeration. Refrigeration is applicable in a rather general way. Antibiotics cannot be, for it is necessary to know rather precisely both the action of each antibiotic and the nature of bacterial spoilage of each individual food item. An illustration may serve to clarify this point. Whereas streptomycin can inhibit bacterial soft rot in certain fresh vegetables(32) and penicillin may inhibit lactic acid production by certain bacteria in milk(4), (20) neither of these appear to have any value in meat preservation(15), (35). In fact penicillin inhibits the growth of gram positive cocci which appear to be competitive with the microflora which usually develops in ground beef and pork(36). It is not too difficult for those who are conversant in basic science related to foods to see how antibiotics can do much good in solving certain food distribution problems. At the same time it must be equally apparent that to be practiced on an industrial scale the use of antibiotics will require newer and more rigid quality control throughout both production and distribution of food.

There are other general considerations which could be discussed; but with the foregoing in mind I should like to relate briefly some of the possible applications of antibiotics to meat since I am most familiar with this field, and I believe meat problems can be used to illustrate many of the general features of this potentially rapid field of development.

In the United States and many countries of the world meals are planned with meat as the main item and perhaps more money is spent on meat than any other food. Meat is quite perishable and meat producing areas are usually long distances from centers of population.

In Ohio a group of workers are quite interested in trying to find out in fundamental terms what a good piece of meat really is(11). Much of this work is cooperative with Professors L. E. Kunkle and V. R. Cahill of the Department of Animal Science and H. H. Weiser of the Department of Bacteriology and the author. When one gets into such a broad and relatively unexplored area as this, it is soon very apparent that much of modern biochemistry, physiology and bacteriology which might be applicable to meat problems is not actually being applied. If it were known what was required to produce tender or flavorful meat or meat which would hold up on freezing, dehydration, etc. then something might be done by the livestock producer or by the meat processor. At the time of slaughter there are many possible ways to process an animal to produce desirable properties in meat. The animal has a built in processing plant in its vascular system, and at slaughter the carcass lends itself to modification by this route. At this time also the musculature is much more responsive to change. However, to embark on investigations based on vascular processing at time of slaughter and before dressing out a major question had to be answered. Why does meat spoil internally if not refrigerated immediately?

Every practical meat man knows that if meat is not chilled out to an internal temperature below 60° F. (15° C.) within 20–24 hours post mortem a large percentage of meat will spoil internally. This necessity for immediate chilling may also be a major limitation in the development of processing methods. It was this approach that led to the investigations on antibiotics in meat.

Lepovetsky *et al.*(25) examined commercially slaughtered beef and found that

most of the bacteria within the meat (not on the surface) were found in the lymph nodes and relatively few bacteria were present in the bone marrow and skeletal muscle. He made 492 isolations and after eliminating duplicates 93 separate strains were left. It was now apparent that most of these organisms would have to be controlled to get around the necessity for immediate refrigeration or for processing fresh meat at slaughter. The most promising approach appeared to be vascular infusion at slaughter since the lymph nodes serve a phagocytic function and have good blood supplies. Two possibilities were antibiotics and lowering pH: Husaini (18) had previously shown that lowering pH altered meat flavor and appearance of carcass. Therefore antibiotics were studied.

Goldberg *et al.*(15) screened six antibiotics by using fresh ground beef. Penicillin, streptomycin and bacitracin had little or no effect in delaying spoilage whereas chloramphenicol, oxytetracycline and chlortetracycline gave encouraging results. These three antibiotics were then screened against Lepovetsky's(25) 93 isolates from beef. Eighty-one organisms were sensitive to chlortetracycline, 77 oxytetracycline and 74 to chloramphenicol. Nine strains were affected by all three antibiotics.

Hot rounds were taken at slaughter and infused via femoral artery with physiological saline containing 55 p.p.m. chlortetracycline(35). The antibiotic solution infused was ten per cent of the weight of the round. Three of 10 control rounds and none of the infused rounds were sour after 48–72 hours post mortem and without refrigeration. In all cases total counts were significantly less in the infused round.

Following the success with excised rounds whole beef animals were infused(35). After stunning in the usual manner the animals are bled via jugular vein and/or carotid artery. The animal is placed on its back as for skinning, the carotid arteries are exposed, one is clamped and the other is pumped. Usually one-third blood volume of infusate is used. This requires about 3 to 4 minutes and the animal is then dressed out immediately by customary procedures. In the original experiments of this kind and in subsequent ones no internal spoilage developed when refrigeration was delayed 48 hours. The surface spoilage under such conditions can be helped by spraying the outside of the carcass with the infusion solution. Unrefrigerated carcasses dry rapidly and the horny surface which develops cuts down bacterial growth.

Meat infused with chlortetracycline appears quite normal. Furthermore whether refrigerated or not it has enhanced keeping qualities as it goes through normal distribution channels. Notably the slime formation is delayed even on moist surfaces.

The question of residual antibiotics and resistant organisms are usually raised when antibiotics in food is discussed. In the Ohio experiments on beef, studies along this line have been carried out. Aureomycin does not appear to be particularly stable. Mr. James Jay in our laboratory found essentially no Aureomycin (0.05 p.p.m.) in infused rounds held 6 and 9 days without refrigeration (meat temperature about 80° F. (26° C.)). Furthermore, when both infused and control paired rounds were held at room temperature 68–95° F. (20–35° C.) until spoiled, preliminary study of over 300 isolates shows no evidence that organisms resistant to the antibiotic preferentially developed. These experiments are continuing.

The infusion technique via carotid artery works quite well for cattle and sheep.

It is somewhat more difficult to apply in swine. Intraperitoneal injection of antibiotics a short time before slaughter offers some possibilities. Since it must be done about one to two hours before slaughter, it requires handling live animals twice. For domesticated cattle the technique is quite simple but for the more untamed variety it is difficult. In our experience it is more difficult than by vascular infusion. Because of the rumen only a small area is readily accessible through which to inject the solution. In fat animals the kidney and surrounding fat partially or even completely block this pathway. Intraperitoneal injection may be more easily applied in swine. If the antibiotics of choice (the tetracycline group) are used in simple aqueous solution large volumes of solution must be injected in order to overcome precipitation of the antibiotic in the peritoneal cavity. This is because of low solubility and may be overcome by complexing the antibiotics with solubilizing compounds. Apparently this is the technique used by Sacchi and McMahan as reported pictorially in the public press of Cuba recently(2). Another problem which comes from intraperitoneal injection is the concentration of antibiotic which develops in the kidney and liver and the tendency to stain the peritoneum. Much work remains to be done.

One might ask why the interest in antibiotics in meat when the ultimate goal of actual permanent preservation is not in sight and in fact keeping quality has been extended only a matter of days. Let us see how important these days really are.

In the United States and similar places where meat is distributed under refrigeration supposedly from the time of slaughter to the time it is consumed, meat is still so perishable it is distributed in large wholesale cuts for cutting and packaging at the retail outlet. This archaic method must still be used in modern supermarkets. Antibiotic treatment of meat could mean in-plant packaging and distribution to the retailer of consumer packaged meat. This means greater economy, and better and more centralized quality control. It means less waste in terms of trimming losses and less deterioration of products in transit and these are no small items for the large packer doing an interstate business. It means better fresh pork sausage and ground beef which are extremely perishable—so much so that fresh pork sausage is sold only in very limited amounts. Lastly this means that consumers could buy a retail package of fresh meat bearing a date and government inspection stamp indicating that not only the meat had been killed under sanitary conditions, but the best in sanitation and freshness had been carried and guaranteed to the consumer—not merely guaranteed to the wholesale or retail market.

What does a few days keeping time mean to countries without refrigeration or at best limited central refrigeration? It means vast supplies of cheaper meat for people who do not live near areas where meat producing animals thrive. It means that live animals do not have to be taken to point of consumption for slaughter. We have received many inquiries along this line. In much of the world capital equipment costs for refrigeration, as we know it in the United States, is itself prohibitive. Power costs are more and in many places power does not exist. In the United States with power at 1 cent a kilowatt it costs about 30–35 cents not including capital merely to chill a carcass of beef. Antibiotics can compete in many countries and effectively so because heavy capital investments are not required. It means that if highways are available meat producing animals can be killed 500 miles away from

point of consumption and trucked to market as carcasses. In some parts of the world it will make it economical to fly meat 1500 miles or more to market. An inquiry from one cattle producing country indicates that antibiotics will open a market in a meat deficient country 1500 miles away.

Defrosted frozen meat is highly susceptible to sliming. Antibiotics in quarters and sides of beef frozen and shipped from Australia, New Zealand or South American countries to the European market might well prevent deterioration in quality which results when these large wholesale cuts are thawed out and retailed without refrigeration.

The whale presents an intriguing problem—that is, he is a warm blooded mammal that is so large that chilling rates are so slow that much potentially edible meat is lost before processing can be completed and hence the interest in the whale as a source of fresh meat is a secondary one. If antibiotics could be gotten into the carcass soon enough then it is quite possible that spoilage of whale meat could be materially reduced and the whale meat itself could be of great economic importance.

Can antibiotics be fed to meat animals and fowl so as to enhance the keeping quality of the flesh? This may be difficult and uneconomic. Broquist and Kohler(7) report that a ration containing 1000 grams chlortetracycline per ton gives an 0.02–0.12 p.p.m. in chicken muscle. Luther *et al.*(27) report that the same level of feeding oxytetracycline produces 1.05 p.p.m. in chicken muscle and 1.26 p.p.m. in pig muscle. One might expect enhanced keeping quality to meat containing 1.0 p.p.m. A series of experiments are currently being conducted by my colleagues A. R. Winter and Timothy Chang. The keeping quality of both meat and eggs from chickens getting rations containing 1000 g. per ton of several antibiotics is being investigated. No significant differences were found in the keeping quality of the chicken meat from controls and from birds getting penicillin, bacitracin or chlortetracycline.

The results on eggs showed quite striking differences as seen in Table 1. Egg whites have quite satisfactory keeping quality compared to yolks inasmuch as the antibiotics avidin and lysozyme occur naturally in the white.

The eggs from a previous feeding trial involving only chlortetracycline clearly confirmed these results. These results on chicken meat and eggs might be expected from the findings of Broquist and Kohler(7) referred to above. These latter authors found that 2000 grams of chlortetracycline per ton of feed gave antibiotic concentrations of 0.15–3.1 p.p.m. in the egg.

TABLE 1

POOLED EGG YOLKS STORED AT 88° F. (31° C.)*

	Millions of bacteria per gram			
Hours	Control	Chlortetracycline	Bacitracin	Penicillin
0	———	———	———	———
6	———	———	———	———
24	0.7	———	———	———
30	80.	———	———	17
49	Putrid	———	———	200 off odor
55	Putrid	———	8	Putrid

* Courtesy A. R. Winter and Timothy Chang.

Fresh dressed poultry has always presented a serious spoilage problem. Because of its size it is slaughtered at point of use in much of the world, but as cities grow this becomes more difficult and almost impossible from the public health standpoint. Much has been done in recent years to improve poultry processing operations, and although improved sanitation has done much to give better dressed poultry to the consuming public, cut up and dressed poultry still deteriorates rapidly. Ziegler and Stadelman(38) using techniques similar to those used by H. L. A. Tarr and coworkers(33) on fish, have shown that poultry which has been dressed, washed and cooled and dipped for 10 minutes in Aureomycin solution containing 10 to 40 p.p.m. will keep significantly longer than control birds. Kohler *et al.*(23) showed that chlortetracycline was the most effective of ten antibiotics (chloramphenicol, erythromycin, procaine penicillin, neomycin, chlortetracycline, streptomycin, magnamycin, polymyxin B, bacitracin and acridone) in controlling the growth of organisms isolated from spoiled poultry. Further work along this line is reported by Gibson *et al.*(12). Kohler(23) confirmed and extended the work of Ziegler and Stadelman(38) by showing that by dipping in chlortetracycline or cooling with ice containing chlortetracycline birds kept longer than untreated controls. On comparing birds treated with chlortetracycline, oxytetracycline or tetracycline they found chlortetracycline most effective. Although not published it is understood that semicommercial type of experiments are being conducted using Aureomycin or Terramycin Arquad, which is a quaternary salt of oxytetracycline. The doubling of the shelf life of fresh poultry meat as shown by these workers can be a great help in making high quality poultry meat available to the consumer. If residual antibiotics are to be avoided in the food as consumed it is of considerable interest to note that Kohler *et al.*(23) found that cooking destroyed the residual chlortetracycline in the chicken meat. There is no doubt that there will be increased interest in antibiotics by the poultry industry the world over. As in red meat and fish some antibiotics can help retard spoilage and this will be a major advance whether or not it is used with or without refrigeration.

The possibilities of antibiotics in food have been of much interest in the dairy industry. The earliest experiments are perhaps those of Curran and Evans(9), (10) who concluded that penicillin and streptomycin were of little value in keeping milk. A few years later Ocklitz and Schmidt(29) reported that chlortetracycline was more effective than streptomycin in preserving human milk for short periods of time. Greene and Bell(16) showed that oxytetracycline and chlortetracycline inhibited bacterial growth in milk for 20 hours. Later Hashida(19) showed that at 30° C. penicillin and streptomycin would keep milk 1 day, chloramphenicol 2 days, patulin 3 days, and oxytetracycline and chlortetracycline 4 days. Shiveler and Weiser(31) showed that chlortetracycline was more effective than penicillin and dihydrostreptomycin in keeping raw milk and still more effective in preserving pasteurized milk. Using flavor and odor as criteria for soundness of milk at 3° C. Shahani *et al.*(30) recently reported the data in Table 2. They also report essentially the same pattern of results for 25 p.p.m. It can be readily seen that the tetracycline group of antibiotics has possibilities in fluid milk supplies. However, in countries like the United States the benefit from their use would be limited, but not so in countries where fluid milk supplies are not refrigerated from the time of milking to consumption. The

TABLE 2

EFFECT OF LOW CONCENTRATIONS OF ANTIBIOTICS UPON THE MICROBIOLOGICAL KEEPING QUALITY OF MILK
(Concentration 1.0 p.p.m.)*

Number of days stored	No Antibiotic		Penicillin		Streptomycin		Aureomycin		Terramycin	
	Raw	Past.	Raw	Past.	Raw	Past.	Raw	Past.	Raw	Past.
0	+	+	+	+	+	+	+	+	+	+
3–4	±	+	±	+	+	+	+	+	+	+
7–8	−	±	−	+	±	±	±	+	−	+
10–11	−	±	−	±	−	±	±	+	−	±(S)
14–15		−		±	−	±	−	±(S)		±(S)
17–18		−		−		−		±(S)		±(S)
21–22								±(S)		±(S)
28–29								±(S)		−
35–36								−		−

+ = Good.
± = Slight off-flavor.
− = Objectionable flavor.
S = Slight stale or storage flavor.
Past. = Pasteurized at 143° F. for 30 minutes.
* Courtesy of K. M. Shahani, I. A. Gould, H. H. Weiser and W. L. Slatter (30).

addition of some antibiotics to milk as it is obtained would add much to its keeping qualities and hence to its more effective distribution. Professor C. L. Blackman of our Department of Dairy Science has been studying world wide milk production problems and he points out a fascinating possibility. In some warm countries farmers milk their cows only once a day because the milk from the second milking would spoil before it could be gotten to the consumer. It is well known that once-a-day milking tends to depress milk production even for the one milking. Consequently, the use of suitable antibiotics might overcome this difficulty and promote greater and much more efficient milk production and thereby make more milk available to the people who really need it.

Since the use of antibiotics has become common in the treatment of mastitis a considerable amount of work has been done on antibiotics in raw milk supplies(17), (37) and this has become something of a problem for the cheese maker since the organisms he uses might be sensitive to the antibiotics present(4), (20).

A problem in cheese production of many types is faulty fermentation apparently due to some forms of Clostridia which produce gassiness and butyric acid. Nisin, an antibiotic produced by certain streptococci will effectively control this kind of spoilage(34). MacClintock *et al.*(28), Berridge(5) and Mattick(6) have shown that nisin may be added directly. This is done commercially in Britain according to Food Manufacture(3) which points out that this is the first example of antibiotics being used commercially in foods. Kooy and Pette(24) point out that antibiotic-producing strains of *S. lactis* may be used as starter cultures in certain cheeses and thus overcome faulty fermentation.

Dairy products which are notoriously troublesome in food poisoning are custards, cream fillings, etc. These are often marketed as bakery items. In my home town of Columbus, Ohio, two years ago a very large baking company distributing to a population area of about 1.5 million people sold some custard and cream filled items causing several thousand cases of acute food poisoning. In perishable foods such as custards, chicken salads, creamed and cream filled foods antibiotics might be useful according to the work of Godkin and Cathcart(14). These authors point out that 70 p.p.m. subtilin or 10 p.p.m. oxytetracycline are effective in controlling for three days at summer temperature food poisoning strains of enterococci, salmonella, and micrococci in custard fillings. An earlier report indicated that 0.1 to 1.0 p.p.m. chlortetracycline and oxytetracycline was ineffective in certain heat resistant bacilli(13).

Since in the foods discussed bacterial spoilage is of dominant importance, antifungal agents have not been discussed. However, the control of bacteria in foods may permit the slower growing yeasts and molds to become more important. Tarr *et al.* have reported this(33) and we have noticed that on moist meat surfaces molds may develop if bacteria are controlled by chlortetracycline. However, this problem does not at this time appear to be of major significance.

Another kind of food spoilage problem which has not been discussed and yet has considerable bearing on the use of antibiotics in foods is insect infestation. Refrigeration is a very effective deterrent to insect growth. In perishable foods such as meat, milk, eggs, etc. antibiotics may retard spoilage but they have no insecticidal value. Consequently, if perishable foods are contaminated with the eggs of certain

insects such as flies and are held at warm temperatures, spoilage due to insects may develop. This points up again that antibiotics are most effective when used under the most sanitary conditions.

The foregoing discussion has been strictly limited to the use of antibiotics in perishable foods—foods which up to the present time do not lend themselves to processes for long time preservation without deterioration, loss of consumer appeal, or extreme cost. This limitation is not meant to indicate that these are the only applications of antibiotics to foods. Indeed considerable interest has been shown in the possibilities of antibiotics supplementing other processing methods—particularly heat sterilization. Experiments along this line have been somewhat disappointing. The requirements of antibiotics for this kind of use are quite different from the requirements for extending the usable life of perishable foods(1), (8), (21), (26).

A field of investigation which may show promise is the possible use of antibiotics in conjunction with radiation. Complete sterilization by ionizing radiations often produces unpalatable foods. If antibiotics and radiation could complement each other in the kinds of organisms to be controlled then the deleterious effects of radiation might be overcome. Much fundamental research is needed in this area.

It is well to point out here that antibiotics have one distinct advantage over refrigeration and radiation and this is the relatively insignificant capital equipment costs required by the processor for processing foods with antibiotics. For refrigerated distribution capital equipment costs are high.

No doubt it is at once apparent that much needs to be done in the entire field of antibiotics in foods. Research in some areas shows great promise yet application of ideas must be proved by extensive development work which is often costly. Food processors who must necessarily be responsible for development and production of new processes are reluctant to go ahead when as yet the basic philosophy of the use of antibiotics in foods—biological control in food processing and distribution—has not been officially approved by regulating agencies. Recently the so-called Miller amendment has modified this situation a bit in the United States. In my field of interest—meats—this deterrent may even be greater than in other fields because of two reasons: one is the cost per unit of working material and the other is that not only the use of antibiotics is a point in question but also the alteration of slaughtering procedures per se is in question. If the experimental material is to be condemned and not salvaged because it is something new, meat costs become prohibitive since the cost of a steer is about 200 times that of a chicken or 4000 times the cost of an egg. Regulating agencies are extremely important to insure the fulfillment of individual and corporate responsibilities. Certainly none of us would want to be without them. Even though their function is primarily one of regulation and not research, they can have great influence on research and hence have a responsibility in actively nurturing and cooperating in new developments. New developments can mean much for public health, nutrition and safety and this is in reality the responsibility of regulating officials.

More than anything else research and development work is needed. Let the facts be known and answers will be available to handle the practical application questions as well as those concerned with public health. Answers to the fundamental questions as to the nature of the action of antibiotics, the mechanism of the development of

resistant strains, etc. would be most helpful. In most of the examples cited above the antibiotic was used at point of harvest only, and this would not seem to promote the development of resistant strains in the food. However, the only real test to verify this is a well controlled pilot commercial operation.

Antibiotics have come into prominence through their medical use. This has tended to cloud basic and fundamental facts concerning their place in the food industry. Only intensive study, research, and development can keep the right course so that public ignorance, hysteria and presumptions do not retard or even stop progress. Progress here means more and better food for more people at less cost.

In this limited review several promising examples of the uses of antibiotics in the food industry have been discussed. At the present time certain antibiotics can retard, but not prevent, bacterial spoilage of perishable nutritious high protein foods such as milk and other dairy products, poultry and eggs and meat. These uses of antibiotics have been illustrated from the point of view of the food industries of different countries. These and many other promising food uses will require much additional research and development and will entail greater individual, corporate and governmental cooperation and this in turn means greater responsibilities to insure the greatest benefit to all.

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Food Preservation

PANEL DISCUSSION

Moderator
James G. Horsfall

Panel Members
F. E. Deatherage — Sven Dyrendahl
Wilson L. Smith, Jr. — H. L. A. Tarr

Summarizer
Emil M. Mrak

Dr. Horsfall: Unfortunately, Professor Marston from Australia, referred to by Professor Deatherage, is not able to be with us. He sent in two or three comments about the Australian problem of antibiotics in food from which I would like to read two or three excerpts at this point. He says first that he hopes that we will convey to the Conference his regrets at not being able to come.

"Australia," says he, "is approximately four weeks by sea away from her main markets for export beef. The main problem which confronts the beef industry here is to extend the 35- to 40-day period during which beef may be held in chilled condition without serious deterioration. An extension of even a few days would be of very great value to us. There is general agreement that appropriate treatment with Aureomycin, Terramycin and chloramphenicol will increase considerably the storage life of meat and fish, whereas penicillin and streptomycin are not effective."

"Large-scale tests conducted in Australia for quite another purpose have provided unequivocal evidence that after initial low survival of microorganisms there can, with repeated use of the bacteriostatic or bactericidal agents, be a 'steady and relentless build-up of survivals.' You can see how important that would be if the beef had to be in transit for several weeks."

"It is not improbable that quite a number of resistant strains occur in the Pseudomonas and Achromobacter bacteria that dominate any population of bacteria that contaminate flesh-products, even a rough evaluation of the chances of success of antibiotics for meat preservation is very difficult indeed in the absence of the precise knowledge which would be deemed essential before long-term, large-scale tests are started. A better knowledge of the rates at which resistant mutants of the important food-spoilage bacteria will arise is especially desirable at this juncture."

"Another point on which experimental evidence is meager is the stability (i.e. continued effectiveness) of antibiotics in contact with meat surfaces. Further study of this is desirable to explain the different findings published in the literature."

Two panelists are left. One, Professor Dyrendahl, is a veterinarian and presumably interested in the meat end of it; and the other, Dr. Smith, is interested in the preservation of plant material in transit. I will ask first for any comments that Professor Dyrendahl would like to make.

Dr. Dyrendahl: Dr. Brierem and Dr. Clausen gave you this morning and yesterday a good idea, I think, of how we in the Scandinavian countries are looking at

the antibiotics and what we have tried to find out in our experiments on these substances, especially in swine.

There has been some research work on the use of antibiotics for the preservation of fish in Sweden during last year at the Institute for Food Preservation in Goteborg, but they are just at the beginning.

As a veterinarian, I am very glad now to have the opportunity to learn a great deal about these impressive things given in the paper by Dr. Tarr and Dr. Deatherage.

My questions must be considered questions posed by a non-specialist.

First, I wish to ask if the biological process has been considered seriously enough if antibiotics are to be used for preservation of food, especially fish. The bacteriological aspects of different antibiotics are known. It is therefore possible to figure out which bacteria can be affected with the aid of these substances. Is it not, however, true that microorganisms, bacteria, or fungi, may increase in the food in question and the result of our treatment with them be not preservation but only a change in the flora? I believe it is very important not to forget the biological connection here.

The problem that concerns the distribution of antibiotics in ice has been studied with the help of some active substances. Is it not a fact, however, that exactly these substances promote the spread of microorganisms? Instead of just a few colonies of microorganisms, one would get the result of millions of such colonies. On my part, I believe that the method of adding antibiotics to the cooling ice for fish seems very striking at first. When one thinks of the problem carefully, however, a great deal of antibiotic must be wasted when the ice melts. The advantage of ice for cooling is that the ice is cheap. Because it is so cheap, one can afford to let it melt and disappear. No distillation is necessary. Methods of reducing the melting of the ice perhaps through some type of insulation would result in economic loss and poorer cooling effect.

As the important thing is the prevention of bacterial growth on the meat by means of solutions of antibiotics, the surface of the meat must be kept damp. Under normal conditions the surface dries, reducing the bacterial growth. To me it does not seem right to moisten the meat the surface of which has already been dried. And what is known about the stability of antibiotics in the meat?

DR. HORSFALL: Thank you Professor Dyrendahl. The next person on the panel from whom I think we should hear works with the use of antibiotics for the preservation of plant as contrasted with animal foodstuffs in the market, and therefore I would like to ask Dr. Smith to give us a short summary of the sort of problems which occur in that field.

DR. SMITH: One of the earliest reports of an antibiotic used on a vegetable is that of subtilin used in the canning of vegetables in experiments conducted in California. At first it looked very effective. By the use of the antibiotic the population of microorganisms was reduced and it appeared that the heat sterilization period during canning could be shortened. In later reports it was not so effective. I don't know of the tests which are going on at the present time in that respect, but I do hope that some of the new antibiotics are being tested. A shorter sterilization time for foods in the cans, could possibly result in better flavor of the product.

With freshly processed vegetables we have three main types of organisms which would cause decay or deterioration. On leafy vegetables the soft-rot bacteria are extremely common, and within 24 to 48 hours at temperatures above 60 degrees F. may completely destroy the product. Industry at the present time is putting these leafy vegetables in transparent sacks, forming an ideal medium for the growth of these organisms. Attempts to control the soft-rot bacteria have been made with different germicidal washes. All attempts, to my knowledge have been relatively unsuccessful. Therefore, the only method remaining for control of bacterial decay in most instances, is refrigeration. Actually, refrigeration, as Dr. Deatherage has reported, is only a method for restraining the organism; it is not sterilizing or killing the organism.

It was suggested that we try some tests with antibiotics to control these bacteria. A few years ago we tried adding streptomycin sulfate at a concentration of 1000 p.p.m. to the water used in washing spinach. Spinach was then packaged and held at 70 degrees. While the checks were decayed within 24 hours, those treated with streptomycin sulfate showed almost no decay within 48 and in certain cases even up to 72 hours.

Further tests with streptomycin wash have been conducted with cole slaw. The average market life of unrefrigerated packaged cole slaw is approximately 12 hours. With the streptomycin wash we held packaged cole slaw, rated completely salable by a panel of judges, for a period of two days. Deteriation of cole slaw is a multiple effect. Not only does it have bacterial decay, but the cut surface of the cabbage leaf will rapidly oxidize, or some other enzymatic process will turn it a dark color. Even in the absence of bacterial decay, the streptomycin wash was sufficient to control this discoloration of the slaw. We have no explanation of that at the present time.

Another type of bacterial decay or deterioration would be the bacterial fruit spots. Dr. Zaumeyer has already talked a little bit about the work done on beans and on apples and pears. I won't go into that.

One of the new reports is from Conover in Florida, who has sprayed tomato fields with streptomycin sulfate several times during the growing season and obtained approximately a 60 per cent reduction of bacterial spot of tomatoes. He also got an increase in the fruit size, an increase in yield, and a decrease in fruit cracking. In this case an antibiotic improved the appearance of the fruit and probably put the fruit in better grade and better eating quality for the consumer.

In addition to the bacterial decays, we have fungus decays of fruits and vegetables. Very little work with antibiotics has been done on these. We have tested rimocidin for the reduction of peach brown rot and peach rhizopus rot over a period of three years. The peaches were sprayed after harvest with rimocidin, and the final decay readings made after six days at 75 degrees. Over a three-year period rimocidin gave better than 50 per cent reduction of both peach brown rot and rhizopus rot. Another new material we tried last year which has proved to be equally effective is canidicidin. It reduced both brown rot and rhizopus rot by better than 50 per cent in rather limited tests. A report from California also has shown that peach brown rot is reduced by post-harvest treatment with canidicidin.

In summary, I would like to say that most of our tests are in their infancy. We do not have good concentrations or low concentrations that meat and fish people have. We will have to make further tests in that respect. We also have very little accurate data on residues.

What we are looking for constantly are new antibiotics which are not of use medicinally, and which will control both bacteria and fungi.

DR. HORSFALL: Thank you very much, Dr. Smith. Dr. Deatherage?

DR. DEATHERAGE: Dr. Dyrendahl asks a question about the spoilage organisms. We are presently engaged in some work on whether the antibiotic-treated meat spoils normally or abnormally. My only comment is that all experiments so far that we have conducted indicated it is just normal spoilage. By that I mean we have made several hundred isolations of organisms from two rounds of beef which have been held at room temperature, one of which had been infused with antibiotics and the other had not. Both had been allowed to hang in summer temperatures until spoiled. I might say we had two rounds which we held for nine days without rotting. The other fell off the hook. We have not found any selectivity there. I think the reason is that the antibiotic disappears to zero under those conditions.

Another question which has been asked here: "Would the antibiotic prolong shelf life and is it economical for eggs?" I doubt if it can be used for shell eggs. It may be used for separate egg meats, prepared for freezing, and so on, where there is a distinct spoilage problem. You can feed chickens sufficient antibiotic to get antibiotic in the egg so the egg yolk will keep a little bit better, but probably it is not economical.

Dr. Coles of South Africa has asked what percentage of Aureomycin is used in water in dipping poultry. We are going to have poultry specialists here later in the day. However, I think the concentration is about 200 parts per million. It is hoped that the amount of antibiotic will reach about two parts per million in most of these experiments on the effects on perishable foods.

DR. HORSFALL: Thank you, Dr. Deatherage.

Ed. Note. The following comments by Dr. McMahan and Dr. Hines were made at the Fifth Session but properly belong in this session and are therefore inserted at this point.

DR. J. RAYMOND MCMAHAN: I want to clarify a couple of points in connection with the intraperitoneal injection methods of preserving carcasses which was mentioned in Dr. Deatherage's paper. Most of the work with this method has been done with oxytetracycline, but the method is perfectly applicable to other antibiotics.

The assertion was made that this is a difficult procedure. We do not regard it as particularly difficult as compared with the infusion method which requires pumping about one blood volume of fluid into the circulatory system. Our procedure can be incorporated into the regular plant operation simply by running the animals through a restraining chute where the injection can be done in approximately one minute per animal.

The volumes of injection fluid are very small. They are not three liters or so, as was said, but are approximately 50 to 100 cc for large animals such as steers and about 15 to 30 cc for small animals such as sheep. The tissue levels we are aiming

for are about one part per million and that is obtained by using about one and one-half grams of the antibiotic per 1000-pound animal.

Residual levels of antibiotic in kidney and liver were mentioned. Those are approximately ten times the level found in lean meat, and therefore would be approximately 10 p.p.m. Whether the small amount of antibiotic remaining after cooking would be of significance has not been determined.

It was mentioned that we use both the hydrochloride and a quaternary ammonium complex of oxytetracycline. We use tartaric acid as a solubilizing agent with the complex. The reason we are using the complex is that it is quite effective against Salmonella in other food applications. We wanted to see if the complex would be effective also in the intraperitoneal injection method.

DR. L. R. HINES: There are three very brief items that I would like to touch upon and summarize.

First, in answer to a question which was raised the other day, that has been covered to some extent by Dr. Reynolds concerning concentrations of antibiotic in the tissues. The concentration of Aureomycin that we find in chicken breast muscle when Aureomycin is included in the feed is shown in Table 1 and the level is indicated in parts per million. The parts per million are roughly equivalent to grams per ton.

In this particular series of experiments, the chickens were administered 100 parts per million in the diet over a rather extended period of time, about two months, in which case we have none in the breast muscle. You can see from the data what the results were at 200 and 1,000 parts. Table 2 shows the uptake of Aureomycin in chicken breast muscle when it is immersed in Aureomycin solutions for two hours. If you will look at the series of columns across the top, 3, 10, 30, 100, and so on, micrograms per milliliter, that is the concentration of Aureomycin employed in this experiment. In all the work we have found 10 micrograms per milliliter to be entirely adequate. That is the area in which we have done almost all of our work.

I would like to call to your attention a very interesting observation, the rapid destruction when this chicken breast muscle is subjected to boiling temperature. You will note that when the material is boiled for a short time, such as 15 minutes, no Aureomycin residues remain in the muscle. Even those which were dipped in concentrations as high as 100 gamma micrograms per milliliter are destroyed. The rest of the data you can see.

TABLE 1

AUREOMYCIN CONTENT OF CHICKEN BREAST MUSCLE FOLLOWING FEEDING OF AUREOMYCIN TO POULTRY OR DIPPING FRESHLY KILLED POULTRY IN AN AUREOMYCIN SOLUTION

Aureomycin in feed p.p.m.	Aureomycin in breast muscle γ/gm.	Concentration of Aureomycin in dipping water p.p.m.	Aureomycin in breast muscle γ/gm.
0	None	1	0.26
100	None	5	0.41
200	0.02–0.06	10	1.9
1000	0.05–0.1	20	2.9

TABLE 2

EFFECT OF BOILING (100° C.) ON RESIDUAL LEVELS OF AUREOMYCIN IN CHICKEN BREAST MUSCLE

Boiling time in minutes	Aureomycin remaining—γ/gm. tissue					
	3 γ/ml. sol.	10 γ/ml. sol.	30 γ/ml. sol.	100 γ/ml. sol.	300 γ/ml. sol.	1000 γ/ml. sol.
0	0.34	2.2	3.4	23.0	32.0	115.0
5	0.65	0.11	0.28	2.6	1.6	8.0
15	neg.	neg.	neg.	neg.	0.45	0.45
30	neg.	neg.	neg.	neg.	neg.	neg.
60	neg.	neg.	neg.	neg.	neg.	neg.

If we wanted to make a ridiculously severe test out of this we could dip the material in a concentration of 1,000 parts per million. You will note there that in 30 minutes it was completely destroyed. We have done this many times. I believe the data are accurate.

There is only one other point that I would care to make, and that is, we have been interested—and I am sure some of you are, since I have had some questions about this—in the type of organisms which are responsible for spoilage in poultry which has been treated with Aureomycin. That is, are they the same or are they different from the organisms which are normally responsible for the spoilage of untreated poultry?

A survey of the literature and our own work would indicate that at the time of spoilage in poultry which has not been exposed to any antibiotic, the spoilage organisms are predominantly Pseudomonas and *C. aerogenes*, and about 95 per cent of them are Pseudomonas. In the case of poultry which has been dipped in antibiotic solution the same organisms are responsible for spoilage. In each case about 95 per cent of the population at the end of 14 days is Pseudomonas.

DR. HORSFALL: Now comes, to my mind, about the toughest job in a session like this, the man who must tell us in a few minutes what we have learned during the course of the last hour and a half. I take pleasure in introducing Dr. Emil Mrak, of the University of California.

SUMMARY OF THE FOURTH SESSION

FOOD PRESERVATION

DR. MRAK: I have tried to summarize these talks and discussions under eight headings.

1. Is there a place for the use of a chemical or antibiotic, such as we are talking about today? Apparently the answer is yes. In a sense we may consider this something like a chemical pasteurizing agent, although Dr. Deatherage tells me that this terminology is not correct because the antibiotic does not kill microorganisms, but merely inhibits them. At least it stops their growth. If this is the case, then where do we really have places to use them? First of all, between slaughter and consumer particularly on meats and poultry. It has been brought out that this is an important area; secondly, between the fishing areas, and the shore and the consumer. Undoubtedly there could be better distribution of fish which would taste like fresh fish

and not the fish we are accustomed to; thirdly, between the farm and the consumer insofar as certain fresh vegetables and fruits are concerned, and fourthly, between the farm and the processor on certain vegetables. Today there is a tendency to remove lima beans and peas from hulls and put them in tubs of cool water (sometimes containing chlorine but most of the time not) or ice water, and hauling these in to the plant for processing. There is a question sometimes whether or not microbial control should be used there.

Certain fruits, particularly strawberries mold rapidly after harvesting. Last year in one area there were great losses due to brown rot occurring in peaches between the farm and the cannery during transportation. Such an agent could be used to extend the life of certain foods in areas where refrigeration is scarce. As indicated this morning, such use would be mostly outside of this country.

Possibly in bakery products. I say "possibly" because some of these foods are not permitted to be sold in the summer in certain areas unless under refrigeration.

We may summarize Point 1, then, by saying the most suitable place for use of antibiotics would be when long-time sterilization is not the goal.

2. What could the use of antibiotics on foods mean to the consumer? Better quality of packaged meats and poultry, better quality and distribution of fish and fishery products, conceivably better processed fruits and vegetables. If we get better fruits and vegetables to the canner and processor, we should get better commodities to the consumer. They should have better flavor, appearance and texture. I'll have another comment on that later. In some cases perhaps the utility value should be better. If the consumer can get items such as chickens cut up and the parts he doesn't want removed, we would improve the utility.

3. What would this mean to the processor? Certainly fewer losses of the commodity during handling and transportation prior to processing. This would result in a better processed product. On the other hand, it would mean to him that his technical men would need a great deal of know-how in using the material.

4. Safety. Little has been said about safety here today. Of course this is a matter which would have to be settled by the regulatory agencies, whether or not they think it is safe. It appears, however, that there is reasonable ground to believe that it would be safe. Dr. Deatherage tells me that in order to obtain a dangerous dose of some of the antibiotics from the standpoint of residues in meats, it would be necessary to eat 2,000 pounds of lean beef a day; and Dr. Tarr tell me that it would be 50 tons of fish a day. It appears, therefore, that we are on the fairly safe side.

On the other hand, if we take commodities which are not cooked, like custards or milk, the story may be different. We need information there.

5. How applied? There have been several suggestions on how the material may be applied. Apparently these will work. The important point here is that it must not replace sanitation. Furthermore, as already indicated, we must have an understanding of the spoilage with respect to each particular product.

6. It appears that there may be possibilities for using antibiotics in connection with radiation sterilization. Perhaps the two together might work. Right now there are many problems in radiation sterilization from the standpoint of off flavors. Secondly, it certainly would help to use them with refrigeration. The use with heat has been at best, disappointing.

7. Questions and possible limitations. The question of flavor comes up. A great deal was said earlier this morning about these materials getting into the plants. I could not help but wonder whether or not they would get into the fruit and, if so, would it affect the flavor. If much was in the fruit, might it affect processing operations? What would be the affect of antibiotics on sauerkraut or the pickling of cucumbers where microorganisms are used in producing the product? This may be a minor point, but it may also be a serious one, and worth thinking about.

What about antibiotics favoring the growth of other organisms, such as fungi and yeast? Some mention was made of this possibility by Dr. Tarr and Dr. Deatherage. I felt however that it was passed over a little hastily. Perhaps another antibiotic in combination with the ones talked about this morning might help this. As indicated earlier this morning by Dr. Campbell, there is an antibiotic for fungi which seems to work. I am told it is not toxic.

What about the possibility of promoting resistant strains in a processing plant? If we used the materials in a processing plant over and over, might we induce the growth of undesirable organisms or might we encourage the development of strains which are resistant to the antibiotic? These questions must be answered. Perhaps we already have the answers, I am not certain.

8. Then, of course, we come to the permissibility of use. If we are not going to be permitted to use antibiotics on foods, then of course we are wasting our time. What about the behavior with respect to vastly different foods? It has been pointed out that in hard water the results are different from those obtained in soft water. Fish, as compared with crabs, give different results.

We might summarize all this by asking: Where do we stand? I would start by saying, "It appears." It appears that we have a good possibility of increasing acceptability for the consumer, a good possibility of increasing the utility value of certain foods for the consumer, pre-packaged foods, moist pack foods, and so forth. There is a good possibility of increasing the stability of foods. Some of you probably have had the experience of bringing weiners or hot dogs home and having them get slimy whereas they weren't slimy when purchased. If the stability factor could be increased by a day or so, it certainly would be valuable.

Safety. I have already mentioned safety. It appears that we have fairly good evidence that we are on the safe side. I am certain it would increase the esthetic value of the foods. If we have slimy sausage we don't want to eat it, even though we think or even know it to be all right. For the processor, if the use is permitted, it appears he can reduce his losses, he can pack better commodities, he can have a better quality pack, perhaps lower cost of operation, he can have better transportation; but he must also realize that he must have the technologist on hand and must also not relax on sanitation.

Finally, what about the Food and Drug Administration and legal agencies? They must make the decision.

DR. HORSFALL: Thank you, Dr. Mrak. I would like to thank this panel—Dr. Smith, Dr. Tarr, Dr. Dyrendahl, Dr. Deatherage, and Dr. Mrak. The meeting is adjourned.

. . . The meeting adjourned at one twenty-five o'clock p. m. . . .

FIFTH SESSION

PUBLIC HEALTH ASPECTS

October 21, 1955

WESLEY W. SPINK, *Presiding*

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EMERGENCE OF RESISTANT STRAINS IN CHRONIC INTAKE OF ANTIBIOTICS. A REVIEW *

A number of reviews dealing with various aspects of the subject of microbial resistance to chemotherapeutic agents have appeared during recent years. Elizabeth McCoy's survey of the literature on *Changes in the Host Flora Induced by Chemotherapeutic Agents*(105) is quite pertinent. More recently Lepper(93) attempted to estimate the extent of the problem of antibiotic-resistant organisms as a cause of bacterial infections. The literature dealing with special aspects has also been considered;—the problem of resistant staphylococci by Prissick(127), Spink(160), and Clough(30), the genetic factors by Bryson and coworkers(21), (22), streptomycin-resistance by Miller and Bohnhoff(108), and the more fundamental mechanisms concerned with the adaptation of microorganisms were reviewed by a number of workers at the *Third Symposium of the Society of General Microbiology*(66). The aspects pertaining to replacement of the host's microflora by antibiotic-resistant species during antibiotic intake and bearing directly on problems encountered in agriculture have been incorporated in some of the extensive reviews on antibiotics in nutrition by Mickelson(107), Braude(17), Jukes and Williams(84), Fidanza *et al.*(53), Stokstad(162), Knodt(89), and Jukes(85). The reviewer is particularly indebted to Dr. Thomas H. Jukes for making available the completed manuscript of his recent monograph(85) and for considerable help from him and his associates in obtaining some of the agricultural literature on the subject and also some unpublished data of his own and of other workers. This review will not consider the interesting problems concerning the mechanisms of development of resistance, or the biologic and biochemical properties of resistant strains; these are dealt with in some of the reviews that were mentioned. Nor will the problem that is even more pertinent with respect to animal feeding and nutrition in general, namely the mechanisms of the growth enhancing effects of antibiotics, be reviewed in detail except as it bears on the problem of the residual antibiotic-resistant flora.

Any discussion of the emergence of resistant strains in vivo inevitably separates itself into two distinct parts, one dealing with the development of resistant strains of the same species and subtype as that originally present and demonstrated to be susceptible before exposure of the host to the antimicrobial agent, and the other

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concerning changes in the microflora resulting from the emergence, or attainment of a position of prominence or dominance, of resistant species following the suppression or elimination of susceptible elements of the microflora. While the distinction is of both practical and fundamental importance, it is not always possible to discern it from many of the available reports because of the manner in which the data are collected or presented. From the point of view of clinical therapeutics and with respect to the individual patient, the first type of resistance is of the greatest importance although the second has also assumed considerable significance. The emergence of a resistant flora, and increases in incidence of resistant strains or species of pathogenic organisms in the general population, or in special groups of individuals are more important for the public health, and probably also in the use of antibiotics in animal feeding or in the processing of food. Since most of the data concerning the in vivo emergence of resistant strains of the same species in the primary host are derived from human clinical observations, and since the reviewer is more intimately acquainted with that phase of the problem, this presentation may, understandably, give greater prominence to experiences in human patients than is perhaps justified in a symposium devoted to problems in agriculture.

Emergence of Resistant Strains of Originally Sensitive Species in the Primary Host

It may be concluded from the observations on the use of sulfonamides and antibiotics in the treatment or prophylaxis of streptococcal infections that strains of group A (*Str. pyogenes*) do not develop resistance as result of exposure to these agents in humans(38). Thus far, strains of significant degrees of resistance to penicillin, the tetracyclines or erythromycin have not been encountered, except for the few strains isolated from infected burns; the latter strains were only moderately resistant and it is not certain whether the same strains were originally present and sensitive before chlortetracycline therapy was started. At the Boston City Hospital, strains of group A streptococci of even slight resistance to penicillin or the tetracyclines have not been encountered thus far(55), and, except for some reports (111), (87) of inadequately classified "hemolytic streptococci," some of which may have been of group A, no authenticated strains of this group have been reported to be resistant to penicillin, erythromycin, or the broad-spectrum antibiotics. Sulfonamide-resistant strains of certain specific types, mostly types 17 and 19, remain the only authentic instances of *Str. pyogenes* of significant resistance to any of the important chemotherapeutic agents.

The studies dealing with antibiotic-resistant staphylococci (only pathogenic strains that are hemolytic and produce coagulase are generally considered) may be summarized briefly:

1. The percentage of penicillin-resistant staphylococci isolated from hospitalized patients in large hospitals in many countries has increased steadily in the past few years so that now nearly three-fourths of all strains are highly resistant to penicillin.

2. Penicillin-resistant strains are found not only in suppurative lesions under treatment with this antibiotic but they are also found in the nose and throat of similar proportions of strains from patients in the same hospital who are not receiving penicillin, and they are frequently found in the feces of such patients.

3. The percentage of penicillin-resistant staphylococci among patients in the hospitals increases markedly during the period of hospitalization; this is true both in patients who receive penicillin in the hospital and in those who do not, although penicillin therapy results in somewhat earlier and more frequent appearance of the resistant strains.

4. The hospital staff and personnel carry staphylococci of the same bacteriophage types as the patients, and a similar proportion are resistant to penicillin.

5. Phage typing of staphylococci from patients and staff indicates that the organisms are acquired by cross-infection from other patients and from the hospital personnel, chiefly the latter.

6. The incidence of penicillin-resistant staphylococci is much lower (about one-half to one-third) among out-patients than they are among in-patients; among the out-patients the incidence is related to the amount of previous penicillin treatment or of previous hospitalization, or both.

7. The incidence of strains resistant to penicillin is still lower in the general population than among out-patients, and this too is related to previous treatment and hospitalization. At the time of admission to hospitals, patients have a higher carrier rate of penicillin-resistant staphylococci than people in the community at large.

8. Although there are no good data on the resistance of staphylococci among patients who are treated at home, there is evidence suggesting that such treatment results in an increased occurrence of resistant strains; this is based on the higher proportion of such resistant strains among patients subsequently coming to the out-patient clinics or admitted to hospitals, as compared with the incidence in the population at large.

9. When studies have been carried out in areas where very little antibiotics are used, the incidence of resistant staphylococci from infected lesions or from carriers is generally very low.

10. Resistance of the staphylococci to the tetracycline antibiotics is generally much lower than to penicillin, but in hospitals where they have been used very extensively, the incidence may approach that of penicillin-resistant strains during periods of intensive use. Usually the incidence of tetracycline-resistant staphylococci is about one-half that of the penicillin-resistant ones.

11. Erythromycin-resistant strains of staphylococci rapidly appear in hospitals where that antibiotic is used; both the degree of resistance and the incidence of resistant strains appears to increase with such use even more rapidly than with earlier antibiotics, and the incidence of erythromycin-resistant strains within a brief period may reach or even exceed that of penicillin-resistant strains during periods of intensive use.

12. The incidence of strains resistant to individual antibiotics is related to the extent to which each is used.

13. The frequency with which strains resistant to multiple antibiotics are encountered is similarly related to the extent to which the particular antibiotics in question are used.

14. Increases or decreases in the amounts and intensity with which any given antibiotics are employed may be associated with changes in the same direction in the incidence of staphylococci resistant to those antibiotics.

15. The simultaneous use of antibiotics in combinations has been recommended as a means of preventing the development of resistant strains of staphylococci. Data reflecting the results of combined therapy are scant but thus far indicate only the possibility of depressing, delaying or avoiding the appearance of resistant strains in individual patients; there is no evidence as yet that the incidence of resistant strains is reduced by combined therapy.

16. The increasing occurrence of staphylococcal infections within hospitals and the decreasing effectiveness of the available antibiotics in the treatment of such infections is being widely appreciated, and in many hospitals is reaching serious proportions.

It is interesting to note that in the early studies on the susceptibility of staphylococci to penicillin, the range of concentrations to which different strains were susceptible, was rather narrow. Indeed, strains of staphylococci resistant to more than 10 units per ml. were not encountered in the first few years(56), whereas in recent years most of the resistant strains grow freely in very high concentrations, usually more than 400 μg. of penicillin per ml., and there are relatively few strains of intermediate resistance(55), (57). Although resistance to similar concentrations of penicillin are attainable by proper subcultures of penicillin-sensitive staphylococci in increasing concentrations of the antibiotic, it is rare to encounter changes of similar degree developing in originally sensitive staphylococci under conditions which reasonably insure the exclusion of new intruders from extraneous sources.

Animal Diseases.—A detailed review of the literature on this aspect was not made. Catron(26), however, noted reports that some organisms become resistant to antibiotics used at therapeutic levels, but under practical conditions, in life-cycle feeding, there was no evidence of drug fastness. Bornstein and Samberg(15) observed that in about one-third of birds that are inadequately treated with streptomycin for infectious coryza, SM-resistant strains of *Hemophilus gallinarum* developed. At the 1954 Antibiotic Conference, Hawley *et al.*(74) noted that none of the organisms cultured from refractory mastitis infections were truly resistant to all antibiotics.

Cross-Resistance.—Cross-resistance between antibiotics has been most clearly and most regularly demonstrated in those antibiotics that have closely related chemical structures, but there are exceptions in which such structural relationships are not evident. The reverse, namely increases in sensitivity to heterologous agents may accompany the development of increased resistance resulting from repeated subcultures in some antibiotics. The use of combinations of antibacterial substances to delay and depress the emergence of resistance in vitro has been demonstrated in various organisms and with a number of antimicrobial agents. Only in the case of *Myc. tuberculosis,* however, has this been clearly demonstrated to occur in vivo during prolonged therapy.

Changes in Microflora During Antibiotic Administration

The material presented thus far has dealt with clearly identifiable organisms, which, for the most part are primary pathogens and the chief targets against which the antibiotics were directed,—mostly during therapy, but also in the prophylaxis

of infections. Although the literature concerning these organisms was by no means exhaustively covered, and that concerning other pathogens was not considered, it seems adequately clear that such organisms only infrequently become resistant within the primary host as a result of adequate antimicrobial treatment of specific infections. Exceptions are *Myc. tuberculosis* to various agents under certain conditions, and, to a less extent, *M. aureus* and certain streptococci mostly during treatment with erythromycin, and various other organisms in addition to *Myc. tuberculosis*, during treatment with streptomycin(1), (44), (58), (2), (23), (72), (117). In the case of *Myc. tuberculosis* moreover, although some data indicate that antibiotic-resistant strains that have developed within a patient during treatment have spread and caused infections in other hosts, this has been demonstrated only infrequently and, at least at the present time does not seem to constitute an important public health problem. On the other hand, the problem of the antibiotic-resistant staphylococci, like the earlier one of the sulfonamide-resistant gonococci and hemolytic streptococci, seems to be the result of the elimination of naturally sensitive strains and the resulting persistence and spread of naturally resistant strains of the same species during intensive and widespread use of the appropriate antimicrobial agents. The substitution of a resistant flora for a sensitive one during antibiotic administration is encountered to some extent during treatment of infections of the urinary tract; for the most part, however, this involves the respiratory and intestinal tract.

Intestinal Flora in Humans.—Most of the studies on the effects of antibiotics on the intestinal flora of humans have been carried out in relation to the preoperative preparation of patients for bowel surgery and have usually involved brief periods of administration. Some observations have been made over periods of 2 weeks or longer, either in the course of therapy or during prophylaxis. The earlier studies were made with poorly absorbed sulfonamides,—some with sulfaguanidine, but most of them with succinylsulfathiazole (sulfasuxidine) or phthalylsulfathiazole (sulfathalidine)—but almost all the widely available antibiotics, both those that are and are not absorbed from the gastrointestinal tract, have been used, with recent emphasis on neomycin. In general, full therapeutic doses have been employed.

The effects of the sulfonamides was reviewed by Riddell(133) and also by Poth (122), (123), (124), who introduced the more important ones of this group(125). Sulfaguanidine was soon abandoned because of considerable absorption and some toxicity, although it produced marked reduction in the number of intestinal organisms, which correlated with reduction in postoperative infections(166). When the other poorly absorbed sulfonamides are given in full therapeutic doses, the streptococci are unaffected, but Clostridia and coliforms drop steadily in numbers over a period of 8 days. The extent of the reduction in the total number of bacteria depends on the original numbers present, usually from 10^8 to 10^6, but coliforms may drop from 10^7 to 1000, or even to 0. Many gram-positive and gram-negative species are reduced in numbers, but Salmonella, including *S. typhosa* are unaffected(88). More recently, the combination of these unabsorbed sulfathiazole derivatives, and another one combining formaldehyde(103), have been recommended because they were found to potentiate various antibiotics in reducing many components of the

intestinal flora(124), (103), (159), (24) in suppressing SM-resistant strains(159). A sulfonamide-resistant flora, however, persists during administration of these drugs(104).

Penicillin given parenterally and orally usually does not reduce the number of bacteria in the feces, but the total count of aerobes and of coliforms may increase (104), and this is not affected by the addition of streptomycin(11). On continuous oral doses, the fecal flora in one study(96) was observed to have fewer gram-positive organisms, particularly streptococci, during treatment, but there was no change in the range of sensitivity of the gram-positive cocci that were found as compared with those encountered before treatment; penicillin-sensitive organisms, however, were infrequent while penicillin was being taken.

Oral administration of streptomycin to reduce the number of bacteria in the bowel was first suggested by Reimann *et al.*(130); they noted that *E. coli* was suppressed or eliminated during the first 2 days of therapy, but this effect did not occur or was lost when SM-resistant strains were present or appeared. Fecal streptococci, Clostridia, Bacteroides and Candida have been noted to appear or increase after the coliforms are suppressed(124), (159), (104), (86), (134). When strains of *E. coli* reappear they are usually highly resistant to streptomycin and the development of resistant strains could be delayed, but not regularly, by the use of streptomycin in combination with sulfonamides(159), (134). Marshall *et al.*(104) noted that fungi, staphylococci and streptococci predominate during treatment, but they and others (86) found that the flora returns to normal after the streptomycin intake is stopped. Riddell *et al.*(134) observed successive replacement of SM-sensitive species by SM-resistant ones so that the total counts remained equal to or higher than the pretreatment levels; thus, an irregular and temporary effect was obtained with streptomycin. Proteus and Pseudomonas were not prominent in their study, Coliforms and fecal streptococci were markedly reduced by the use of streptomycin in combination with bacitracin and polymyxin by mouth; the combination reduced the coliform counts faster than streptomycin alone; but did not prevent the development of SM-resistance in the coliforms that remained(168).

During parenteral administration of streptomycin in the treatment of tuberculosis, Hamburger and coworkers(70), (71) observed in most cases the appearance of strains of *E. coli* and *A. aerogenes* that were highly resistant to streptomycin. Sensitive strains returned after the treatment was stopped and gradually replaced the resistant ones, which disappeared completely after weeks or months. In occasional patients a SM-sensitive flora returned during treatment or the resistant forms were observed only transiently(71).

The effect of chlortetracycline on the fecal flora was studied by a number of workers(124), (104), (11), (163), (97); no consistent alterations occurred. Coliforms were usually rapidly reduced(104), (97), (145) and returned to pretreatment or higher levels after administration of the antibiotic was stopped. Total aerobes followed the same pattern(52). Resistant staphylococci may appear(124), (97), (145), (59) and Proteus species often occur and may increase(130), (124), (104), (97), (145) and occasionally assume pathogenicity and give rise to urinary tract infections(130), (97). Although a resistant flora, consisting of fecal streptococci, fungi, Proteus, Pseudomonas, and occasionally Paracolon may prevail during the course

of treatment(104), (11), (163), (97), the development of resistant strains was not noted(25). Administration together with sulfonamides may enhance the drop in coliform and aerobic counts(103), (24).

Oxytetracycline has been found by most observers to produce similar changes (124), (24), (97), (75), (10), (95), (113), (165). It was early observed(75) that oxytetracycline is readily excreted into the bile and usually concentrated there in patients with normal liver function. After oral administration, large amounts appear to be unabsorbed and are excreted into the feces, so that up to 2.5 mg. per ml. of feces may be recovered on a dose of 1.0 to1.25 gm. every 6 hours. Remarkable alterations in the bacterial flora resulted; Clostridia, streptococci and coliform bacteria disappeared and the remaining forms were resistant yeasts, Candida and micrococci(75). Other workers also noted that coliforms and fecal streptococci may be reduced or cleared early(10), (95), (77), (78), (39), (19), but they may sometimes persist(10) and in some instances even increase during treatment(77), (78), (39), (19). Yeasts, Proteus, Pseudomonas and resistant micrococci in various proportions or combinations may supplant the normal flora(95), (39). Resistant and pathogenic micrococci may become particularly prominent and in some cases supplant the entire fecal flora and produce severe diarrhea, which in some instances has been associated with a pseudomembranous enterocolitis(79), (81), (42), (36), (43), (131). The latter complication has been observed more frequently with oxytetracycline than with chlortetracycline(59), (75), (43). The effects of oxytetracycline on the fecal flora may be uncertain and variable(113), and the entire normal flora may be maintained(8) in some patients.

A case described by Finger and Wood(54) has been offered as illustrating another way in which the effects of antimicrobials on the fecal flora may be manifested. In their case a strain of *Salmonella muenchen* that was moderately resistant to oxytetracycline apparently increased in number during treatment of a carrier with oxytetracycline as the other elements of the fecal flora were suppressed. This resulted in an acute enteritis which subsided shortly after the antibiotic was withdrawn and the number of Salmonella organisms decreased. This case is of interest in view of the demonstration by Bohnhoff *et al.*(109), (13) that preliminary oral feeding of streptomycin to mice increased their susceptibility to subsequent oral inoculation of *S. enteritidis* that were resistant to streptomycin; this effect decreased as the interval between streptomycin treatment and inoculation increased. The increased susceptibility was interpreted as resulting from the disturbance in the natural flora, which is presumed to suppress the Salmonella, since no stimulation of the strain by streptomycin could be demonstrated in vitro or in vivo.

Many of the changes in the intestinal flora during chloramphenicol administration are similar, though less striking than those seen with the tetracycline antibiotics(24), (104); some workers observed no decrease in flora, and in an occasional patient *E. coli* may remain predominant throughout, whereas in others it may become predominant after 10 days(104). In children, a marked decrease in gram-positive and gram-negative bacteria has been noted with a concomitant increase in yeasts and fungi, including various species of Candida other than *C. albicans*(106). In other patients, however, even in combination with dihydrostreptomycin, chloramphenicol may not decrease and may actually increase the total bacterial counts,

the coliforms and pathogenic streptococci being markedly depressed; there is an overgrowth of the yeasts, without, however, producing any untoward effects(14).

Erythromycin, in one study in newborn infants(63), did not appear to affect the coliforms in the gut, and did not increase the fungi or staphylococci.

Neomycin is now considered by many workers to be the most effective agent in reducing the number of intestinal organisms in man(124), (24), (60), (61). In an early trial, Poth(124) observed rapid elimination of intestinal bacteria; indeed, within 30 minutes after flooding the gastrointestinal tract with a 1 per cent solution of neomycin he was unable to grow any bacteria from a piece of resected bowel. The agent, however, is not active against Shigella organisms(124), and resistant strains of *Aerobacter aerogenes* have also persisted(124). Clostridia likewise were found to persist in most cases after the coliforms were eliminated and the numbers of enterococci, Pseudomonas and Proteus were reduced(150), (37). Bacteroides (81) and yeasts(79), (61) were unaffected, but complications attributable to overgrowth of yeasts were not encountered by Poth(124) in 526 patients treated with neomycin orally; others have not noted overgrowth of yeast or fungi(37). Fog(60), (61) reported more striking effects, both clinically and bacteriologically from a combination of neomycin with bacitracin, and others(24), (126), (9) noted a more marked and more prolonged effect when neomycin was used together with sulfathalidine. On the other hand, Pettet *et al.*(121) at the Mayo Clinic reported 8 cases of enterocolitis, associated with staphylococci, among 71 patients prepared for abdominal surgery with a combination of neomycin and oxytetracycline, but none among 72 similar patients given neomycin alone. Febrile complications, wound infections and fecal fistulas were frequent in both groups, but peritonitis occurred in only 1 patient who was treated with neomycin alone.

It seems clear that, in spite of Poth's early failure to obtain growth after washing the bowel with 1 per cent neomycin, that sterilization of the bowel contents is not achievable, except perhaps locally and for very brief periods. Such successes as may have been achieved may be due to the low incidence of neomycin-resistant organisms in the early experiences, to the presence of large residuals of antibiotic in the material being cultured, or to the concomitant use of mechanical cleansing of the bowel with the use of castor oil and enemas(35), (126). Such results may not be obtainable regularly and for long periods since a resistant flora does develop. Poth(124) has recommended preserving neomycin for the unique purpose of preoperative preparation of the bowel in order to minimize the spread of resistant strains or species, but others are also interested in using this agent for various topical infections because of its antibacterial spectrum and its low sensitizing properties. In general, however, the major problem has been the replacement of sensitive by resistant species, and the development of resistance in originally sensitive strains has been a minor factor, except with streptomycin and has not been observed with neomycin(150), (35). The development of cross-resistance between neomycin and streptomycin has been demonstrated in vitro(69), (116), but such occurrence in vivo has not been studied.

The relation of antibiotic therapy to various nutritional deficiencies in man has been a controversial subject since Ellinger and Shattock(49) described a case of nicotinamide deficiency after oral administration of penicillin. A similar case was

observed while on sulfadiazine(150). Other vitamin deficiencies were ascribed to treatment with chlortetracycline and chloramphenicol(67), but malnutrition(169) either underlying or related to the infection under treatment, may be an important factor, or other pharmacologic and biochemical factors may play a role. This aspect will not be considered here but many of the conditions described as deficiencies and ascribed to antibiotics(161) are open to challenge. It may be of interest to point out, however, that the negative nitrogen balance and increased urinary riboflavin excretion observed during chlortetracycline in some men(64), (52), (65) could not be correlated with the fecal flora, since greater reductions in the same types of organisms were achieved in some of the same subjects by the use of bacitracin together with polymyxin without producing similar nutritional changes(65).

Changes in Intestinal Microflora of Animals During Antibiotic Feeding.—This subject is intimately related to the problem of nutrition of animals; it has been dealt with extensively by many workers and is considered in detail in the reviews referred to in the opening paragraph. Among the major differences between the data in animals and in humans are, of course, the nature and pathogenicity of the different microflora, the marked differences in the dietary constituents, and the great divergence in the dosage and duration of antibiotic administration. The more important problems directly concerned with animal nutrition are outside the province of this review and only those aspects directly related to the microflora need be considered. The literature on the biologic importance of the intestinal microflora and particularly its relation to nutrition was reviewed in 1949 by Johansson and Sarles(82).

McCoy(105), in her review aptly pointed to the fact that the dosages of the antibiotics used in animals are incalculably low compared to those used by surgeons for intestinal antiseptics but she suggested this as an explanation for the temporary effect of the antibiotics in reducing the flora in animals. However, many of the effects are temporary even with the larger doses used in man, and some of the effects in animals may be permanent,—at least as long as the antibiotic administration or feeding is continued. Differences in the character of the flora that is inhibited, or of that which returns, are often difficult to evaluate, but both in humans, as already noted, and in animals the predominant residual flora during continuous oral administration is resistant to the agents being used(51), (157), (161), (46), (83), (3), (119), (151), (80), (120).

The principal work has been done with chicks, turkeys, swine and the laboratory rat; more recently, work has also been done with ruminant animals which present a different problem with respect to their flora. The antibiotics most widely employed have been chlortetracycline, penicillin, and later oxytetracycline; but others have also been studied and used alone, or in various combinations, or together with those mentioned. Most workers have dealt with broad groups of microorganisms, namely, the coliforms, lactobacilli, total aerobes, anaerobes (chiefly Clostridia) and enterococci, and to some extent the yeasts. McCoy(105) in preparing her review went to great trouble to chart the rise and fall of the various groups of organisms in response to antibiotic feeding in the principal animals studied but was unable to arrive at any generalization. This she interpreted to be consistent with the contro-

versial character of the changes in flora in relation to the antibiotic growth responses. A few changes have been observed frequently and may be mentioned briefly.

The coliform counts have been reported to show an initial decrease, mostly with the use of streptomycin and the broad spectrum antibiotics(51), (112), (16), (73), (100), (152), (62) followed later by an increase(83), (3), (119), (112), (73), (152), (62), (155), (68), (128), (129), (132). The secondary coliform flora has been shown, in some of the studies to be resistant to the antibiotics used(51), (157), (161), (83), (119), (161), (80), (120), (62), (33), (34), (4). Since the coliform group of organisms may be concerned with vitamin synthesis(82), strains of *E. coli* and *A. aerogenes* from the ceca of antibiotic-fed chicks have been fed to other chicks, with or without penicillin. Whereas the feeding of such organisms(155), (135), (12), (139), or increasing their numbers in the cecum by dietary means (139) produces some stimulation of growth, this is further enhanced by adding the antibiotic(155), (139–142), (5), (6). The occurrence in increased numbers of certain fungi, or feeding them, likewise resulted in improved growth, but this was not true with all strains(129), (153).

The groups of lactobacilli and enterococci, considered together as lactic acid bacteria, require rather than synthesize vitamins. Their response to antibiotics has also been variable and has not always correlated with the growth effect(83), (3), (119), (155), (34), (102), (146), (147), (40), (91).

The anaerobes, particularly *C. perfringens* have received special consideration because their toxins could be considered as a factor in the health of animals and growth effects have sometimes been attributed to a reduction in this factor. Sharp decreases in the clostridial counts under the influence of antibiotic feeding have been noted in turkeys(154), chicks(170), (171), (47), (48), rats(68), (146), and pigs(154), (115), in the latter particularly when combined with proper carbohydrates in the diet(119). These and other organisms have been implicated in infections in certain animals. Merchant (quoted by Sieburth *et al.*(154)) showed that enterotoxemia of sheep is caused by *C. perfringens,* which in some studies has been inhibited by penicillin and Terramycin in turkeys and by Terramycin in pigs (154). By contrast, the toxicity of oral chlortetracycline for guinea pigs has been correlated with an overgrowth of *Listeria monocytogenes;* this effect is not observed with the feeding of penicillin or chloramphenicol, or with the subcutaneous injection of chlortetracycline or oxytetracycline(136–138). The importance of Clostridia, however, has been questioned by a number of workers(170), (92), (158); for it is particularly difficult to reconcile this with the failure to influence the growth of chicks by the feeding, or cloacal administration of clostridial cultures or their toxins(170). Nor is it consistent with the observation that a good growth enhancing effect may continue even after the numbers of Clostridia increase(158).

Proteus(152), (146), (147), (18) and yeast(153), (128), (129), (90) have been noted to be increased by feeding antibiotics, particularly those with a wide spectrum. *E. coli* and others of the normal coliforms were found to prevent unusual increases in Proteus(128), (132) and also to prevent emergence of yeasts(132), (118), but Proteus antagonism was not found by Scaletti *et al.*(147) whereas Sieburth *et al.*(153) also found that overgrowth of Proteus antagonized and depressed the growth of yeasts(153).

The correlations between the bacterial flora and the antibiotic response in ruminant animals has been irregular and the findings even more complex(89), (85), (32), (98), (114), (29), (101).

The gross changes in the various constituents of the intestinal flora as reported in 29 different studies * involving chicks, turkeys, poults, ducks, pigs, rats and lambs fed various antibiotics, but principally penicillin, chlortetracycline and oxytetracycline have been tabulated by Jukes in his most recent review(85). The material in his table has been rearranged in order to indicate the frequency with which the various changes have been observed in different studies. In Table 1, this has been done to bring out the differences in the effect, on the one hand, of penicillin which in vitro may be considered to have little or no direct effect on the Gram-negative organism, particularly the coliforms, and on the other hand the tetracyclines or other antibiotics, such as combinations of penicillin with streptomycin or various other combinations that may be expected to affect a wider spectrum of bacteria.

It is seen from Table 1 that increases in coliforms were encountered both with penicillin and with the antibiotics having a broader spectrum of activity, although perhaps less regularly with the latter. Lactobacilli were more regularly reduced by penicillin, and variable results have been reported from the use of the other agents. Total aerobes were irregularly affected and were more often found to be reduced by penicillin, whereas the anaerobes were most frequently reported as reduced by all antibiotics, although not by all workers. Likewise, enterococci were more frequently recorded as reduced by penicillin, but increased by the tetracyclines or by various antibiotic combinations.

The same material has been summarized in another manner in Table 2 to bring out any possible differences related to the various animal species studied. Because of the small number of studies, the pigs and rats have been considered together and the studies in chicks, turkeys, poults, and ducks were also pooled; the 2 studies on

* References (46), (83), (3), (16), (155), (68), (132), (34), (4), (141), (102), (146), (147), (154), (48), (115),(92), (18), (90), (41), (148), (94), (143), (164), (50), (45), (156), (7), (144).

TABLE 1

EFFECTS OF FEEDING VARIOUS ANTIBIOTICS ON THE NUMBERS OF VARIOUS INTESTINAL MICROORGANISMS IN ANIMALS*

	Observed effect from antibiotic feeding †								
	Penicillin			CTC or OTC‡			All studies		
Intestinal organisms§	+	−	0	+	−	0	+	−	0
Coliforms	7	1	0	6[1]	2	2	14	3	2
Lactobacilli	0	5	1	4	4	3[1]	4	9	5
Aerobes	1	3	0	3[1]	2	1	5	5	1
Anaerobes	1	4	0	1	7[1]	1	2	12	1
Enterococci	1	4	1	4[1]	2	1	6	6	2

* Summary of 29 studies adapted from Jukes(85).

† Numbers of studies demonstrating the indicated effects, viz., + = numbers of organisms increased; − = numbers decreased; 0 = no effect.

‡ CTC = chlortetracycline; OTC = oxytetracycline. Includes combinations with penicillin. Superscripts indicate number studied with streptomycin alone.

§ Increases in Proteus were noted in 3 studies; — once each with oxytetracycline, penicillin + streptomycin, and "various antibiotics."

calves were omitted from this table. It is seen that increases in the coliform counts were regularly demonstrated in the pigs and rats and somewhat less regularly in the birds. Decreases in lactobacilli were observed regularly only in birds fed penicillin. The total aerobes and the enterococci were most often observed to decrease in birds fed penicillin and increased when antibiotics with broader spectrum were fed; there were too few studies in pigs and rats dealing with this phase. Anaerobes were observed to be similarly affected in both groups of animals and by all the antibiotics, but there were exceptions.

An excellent summary and appraisal of the results of his extensive experience with feeding antibiotics to swine was presented by Catron in 1953(*27*), listing separately the results which seem to be fairly well established and the implications that could be drawn from research but which have not been definitely proved. Only the parts relating to the microflora need be noted here. In the first category he lists: (1) Antibiotics in swine ration control a high per cent of nonspecific enteritis under both experimental and practical farm conditions as well as in challenge experiments. (2) The response of pigs to antibiotic feeding seems to be in proportion to the disease level; this has also been confirmed by poultry research and by studies in disease-free animals. In the category of inferences, Catron lists: (1) Bacteriological studies of feces and intestinal tract content indicate that antibiotic feedings inhibit certain classes of microorganisms and stimulate others, and, contrary to earlier opinion, the total bacterial counts actually increase following an initial decrease in number. (2) Evidence indicates that the activity of antibiotics is confined to modifying the intestinal flora, thereby, (a) permitting the synthesis and/or sparing of critical nutrients needed by the host (nutritional effect), and/or (b) inhibiting pathogenic, semi-pathogenic and/or toxin-forming microorganisms injurious to the host (disease control effect).

TABLE 2

EFFECT OF ANTIBIOTIC FEEDING ON NUMBERS OF INTESTINAL MICROORGANISMS IN ANIMALS*

Intestinal organisms	Antibiotic	Number of studies reported†					
		Pigs and rats			Poultry		
		+	−	0	+	−	0
Coliforms	Penicillin	2	0	0	5	1	0
	Others*	3	0	0	4	2	1
Lactobacilli	Penicillin	0	0	1	0	5	0
	Others	2	1	0	2	3	3
Aerobes	Penicillin	0	0	0	1	3	0
	Others	1	1	0	3	1	1
Anaerobes	Penicillin	0	1	0	1	3	0
	Others	1	4	0	0	4	1
Enterococci	Penicillin	0	0	1	1	4	0
	Others	1	0	0	4	2	1

* Summary of 27 reports tabulated by Jukes(85). Two studies on calves omitted.

† Oxytetracycline or chlortetracycline (with or without penicillin), streptomycin, or "various antibiotics."

The evidence supporting the disease control concept was reviewed by Jukes(85), who also summarized the reports on the relation of "clean" and "dirty" environments to the antibiotic growth effect. Differences in growth effects of antibiotics in new or clean, as compared with old, and presumably contaminated environments has received considerable attention(76), (31) and the loss of the growth-stimulating effect of continuous antibiotic feeding over long periods in the same environment has been clearly demonstrated by Waibel *et al.*(167) and by Catron(28). Such findings have been interpreted as evidence of the primary importance of the microflora (27), whether by eliminating infection or by otherwise favorably altering the microflora. The failure to obtain any growth stimulating effect in a germfree environment(99) lends further support to this view.

These findings may also be interpreted as indicating that the antibiotic-resistant flora which emerges during antibiotic feeding is a favorable one; the growth-enhancing effect of feeding such residual flora or cultures of organisms from antibiotic-fed animals, as cited earlier would support this view. On the other hand, if pathogenic organisms, such as Salmonella in mice(13), (110) or *Listeria monocytogenes* in guinea pigs(138) are present and are not inhibited but are enhanced by the feeding of certain antibiotics, then those antibiotics will have a deleterious rather than beneficial effect; this appears to be the principal way in which the emergent resistant flora in human therapy and prophylaxis has exerted unfavorable effects.

Another important feature of antibiotic feeding in animals is that it is essentially life-cycle feeding, carried out either to enhance early growth and development or for fattening in preparation for market. Epidemiologically this provides advantageous circumstances for the maintenance of any type of flora which the prevailing local conditions may favor, by constantly introducing large numbers of susceptibles, while at the same time removing essentially equal numbers of those already adapted to the flora. If the conditions favor the establishment of a pathogenic or injurious flora, then a "high disease level" will prevail; conversely if the conditions favor establishment of an advantageous flora, the animals should thrive. Obviously, antibiotic-feeding in general, has resulted in the establishment of a favorable flora. This could serve to explain the loss of the growth-stimulating effect of antibiotics after continuous feeding in the same area over long periods, and also the increasingly favorable effects that are concomitantly observed in control animals kept in the same environment on similar diets but without antibiotics(167), (28).

It would be interesting to observe animals in these environments following withdrawal of all antibiotic feeding. Careful studies of the returning microflora in such surroundings may uncover some of the unfavorable members of the flora which, if the deductions are valid, should make their appearance when and if there is a gradual reduction in the rate of growth or feed efficiency. Moreover, the new and returning flora should be susceptible to the antibiotic which in the same environment previously yielded growth-promoting effects. Alternatively, or at the same time, the antibiotic-resistant organisms which may have been responsible, at least in part, for the earlier favorable effects, may be reduced or eliminated. The favorable effects of feeding some of the latter types of strains has been demonstrated to some extent, as already noted. It would be of interest to learn whether any newly introduced or returning strains could be shown to be deleterious when fed without antibiotic supplements

to control animals that had been raised along with those receiving antibiotic-supplemented rations.

The recent studies on the extension of the shelf-life of poultry by dipping in antibiotics has some interest in relation to the residual antibiotic-resistant flora following antibiotic feeding. An experiment performed at the Lederle Laboratories is of interest(20). Two groups of chickens, one that had been fed on a ration supplemented by 100 p.p.m. chlortetracycline and the other not supplemented were slaughtered and dressed and one-half of each group were then dipped in water containing chlortetracycline 10 μg. per ml., after which all were properly wrapped and refrigerated at 40° F; after 7 days the birds that had not been dipped in water containing chlortetracycline 10 μg. per ml. prior to wrapping and refrigerating showed several hundred thousand times as many bacteria as those that had been dipped. At the end of 2 weeks those that had not been dipped were putrid while those that had been dipped appeared fresh and edible. The chlortetracycline-fed birds spoiled just as readily and had just as many organisms per gm. of flesh as those raised without antibiotics, indicating that the organisms responsible for the putrefaction were susceptible to the inhibiting effect of the chlortetracycline in the dipping water. This suggests one of the following possibilities with respect to the organisms concerned in the spoilage: (1) these organisms appeared in the birds between the time of antibiotic feeding and the time of slaughter, or at the time of the dressing and wrapping, (2) they were susceptible to the concentrations of chlortetracycline in the dip but not to the concentrations provided during feeding, (3) the organisms were susceptible to the concentration of antibiotics in the bowel and were being inhibited in the intestines of the birds but were not susceptible to the concentrations present in the flesh which was infected during the dressing process. It should be possible to determine which of these possibilities is the correct one.

Discussion

For those of you who may take the trouble to read this review I might say that there has been no pretense of making this a complete review. And to those from foreign, and particularly non-English speaking lands, I must plead guilty of gross neglect with respect to the foreign literature, especially in languages other than English. This, however, must not be interpreted as belittling the importance of such contributions, but rather as a measure of my own limited competence in those languages and of the even more limited availability to me of the foreign literature. Indeed, I regret to say that only very limited access was conveniently available to me to the rich agricultural literature even in the English language; I was saved only by the kindness and generosity of Dr. Thomas H. Jukes and his colleagues, particularly Dr. H. P. Broquist, for making much of that literature available to me.

In this review, particular emphasis was placed on the distinction between two types of resistance;—one is the type that occurs in originally sensitive organisms that have acquired resistance within the original host during the course of administration of antibiotics; this is contrasted with the changes in flora during similar exposure to antibiotics, whereby sensitive strains and species are eliminated and resistant ones emerge. The distinction is highly pertinent, for, in the former instance,

therapy or prophylaxis of the infection by the causative organisms against which the antibiotic is directed becomes invalidated, and if this occurs during the course of therapy before a cure has been accomplished, the treatment may not be successful. On the other hand, changes in flora during antibiotic administration need not necessarily be harmful; moreover, as was pointed out in this review, it even appears to be useful in animals that are given small amounts of antibiotics as feed supplements.

In the therapy of humans, and sometimes even during prophylaxis, the emerging resistant species may be pathogenic, or may possibly acquire pathogenicity, either because of more favorable conditions of competition against other bacteria, through removal of what might be termed "antagonistic species," or because of some special susceptibility of the host under the particular circumstances, or possibly because the numbers of these organisms increase so that they approach and surpass the minimum infecting dose.

Of particular interest is the rather small number of species of bacteria in which the factor of increasing resistance of originally sensitive strains of the original causative organism of the disease results from therapy of the original infection. Resistance developing in *Mycobacterium tuberculosis* during treatment with antituberculous drugs, especially when used singly, is the best example, as well as the one most thoroughly documented. Even in the instance of *Micrococcus aureus,* this factor has not been shown to be the major or primary factor in the increasing number of resistant strains. Organisms (that is, staphylococci) from foci not readily subject to reinfection from other sources, as in unoperated cases of osteomyelitis, or in bacterial endocarditis, ordinarily do not exhibit increasing resistance in successive strains cultured from the blood; in the great majority of cases they usually retain their original susceptibility to the antibiotic used for therapy. There are exceptions, of course, as in cases of bacterial endocarditis in which staphylococci and various streptococci have exhibited steadily increasing resistance to erythromycin during treatment with that agent alone.

The problem of antibiotic-resistant staphylococci, as pointed out in this review and by many other workers, is one of cross-infection or superinfection by antibiotic-resistant strains acquired in hospitals in almost all instances. The incidence of strains of staphylococci resistance to any single antibiotic or any group of antibiotics in any given hospital appears to be directly related to the intensity with which those antibiotics are used in that hospital, and is also associated with a similar incidence of carriers of such resistant strains among the staff and service personnel of that hospital. To be sure, the resistant staphylococci may have acquired their unusual degree of resistance as a result of earlier exposures to the antibiotics in question; this has been clearly demonstrated in the case of erythromycin, and is probably also true of the tetracyclines and of streptomycin. With respect to penicillin-resistance, however, this property appears to be a naturally occurring one, peculiar to certain strains and associated with the ability of such strains to produce the penicillin inactivating enzyme, penicillinase. The development of steadily increasing resistance of staphylococci (and some other organisms) during treatment has been clearly demonstrated in patients receiving erythromycin. Organisms other than staphylococci and tubercle bacilli have been noted, of course, to develop resistance to streptomycin during treatment with that antibiotic; this may occur both in man and in ani-

mals, either gradually, or in a single large step, or by both mechanisms in the same host, just as it has been shown to happen in vitro.

The effect of bacterial resistance on the future of antibiotic therapy may depend, to some extent at least, on the recognition of the types of resistance so emphatically distinguished here. The emergence of resistance in pathogenic bacteria within the original host during treatment would call for certain measures, such as the use of antibiotics in proper combinations or switching from one effective agent to another until a cure is obtained. This aspect is well illustrated in the treatment of tuberculosis, and, more recently, of staphylococcal infections. Changes in flora, on the other hand, may perhaps be tackled best by searching for highly specific anti-microbial agents which would affect only the individual pathogenic species (or perhaps related ones as well) and which would still permit the commensals to retain their usual interrelationships. Alternatively, it may be possible to discover new antibiotics which would eradicate, or at least inhibit, those species which have been emerging and spreading and have been the cause of antibiotic-resistant infections. The spread of resistant species that are undesirable also calls for the development of techniques of prevention; such methods, have, thus far, been either inadequate or not pursued with sufficient vigor.

The nosocomial aspect of antibiotic-resistance as illustrated by the spread of resistant staphylococci and by the increasing occurrence of such resistant species as Proteus, Pseudomonas, Aerobacter and possibly Candida, should not imply that the problem will necessarily remain one confined to hospitals. The large numbers of persons receiving hospital treatment who are discharged into the community, and the increasing numbers of patients receiving antibiotics at home or on an ambulatory basis, are certain to have their effects in the community and may constitute a public health problem. Some evidence suggesting this possibility was cited with respect to staphylococci and tubercle bacilli, in this review.

On the other hand, as pointed out by Catron, Hanson, Waibel and others, and already brought out in earlier sessions of this Conference, the spread of antibiotic-resistant species of bacteria among animals where antibiotic feeding has gone on continuously for long periods may result in improvement in the health and growth of animals coincident with a decline in the apparent effectiveness of antibiotics in promoting growth in the same environment. This may be one of the salutory effects of the emergence of a resistant microflora, but it may be only temporary, continuing only as long as the antibiotic feeding is kept up, and may be lost some time after the antibiotic feeding is discontinued.

Among the major differences between the data in animals and in humans are, of course, the nature and pathogenicity of the different microflora, the differences in dietary constituents, and the great diversity in the dosage and duration of antibiotic administration. McCoy suggested that the incalculably lower doses used in animal feeds when compared with those used by surgeons for intestinal antisepsis might explain the temporary effect of the antibiotics in reducing the flora in animals. However, even in man most of the effects are temporary, even with large doses, and some of the effects in animals may be permanent, at least so long as the antibiotic feeding is continued. Differences in the character of the flora that returns are often difficult to

evaluate, but both in humans and animals the predominant residual intestinal flora during continuous oral administration is resistant to the agents being used.

McCoy in her review stated that she went to great trouble to chart the rise and fall of the various groups of organisms in response to antibiotic feeding in the principal animals studied but was unable to arrive at any generalization. At yesterday's session, Combs attempted to do the same for the poultry studies, with similar conclusions. This McCoy interpreted to be consistent with the controversial character of the changes in flora relation to the antibiotic growth responses. Some of these changes, however, appear to be consistent, at least so far as they have been obtained regularly by various workers.

Summary

An attempt was made in this review to gather some of the available evidence for changes in resistance of bacteria resulting from administration of antibiotics and for the significance of those changes. The data relate chiefly to the uses of antimicrobial agents for the therapy and prophylaxis of disease in humans and for their growth-promoting effects in animals.

The data in humans relate both to changes in the susceptibility of certain originally sensitive pathogens as well as to changes in the microflora within the original host, and also concern the dissemination of resistant organisms during widespread usage.

In the case of streptococci, differences in susceptibility appear to be related to specific species or types; this was clearly demonstrated for *Str. pyogenes* types 17 and 19, which were resistant to sulfadiazine and accounted for epidemics during widespread use of sulfonamide prophylaxis. There was no evidence for resistance acquired either to sulfonamides or to any antibiotics in originally sensitive strains of *Str. pyogenes*. In the *Str. viridans* group and among enterococci there has been no change in the range of resistance even during prolonged treatment or prophylaxis with antibiotics, although the proportion of strains in the less susceptible end of the normal range may increase. Resistant strains developing in originally susceptible ones have been remarkably infrequent except in cases of bacterial endocarditis treated with erythromycin.

Micrococcus aureus has been the most troublesome. Numerous studies in hospitals and in other groups have indicated a marked increase in the incidence and spread of pathogenic, penicillinase-producing and penicillin-resistant strains which have also become increasingly resistant to all antibiotics that have been extensively used. The evidence suggests, however, that only in the case of erythromycin has the increase in resistance of originally sensitive strains within the original host during treatment been frequent. The spread of resistant strains of *M. aureus* constitutes an important health problem. The incidence of resistant strains of *M. aureus* to any antibiotic appears to be related to the frequency and intensity with which it is used.

Mycobacterium tuberculosis is probably the only other instance in which resistance developing within the original host can be frequently demonstrated. This has occurred with each of the three principal antituberculous drugs, viz., streptomycin, para-aminosalicylic acid and isoniazid. The development of resistance has been delayed and depressed but not prevented by the use of combinations of effective agents

in proper dosage regimens. Resistant strains in previously untreated patients are appearing; at present they do not constitute a serious problem but prolonged and extensive domiciliary and ambulatory treatment in chronic cases with persistent organisms may constitute a public health hazard.

The evidence with regard to *Neisseria gonorrhoeae* indicates that sulfonamide-sensitive strains were suppressed while sulfonamide-resistant strains increased in prevalence during the widespread use of these agents, with concomitant loss of effect from such therapy. There is no substantiated evidence of any increased resistance to penicillin or other antibiotics among strains of gonococci in spite of the extensive use of these agents. Penicillin-resistant or sulfonamide-resistant strains of *N. meningitidis* likewise have not been encountered.

Strains of Salmonella or of Brucella have, with rare exception, not been noted to increase in resistance during prolonged treatment with any antibiotic including streptomycin.

Changes in flora of the urinary tract of patients with chronic and complicated infections, with replacement of sensitive by resistant species, have followed treatment with various antimicrobial agents. Only during treatment with streptomycin, however, have originally sensitive strains been frequently observed to acquire high degrees of resistance.

In the respiratory tract also, the continued use of various antibiotics, either in large doses therapeutically or smaller doses prophylactically, has resulted in the substitution of a flora resistant to the antimicrobial agents that are used. Small doses of penicillin, as used in the prophylaxis of streptococcal infections or when incorporated in dentifrices have not significantly changed the flora or the range of susceptibility of the usual organisms encountered, although the proportion of strains in the less susceptible part of the normal range have increased at the expense of the less susceptible ones. When broad spectrum or multiple antibiotics are used, a more resistant type of flora, including Proteus, Pseudomonas, resistant staphylococci and fungi may emerge and may result in superinfections with such organisms.

In the intestinal flora of humans, studies have been made during and after the preoperative preparation of patients for surgery of the bowel, using large doses of poorly absorbed sulfonamides, or antibiotics, or combinations of both, coupled with mechanical cleansing and purging. These procedures have resulted in various degrees of lowering of the total aerobic counts, particularly the coliforms. This, however, has been temporary and the total counts have returned to the original or higher levels in a few days even during continued administration of the antimicrobials. The resulting flora of the bowel is then resistant to the agents used, and pathogenic strains may become established, either in the bowel or elsewhere. Proteus, Pseudomonas, Aerobacter, *M. aureus* and various yeasts and fungi may emerge. Some of these, particularly *M. aureus,* have been responsible for serious disease.

The data in animals relate primarily to changes in the microflora of the intestinal tract during antibiotic supplementation of the feed. Although much smaller doses are involved, when compared with those used in humans, their use is accompanied by varying changes in the intestinal flora depending on many factors, such as the different animals employed and the diets and antibiotics studied. The total bacterial counts, however, are generally not reduced, except temporarily during the first few

days, and may then frequently be increased above the levels found in control animals. The species that persist and increase are predominantly resistant to the antibiotics being fed. The residual flora, however, appears to have a favorable effect on the nutrition of the animals. In contrast to the human experience, disease-producing strains have not been found to emerge among the types of animals that are raised primarily for market on antibiotic-supplemented feeds. Pathogenic organisms, however, have been observed to become prominent and apparently injurious in mice treated orally with streptomycin and in guinea pigs fed Aureomycin, but larger amounts of these antibiotics have been used as compared with those added to feeds for growth-promoting effects.

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ANTIBIOTIC RESIDUES

From a public health standpoint, antibiotic residues may be defined as those quantities of antibiotics found in or on foods intended for human consumption. This would include the small amounts of antibiotic drugs left in animal tissues after feeding for growth promotion and the prophylaxis or treatment of animals. Such residues may be present in the blood, tissue, or the products of treated animals such as milk or eggs. There also may be trace amounts remaining on plants after use for disease control. Furthermore, such residues may be found following the use of antibiotics as food preservatives.

Although primarily concerned with antibiotic residues in foods intended for human consumption, in a broader sense the U.S. Food and Drug Administration is interested in the protection of the people who handle the various preparations recommended for use in or on food products. This public health hazard is regulated by the Department of Agriculture in close liaison with the Food and Drug Administration. For example, in applying certain antibiotic crop sprays or dusts, the directions for use must ensure that proper precautions be included in the directions for use, such as avoidance of contact with skin, eyes, or lungs; the use of dust masks, gloves, and thorough washing of personnel and equipment after use.

Reason for Public Health Significance.—There are several ways that antibiotic residues may jeopardize the public health: (1) They may sensitize a nonsensitive person; (2) Cause an allergic reaction in a person already sensitive; (3) Alter the oral or gastrointestinal flora; and, finally, (4) Cause the emergence of resistant microorganisms.

Possible Sources of Residues.—There are several ways in which antibiotics may reach the consumer:

1. From the flesh, eggs, or milk of animals given antibiotics orally or by injection for nutritional, therapeutic, or prophylactic purposes or processed foods derived from them.
2. As a result of application to crops destined for human consumption.
3. From their use in preservation of foods.
4. From handling or exposure to antibiotic preparations such as feeds, supplements, sprays, or dusts.

The extension of uses of antibiotics to promote growth, as therapeutic and prophylactic agents in both large and small animals, as pesticides in crop sprays,

and their imminent use as food preservatives, has vastly increased the possibility of human contact with antibiotic residues. The following table lists some of these uses and undoubtedly the list will be extended as time goes on.

Extension of Uses of Antibiotics

In Nutrition.—Swine, chicks, poults, calves.

In Animals.—Therapeutic, prophylactic uses. (Cows, beef cattle, calves, swine, chickens, turkeys, mink.)

As Crop Sprays.—Blight: Apple, pear, walnut, peach, beans. Bacterial Diseases: Tobacco, tomatoes, pepper, cherries, spinach, lettuce, potatoes.

As Food Preservatives.—Vegetables, hamburger, cream fillings, fish, fish fillets, shrimp, beer fermentation, beef carcasses, chickens, etc.

The actual or potential introduction of antibiotics into the food supply poses the same problem as does the use of any other chemical, and this growing use of chemicals in food is one of the most serious problems facing the Food and Drug Administration at this time.

There is very little information available in the published literature concerning the presence of antibiotics in foodstuffs. This is not surprising since the use of these drugs as crop sprays is not practiced on a large scale as yet, being used to a limited extent in the prevention of blight in such products as peaches, apples, walnuts, and pears. Thus far, also, no antibiotic is presently used as a food preservative or additive. As long ago as 1948 it was noted that when milk from cows treated for mastitis by intramammary infusion was mixed with antibiotic-free milk for making cheese, failure to produce a satisfactory cheese product often resulted. This was due to the inhibition of the starter culture used in cheese manufacture by the antibiotic present in the milk. Realizing that antibiotics might also reach the consumer via the milk supply, the Food and Drug Administration required producers of antibiotic preparations for mastitis to insert a statement in the circular accompanying each package of drug as follows: "Important: Milk from treated segments of udders should be discarded or used for purposes other than human consumption for at least 72 hours after the last treatment." This three-day period during which the milk was to be discarded was made effective on August 21, 1951, and was based on information available at that time. The American Dairy Science Association, through their Resolution Committee on antibiotics, also recommended that no milk be marketed if obtained from a mastitis treated quarter within 72 hours (six milkings) after the last treatment.

Investigations conducted in Food and Drug laboratories showed that following current methods of antibiotic administration, penicillin could be detected in cows' milk for six days after treatment, chlortetracycline for five weeks, oxytetracycline for four days, and streptomycin for one day. An interesting outgrowth of these studies was that following intramammary infusion of chlortetracycline or oxytetracycline in two quarters, small amounts of the drug could be detected in the two untreated quarters. Although the great bulk of the drug was excreted during the first few hours following treatment, for several days thereafter small quantities could be detected in the milk. Additional studies showed that chlortetracycline or

penicillin could be found in the blood serum, tissue, and eggs of chickens fed 50, 100, or 200 p.p.m. of drug. The small amounts found in chicken tissue were destroyed following frying. Furthermore, when the antibiotic containing feed was withdrawn and normal feeding begun the antibiotic disappeared from the tissue or eggs within a few days. After hard-boiling eggs containing antibiotics, no activity could be detected in the great majority of those tested. Storage under refrigeration for considerable periods did not diminish the concentration of drug.

In late 1952 and early 1953 the Food and Drug Administration began to receive inquiries concerning the use of antibiotics as food preservatives. Experimental work was then being conducted to explore the potentialities of antibiotics as preservatives for canned foods, custard fillings, beef, freshly caught sea food, cow and human milk, and other products. In order to obtain information of a practical nature, a limited field survey was conducted in January 1954. A variety of dairy products were tested, including fluid milk, powdered milk, evaporated milk, ice cream, butter, cheese, shell eggs, and broken eggs. Ninety-four quarts of milk were tested for antibiotic activity, and 3.0 or 3.2 per cent were found positive for penicillin. No other proven antibiotics were found in any of the other products.

After this first survey was completed, it was decided to make future surveys at appropriate intervals. Larger and larger quantities of veterinary preparations were being used, dosages were increasing, and it appeared that some individuals were marketing milk from treated cows too soon after treatment. Furthermore, in some instances it was believed that unscrupulous individuals were deliberately adding penicillin to milk to upgrade it.

A second and more extensive survey was conducted in December 1954 through January 1955. In the first survey, seven Food and Drug districts were covered, while in the second all sixteen districts, covering geographically virtually the whole country, submitted samples. Each district sent a minimum of 25 quarts, representing as many dairies, of fresh fluid milk. Each sample was examined for its content of penicillin, streptomycin, bacitracin, oxytetracycline, chlortetracycline, and tetracycline. In all of the positive samples except one, penicillin was the only antibiotic found. A total of 474 samples were tested and 55 (11.6 per cent) found positive for penicillin. In every instance the penicillin activity was abolished by penicillinase, an enzyme which specifically inactivates penicillin. Noteworthy was the small amount found ranging from a low of 0.003 unit/ml. to a maximum of 0.08 unit/ml. Thus, a person drinking a quart of milk could ingest about 80 units of penicillin and much of this would be inactivated by the acid of the stomach, or the action of the intestinal flora. The small amount that is absorbed is probably insignificant and could hardly be detected by microbiological methods.

The marked increase in the number of positive samples in the year separating the two surveys caused some concern, and it seemed advisable to obtain some opinion on the possible public health significance of these quantities of penicillin in market milk. Consequently, a number of nationally recognized experts in the fields of antibiotic therapy, pediatrics, and allergy were sent the results of our findings and asked to express an opinion on the possible public health significance of these quantities of penicillin in market milk. It was recognized that little evidence was available upon which to base an opinion and that it would be necessary for these authorities

to arrive at their conclusions from experience in their respective fields. Answers to the following questions were requested:

1. Are these amounts of antibiotics in milk dangerous for the consumer to ingest on the basis of his daily consumption?
2. Specifically, do you think these amounts may:
   a) Sensitize a nonsensitive individual?
   b) Cause a reaction in the exquisitely sensitive individual?
   c) Cause emergence of resistant microorganisms?
   d) Change the normal intestinal flora?
   e) Change the normal oral flora?

Of 31 replies received, the majority answered "no" or "probably not" to all questions except 2*b*. The great majority were of the opinion that the ingestion of the amounts of penicillin found in milk might conceivably cause a reaction in an extremely sensitive individual.

It is thus an accomplished fact that penicillin is present in small amounts in our milk supply. Further knowledge of the presence of antibiotics in the food supply was obtained recently when it was discovered that tens of thousands of chickens were being injected in the neck tissues with a preparation which left an insoluble residue of active drug. No antibiotic was found in tissues remote from the injection site, but in many cases the drug was found in that portion of the breast adjacent to the neck. It was further found that when such antibiotic containing tissue was baked or fried the concentration of drug was not appreciably diminished, due to its insoluble state.

The possibility of the exposure of large segments of the population to a multiplicity of antibiotics is a matter of some concern. The problem that confronts us today is no different in principle than that facing us in the days of the first Food and Drug Act of June 30, 1906. The main difference is the vastly increased number of new chemicals now used or proposed for use in food for man and animals. The Federal Food, Drug, and Cosmetic Act regulates food within its jurisdiction which contains added antibiotics. The manner in which the antibiotics are added determines which of two sections of the law is involved:

1. If the antibiotics are used as pesticide chemicals (i.e., to control bacteria, molds, blights, etc., on raw agricultural commodities) so that residues remain, safe tolerances should be established for the residues under section 408 of the law. This is the latest amendment to the Federal Food, Drug, and Cosmetic Act, commonly known as the Miller Pesticide Chemicals Amendment (Public Law 518, 83rd Congress).

2. If antibiotics are used in or on food which has been processed, fabricated, or manufactured by cooking, freezing, dehydrating, or milling, the "Miller Amendment" does not apply, and application for a tolerance must be made under section 406 of the Federal Food, Drug, and Cosmetic Act. The granting of a tolerance under section 406 requires that the applicant show that the chemical he wishes to use on a certain particular food is required in the production thereof or cannot be avoided by good manufacturing practice. This is difficult to do.

Before the passage of the Miller Amendment, the Food and Drug Administration had given careful consideration to the entire problem of the projected use of anti-

biotics as food preservatives and in February 1953 a statement of policy was issued by the Secretary of Health, Education, and Welfare concerning the direct or indirect addition of antibiotic drugs to foods for human consumption. In effect, the statement was as follows:

The presence of antibiotic drugs in foods intended for human consumption or the direct or indirect addition of such drugs to such foods may be deemed an adulteration within the meaning of section 402 of the Federal Food, Drug, and Cosmetic Act. This statement of policy regarding antibiotic drugs put them in a class apart from most other pesticide chemicals until the passage of the Miller Amendment. The status of this statement of policy became subject to change only with the passage of the Miller Amendment. Now, a person may submit a petition to the Department of Health, Education, and Welfare requesting that a safe tolerance be set for residues of an antibiotic in or on specified raw agricultural commodities. (Certain technical requirements must be met before such a petition is filed.) If the Secretary of Agriculture certifies that the antibiotic is useful for the purpose for which a tolerance is requested, the Commissioner of Food and Drugs will establish a safe tolerance for the antibiotic residues which will protect the public health. If he concludes that no antibiotic residue should remain, he may set the tolerance at zero. If he establishes tolerance greater than zero, this would constitute a change in the policy stated in February 1953 and appropriate modification of the policy statement would be made. (If the manufacturer finds that use of an antibiotic as a pesticide chemical leaves no antibiotic residue on the food, then no petition would need to be submitted for a tolerance, since under the Miller Pesticide Chemicals Amendment, a pesticide has the equivalent of a zero tolerance until a higher one is established.)

The foregoing discussion serves to emphasize the fact that the addition of antibiotics to the food supply should be approached with the utmost caution. It may well be in the public interest to reserve this valuable class of drugs for the purpose for which they originally were developed, that is, the prevention and treatment of disease.

Public Health Aspects

PANEL DISCUSSION

Moderator

Wesley W. Spink

Panel Members

R. Braude Harry Eagle Maxwell Finland
William A. Randall F. Ruiz-Sanchez
Robert L. Squibb

Summarizer

Chester S. Keefer

Dr. Spink: For two and a half days you have heard about the use of antibiotics in plants and in animals. You have heard about the underlying biological mechanisms involved. You have heard about the economic advantages. So it is appropriate after these discussions to inquire what about man when he ingests these foods? In human medicine the antibiotics when given for therapeutic purposes do cause undesirable side effects. These are manifestations of the toxic action of the drug as well as a manifestation of the hypersensitivity of the human host to these drugs. Will the dissemination of antibiotics among plants and animals result in increased incidence of the appearance of antibiotic-resistant microorganisms? There is one other question you might ask. Is there any evidence that the feeding of antibiotics to human beings causes an acceleration of growth? These are some of the questions that this panel hopes to discuss this afternoon.

We have heard Dr. Finland discuss the problem of antibiotic-resistant microorganisms. This phenomenon occurs in man. It apparently occurs in animals. Related to this problem, of course, is the question of the pathogenicity of those organisms which do become resistant, and I think Dr. Finland pointed out quite clearly that the over-all problem in this country and in many other areas is the problem of the staphylococcus, particularly in the hospitals. I have learned from some of my veterinary friends that in the treatment of mastitis with penicillin, penicillin-resistant staphylococci are being excreted by the treated cattle, in milk.

This problem of resistance is an important one in human medicine. Whether it will be of concern from the viewpoint of adding antibiotics to food and the dispensing of foods is another matter.

At this point it probably would be appropriate to have one of our distinguished authorities in this country discuss this problem of resistance with relation to time and to the amounts or concentrations of penicillin or any other antibiotic necessary for the appearance of these resistant strains. Dr. Harry Eagle, of the Microbiological Institute, perhaps would be willing to discuss this problem.

Dr. Eagle: I think in considering the problem as to whether with the presence of antibiotic residues in food there is the danger of emerging resistance, we ought to break the question down, as Dr. Finland indicated, into two parts.

In the first place, what is the chance that bacteria which begin as sensitive bacteria will become resistant under the impact of small concentrations of antibiotics in the food?

The second question, what is the answer if entire species of bacteria will be suppressed and replaced by other species which were resistant a priori?

For the first question, as to the possibility or probability that initially sensitive bacteria will become resistant to the antibiotic as a result of exposure: Bacteria become resistant by virtue of the fact that in a large bacterial population there are a few organisms, probably mutants, which in the presence of the antibiotic grow out at concentrations which suppress the preponderant normal population. However, in order for this to happen there are certain essential pre-conditions:

The concentration of antibiotic must be precisely that concentration which will inhibit the normals and not inhibit the mutants. If it is higher, both are killed or inhibited to the same degree. Even if there is not sterilization, the few organisms which remain will have the same proportion of normal and resistants as did the original population. The presence of a very high concentration, then, does not make for the selective propagation of resistant mutants. It cannot be too low, because if it is too low both the normal and resistant organisms will grow out at the same rate.

What are the chances that with the ingestion of antibiotic residues in food we will have maintained precisely that concentration which will inhibit the normal components of a given species and not the resistant mutant? A priori, I would judge that they were very small, indeed, astonishingly small. There are only a very few species in which the difference in resistance between the normal and resistant mutants is sufficiently great to suggest even the possibility that that could happen. As already indicated, staphylococcus is one of them.

The second possibility, that antibiotic residues will inhibit entire sensitive species and permit the selective propagation of those species which were resistant at the very outset, is a more real one. The mechanism is exactly the same. Here, also, a priori the number of species which are sensitive to the very low concentrations which we are here discussing is extremely small.

Mr. Chairman, although you could get the opinion of dozens of people who have worked with the antibiotics, I don't think there are sufficient data available to permit one to make the ex cathedra statement that antibiotic residues at such-and-such a level will or will not permit the emergence of resistance in a large human population. My personal opinion is that they won't to any important degree. I submit that this is a sufficiently important problem and one which is sufficiently difficult to adjudge a priori that the human experiment is indicated on a large scale and under controlled conditions.

DR. SPINK: Thank you, Dr. Eagle. Does anyone else on the panel wish to express an opinion about this? Would anyone in the audience care to express an opinion at this time about this problem of the emergence of antibiotic-resistant organisms as a result of these residues in food?

I might say this problem has engaged our interest. I am in thorough agreement with Dr. Eagle that the evidence at hand does not indicate that it is a large problem. Dr. Sanchez?

DR. FRANCISCO RUIZ-SANCHEZ: Mr. Chairman, after listening to the conference

about the use of antibiotics in agriculture, I believe that it is too early yet to judge whether the use of antibiotics in food could result in the emergence of resistant strains of germs.

DR. RUIZ-SANCHEZ (Through the Interpreter, Dr. Fribourg): I do not know of any experiments nor have I personally done any experiments on the consequences that would be brought about through the chronic ingestion of very small amounts of antibiotics. At this moment I am talking as a clinician and not as a laboratory person. My personal experience in the handling of antibiotics with the sick in hospitals is what I am going to use as a basis for these comments.

Prolonged contact with antibiotic as it is used in its elaboration or intake can result in states of sensitivity known by all. This is particularly frequent in the handling of streptomycin.

The possibility that these states can develop in persons who handle antibiotics for any length of time in the preservation of food, in the prophylaxis of disease in animals, in the protection of crops, et cetera, is perfectly acceptable.

The possibility that very small quantities ingested for a long period of time may produce sensitivity is more remote, particularly in the case of penicillin, which upon its being introduced in the stomach suffers the modifications which are imposed upon it by the gastric juice.

Nothing very certain can be affirmed, either, as to whether such small quantities are capable of producing disagreeable reactions in individuals previously sensitized. The fact that it reaches the organism through the digestive tract decreases these possibilities in the case of penicillin. We might also include as a possible mechanism of desensitization the producing of reactions of even slight intensity which would not produce any clinical symptoms. Much would depend upon the clinical aspect which would be produced by the previous state of sensitization, whether it be a true anaphylaxis or a state of allergy.

The possibility of creating resistance in the pathogenic flora depends on the mechanism which would be accepted as the cause for the resistance observed. (*a*) If it is a question only of the survival and development of the naturally resistant flora and the disappearance of the sensitive flora, it would not seem probable that such small amounts of antibiotics would represent a danger and lead to the appearance of resistance conditions. (*b*) If the resistance conditions can be explained through the mechanism of biological mutations, implying sudden variation and modification of the genetic structure of the microorganism then this would make possible the transmission through heredity of the newly acquired properties. However nothing definitive can be stated. (*c*) It is very difficult to accept from the clinical viewpoint the third mechanism which would explain the phenomenon of resistance as a mere tolerance phenomena or custom with respect to the germs, resulting from the prolonged contact with very small doses of antibiotics. In any case, this would have to be clearly demonstrated.

With respect to the possible alterations of the normal flora of the mouth and of the intestines, I do not believe that this will happen. Studying the treatment of human brucellosis with doses of tetracycline or chloramphenicol as low as five milligrams per kilogram per day during several weeks, doses which represent quantities much larger than those which could be ingested with food, we have not found any

consistent modifications of the mouth or intestinal flora, no predominance of any bacterial species, staphylococcus or *Pseudomonas aerogenes,* Proteus or *E. coli* or fungi such as *C. albicans.* Neither have we observed stomatitis, vaginitis or proctitis, which are frequently observed with larger doses of antibiotics.

In conclusion, the chronic ingestion of antibiotics with food and in very small doses is relatively a new fact, the consequences of which are not yet known in human medicine. Even though a priori we can accept that they will not produce major consequences, only observation and experimentation will be able to give us the last word on this question.

DR. SPINK: Thank you very much, Dr. Ruiz-Sanchez. I think it would be appropriate at this time to hear from Dr. Squibb concerning his remarkable series of observations in Guatemala on the feeding of antibiotics to groups of malnourished children, not for a month but for years. May we hear from you at this time, Dr. Squibb?

DR. SQUIBB: For the past seven years we have been carrying out a cooperative program in agriculture and human nutrition. While I will use the pronoun "we," I should like it understood that the data I shall present are from human nutrition studies carried out by my colleague, Dr. N. S. Scrimshaw and his co-workers, at the Institute of Human Nutrition. I might also add that our cooperative program has been termed by Dr. William J. Darby and our colleague in the audience, Dr. Leonard A. Maynard, an "interdigitated" one.

The country of Guatemala, location of our program, lies in tropical America just below Mexico. Sixty-five per cent of its two and a half million inhabitants are pure Maya Indians, eating diets which have been little changed for over four hundred years. Over ninety per cent of this diet is of vegetable origin; 75 per cent of it comes from corn largely in the form of tortillas. I might say that both the animals and the humans in this area suffer from acute malnutrition. Here, then, is an area where antibiotics should work.

Our data from humans and animals have not always shown the response which previously has been reported here at this session. For example, I think it may be of interest to Dr. Catron that our runt pigs just don't grow when we add Aureomycin to the diet; but when we substitute overripe bananas for the corn, the growth response is immediate. We need to do much more work on this phenomenon.

We have also studied the serum levels of some seven to twelve blood constituents of pigs and poultry and of humans fed antibiotics, and to date have not observed any adverse effect of the antibiotics on these blood constituents.

With the usual antibiotics we have observed an increased growth and feed efficiency in swine and poultry; however, in most cases their use has not proved economical.

Now let us examine some of the data observed in children. The diets of the children in these areas average only five to ten grams of animal protein per day although in many cases the total protein intake is sufficient. We have also observed both in animals and in humans, deficiencies of vitamin A and riboflavin.

In the trials to be discussed this afternoon, children were fed 50 mg. of Aureomycin per child, per day. Penicillin was fed in the same dosage. While there have

been a considerable number of trials since 1950, I will present data only from some of the very latest work which has been in progress for two years.

Data for the first five months of this series of experiments is contained in Table 1. It shows the results in a control group, a group fed Aureomycin, and one fed penicillin. Measurements were made on weight and height. As you can see, a significant increase of height and weight was observed in children receiving either Aureomycin or penicillin.

The data in the next two tables (Tables 2 and 3) cover a two-year period of continuous feeding of children in the same experiment as the preceding. This study was carried out in a little village called Santa Catarina. The first of the two shows height data. You can see that although in the first six months there was an initial effect from Aureomycin and penicillin, during the second six months no effect was observed. During the third six months there was only a suggestion of gain from Aureomycin which was not significant; and during the fourth six months, no effect of either Aureomycin or penicillin.

Weight data for the same experiment is shown in Table 3. The initial response was favorable for both Aureomycin and penicillin. However, there was no significant change in succeeding six months' periods.

Data from a duplicate study carried out in the little village of San Antonio Aguas Calientes is shown in Table 4. Once again you can see the data for the control groups of children fed Aureomycin, and for those fed penicillin. Aureomycin again appeared to cause initial increase on rate of gain in height. However, there was no effect of penicillin in any of the periods or of Aureomycin in the second, third and fourth

TABLE 1

AVERAGE MONTHLY GAIN IN HEIGHT AND WEIGHT

(Santa Catarina Barahona)

Feb. 53—Oct. 53	N	Ht. cms.	Wt. kilos.
Control	35	.43	.17
Aureomycin	37	.50*	.22*
Penicillin	39	.51*	.26*

* Approx. L. S. D. $_{.05} = .05$ for Ht. and Wt.

TABLE 2

ANTIBIOTIC ADMINISTRATION
RURAL GUATEMALAN SCHOOL CHILDREN

(Santa Catarina Barahona)

Monthly gain in height in cms.*

Group†	1st 6 mos.	2nd 6 mos.	3rd 6 mos.	4th 6 mos.
Control	0.43	0.52	0.38	0.51
Aureomycin	0.50	0.53	0.41	0.55
Penicillin	0.51	0.53	0.38	0.54

* Approx. L. S. D. $_{.05} = .05$.
† Groups ranged from 28–39 children.

TABLE 3

ANTIBIOTIC ADMINISTRATION
RURAL GUATEMALAN SCHOOL CHILDREN

(Santa Catarina Barahona)

Monthly gain in weight in kilos.*

Group†	1st 6 mos.	2nd 6 mos.	3rd 6 mos.	4th 6 mos.
Control	0.17	0.19	0.23	0.15
Aureomycin	0.22	0.18	0.23	0.16
Penicillin	0.26	0.19	0.21	0.19

* Approx. L. S. D. $_{.05} = .05$.
† Groups ranged from 28–39 children.

periods. In the third period there was a tendency toward depression which was not significant, however.

The effect of antibiotics on weight in the same village is shown in Table 5. Again there was no significant effect from Aureomycin or penicillin, but there was a tendency for penicillin to have a depressing effect, in the second period.

Reviewing all of the studies covering a five-year period, I would like to quote Dr. Scrimshaw: "Under certain conditions of low growth rate and poor hygiene, Aureomycin appears to have a slight initial stimulatory effect which is transitory

TABLE 4

ANTIBIOTIC ADMINISTRATION
RURAL GUATEMALAN SCHOOL CHILDREN

(San Antonio Aguas Calientes)

Monthly gain in height in cms.*

Group†	1st 6 mos.	2nd 6 mos.	3rd 6 mos.	4th 6 mos.
Control	0.34	0.51	0.45	0.54
Aureomycin	0.44	0.51	0.45	0.54
Penicillin	0.36	0.51	0.41	0.45

* Approx. L. S. D. $_{.05} = .06$.
† Groups ranged from 17–42 children.

TABLE 5

ANTIBIOTIC ADMINISTRATION
RURAL GUATEMALAN SCHOOL CHILDREN

(San Antonio Aguas Calientes)

Monthly gain in weight in kilos.*

Group†	1st 6 mos.	2nd 6 mos.	3rd 6 mos.	4th 6 mos.
Control	0.24	0.18	0.18	0.14
Aureomycin	0.25	0.18	0.26	0.12
Penicillin	0.18	0.17	0.19	0.15

* Approx. L. S. D. $_{.05} = 0.06$.
† Groups ranged from 17–42 children.

and of no quantitative importance. Even under adverse conditions, it is of no public health importance as measured by growth response. As to the effect of penicillin, it is highly variable and certainly not of practical significance."

Therefore, just what is the public health significance of these antibiotics? First, there has been a continuous feeding of Aureomycin for a two-year period, at a level of 50 mg. per child per day, without any apparent toxic effects. As to the data on which this statement is based, there are first of all the extensive hematological studies which have been carried out on many children. Seven to twelve blood constituents have been determined. In addition, clinical observations have been made by at least two different physicians. No gross or microscopic changes were observed in the feces. However, I might add that there are no adequate data on morbidity and that no detailed bacteriological studies have been made.

I would like to mention one thing which I don't believe has been given adequate attention in this discussion, and that is the fact that the degree of environmental stress is highly important. Environmental stress may markedly affect the growth response in animals or the mode or modes of action and hence the over-all value of antibiotics.

DR. SPINK: Thank you very much, Dr. Squibb. It is an exceedingly significant piece of observation that one can give antibiotics over a period of days, weeks, and years to a group without any significant changes or ill effects. I would like to make one point, however. This was done in children. Children always tolerate sulfonamides and antibiotics much better than adults. Whether that has any significance or not, I do not know, but it is a variable.

Are there any other comments from the panel at this point? I would like to call on Dr. Braude, our guest from England, who is not in the field of public health, who is not a physician. On the other hand, he is sitting on a panel with physicians and public health individuals, and represents the animal industry. I would like to have his reactions concerning the use of these agents in food from the public health point of view.

DR. BRAUDE: I am an animal husbandry man, and the only reason I can think of why I was put on the panel dealing with public health aspects is, I believe, the fact that it was not until September 1953 that the British authorities released antibiotics for practical application to be used as growth promoters for pigs and poultry in my country. One of the major reasons for this delay was the concern about the effect such an application may have on the consumer of animal products which come from animals which were fed antibiotic supplements.

We in Britain have a rather different approach to matters of this kind. However, there is a lot to be said in favor of the American attitude of jumping in head first and finding out afterwards how deep or shallow the pool was. But this attitude involves such risks that many people may consider they should not be taken where human life may be involved.

The British authorities, rightly in my humble opinion, insisted on material proof before considering any action, and a very convenient situation they were in. All that was necessary was to watch the American public health statistics.

As far as I am aware, not a single death in the United States was ever attributed to consumption of products from animals treated with antibiotics. What was per-

haps even more significant was the fact that in spite of the very widespread use of antibiotics, not a case could be quoted of any adverse effect on the consumer. After two years of experience in Britain, our evidence is very much the same; and we all know that powerful interests were searching all the time to find contrary evidence.

Looking back on this matter, I cannot express my feelings better than to paraphrase the famous statement and say, "How much has been talked by so many with so little scientific evidence to back up their views."

I think the time has come when we must accept the view that public health is not in any way affected by the use of antibiotics as growth promoters. We must put the onus on the opponents of this view to produce scientifically sound evidence if they wish to challenge this situation.

There was a time when I was worried about some of the health aspects involving the animals themselves. After all, nobody would wish to jeopardize in any way the health of the animal in order to make it grow a bit faster. All the stories about the danger of undermining the efficacy of veterinary treatment and about the possibility of resistant strains developing which would interfere with such treatment have never really materialized and, in my view, need not be further considered unless challenged on the basis of reasonable scientific evidence involving animals, and not fungi, with due respect to the most interesting paper of Dr. Christensen which we heard the other day.

I am often amused by some of my veterinary colleagues who are worried about the small amounts of antibiotics which may pass to the milk of the sow if she is fed antibiotics at the low levels recommended for promotion of growth, while being apparently completely unperturbed about treating mastitis in dairy cows with enormous doses of antibiotics which for quite a considerable time persists in the milk used for human consumption. I can assure Mr. Randall that there are only a very few farmers who intentionally or unintentionally read legal notices on these points.

Incidentally, talking about sow's milk, I cannot pass the opportunity of just mentioning a topic which somehow was missed at previous sessions, namely, that we now have reasonable evidence indicating that it would be unwise to produce sow's milk substitutes for artificial rearing without including antibiotics in it. I have no hesitation to say that the whole matter of artificial rearing was brought nearer to a satisfactory solution by the advent of antibiotics, and I can see the possibility that in the near future our orthodox method of rearing pigs may call for very substantial alteration.

I would like to conclude with two general remarks concerning research on problems connected with the feeding of antibiotics to animals, particularly pigs.

One was already emphasized by my colleague, Dr. Kon, namely, that all the bacteriological evidence, especially on the intestinal flora, must be taken with a pinch of salt.

I must apologize, being very cynical on this matter, but until I had a little to do with this subject I certainly had much more respect for billions and millions as values. A few naughts do not seem to matter in this field. My confidence in the bacteriological methods employed at present in this field was completely shattered when I saw what terrific variations can be encountered in normal animals, variations

between different animals or even within the same animal in different samples taken at short intervals.

I must apologize for bringing in the rather unpopular subject of statistics. Believe me or not, I have no soft spot for statistics, although I follow them, but these interactions very often have a lot of meaning, unfortunately not always.

This brings me to my final point, which has a bearing on all our experimental work with antibiotics, even including that on the public health aspects. It seems to me there are so many factors involved, so many interactions, that it is absolutely futile, in my opinion, to attack the outstanding problems with tests on only a few animals, a habit which many of us, including myself, so often indulge in. What is needed is a concerted effort on a large scale which would allow proper interpretations of the results and avoid much confusion and controversy.

DR. SPINK: Thank you, Dr. Braude. We have a number of questions from the floor, and they all seem to be devoted to Dr. Randall's remarks.

The first one: "Would you please go over the differences between Section 406 and Section 408 of the FDA and Miller Act?"

DR. RANDALL: Perhaps I could just say briefly that Section 406 is the section of the Act which permits a petitioner to obtain tolerance for a pesticide chemical in a food, but he must show that this particular chemical is necessary in the production of the food.

That is pretty hard to do. If you have a pear tree and you end up with one pear at the end of the season, you still can produce pears. Also, not only did it have to be absolutely needed but it could not be done without using the best of manufacturing processes. I think you can imagine that this was most difficult. As I said, since 1938 there has been one most inconsequential tolerance granted under this section of the Act.

It was in order to make it easier for people to obtain tolerances that the Miller Amendment was passed, Section 408. This sets up a regular procedure whereby a person may go through rather devious channels, though, even now, however all he has to show to the Department of Agriculture is that it is useful. If the Department establishes the product as being useful for the purpose for which it is intended, then the Food and Drug will set up a tolerance. That is all there is to it. So fundamentally it is very simple.

DR. SPINK: Here is another question directed to Dr. Randall: "Were the milk samples" which you tested, I assume, "pasteurized?"

DR. RANDALL: The vast majority of the samples were pasteurized. I might add that we have known for a long time, and so have other people, that pasteurization doesn't abolish even minute quantities of penicillin which may be present in milk. It may diminish it to a certain extent, but in our hands, at least, we have never been able completely to eliminate by the usual pasteurization process minute quantities from milk.

DR. SPINK: Here is another way of disposing of the problem of penicillin in milk, given by someone. It is not signed.

"From public health considerations, should not the milk from mastitic udders be discarded irrespective of whether an antibiotic or other drug has been used?"

DR. RANDALL: I quite agree with that. I think it should be. The reason for that

cautionary statement was that very often in a few hours following the treatment of an infected quarter, the milk will begin to flow and look normal. I am quite in agreement that the milk from an infected quarter, from mastitis, should not be added to the milk supply. But if you treat it with a drug it very quickly clears up. Furthermore, that wasn't the actual reason for the cautionary statement. The reason for the statement was to avoid getting an antibiotic in the milk which might be used for human consumption.

DR. SPINK: Do you agree with that, Dr. Braude?

DR. BRAUDE: I just wonder what proportion of milk produced in this country is from cows which are definitely known to be free of mastitis. As far as I know, in England there is quite a heavy infection in the country, and I would hesitate to make the recommendation that milk from cows with mastitis should not be placed in general circulation.

Just one further comment on that. I know of no evidence to indicate that milk from cows suffering from mastitis is harmful to the human.

DR. SPINK: If it is pasteurized.

DR. BRAUDE: Yes. After all, most of the milk is pasteurized anyhow.

DR. SPINK: Dr. Finland: "Is there any evidence that micrococci developing resistance to antibiotics become more virulent?"

DR. FINLAND: I don't think there is evidence for that, but there is evidence that the virulent staphylococci which emerge are resistant. I think you would have to make that distinction. That is what we have been emphasizing here.

DR. SPINK: Dr. Braude: "Do your remarks apply to penicillin-sensitive humans?"

DR. BRAUDE: I just hesitate to answer that after what we heard from Mr. Randall this afternoon. I just wonder whether it would be reasonable to expect legislation to be passed to protect the very minute fraction of the population, legislation which would take the benefit away from milk.

DR. SPINK: I don't know that we could get the answer, but I have been very much interested in this whole problem of hypersensitivity to antibiotics. I would just want to venture the guess that if one could do a control experiment over a long enough period of time with enough individuals, probably more individuals would be found sensitive to the meat they are ingesting than to the antibiotic which might be there.

Dr. Squibb: "Is there any evidence of reduction in the incidence of non-lethal diseases in groups of children receiving antibiotic versus the controls?"

DR. SQUIBB: These data are not known as yet, but they are under observation. We are summarizing the entire five years' work at the present time. There is nothing on which I can make a statement now. Dr. Maynard is in the audience. He was on our technical advisory commission which reviewed this work. Maybe he can supplement what I have said just now.

DR. L. A. MAYNARD: I have no statement I can make at this time.

DR. SPINK: Thank you.

DR. SQUIBB: We are in accord, then.

DR. SPINK: Another question: "Is it not true that some foods naturally contain low levels of antibiotics? Is, then, the use of low levels of antibiotics for food preservation such a radical development as is sometimes supposed?"

I don't think there is any question that both animals and humans probably for centuries have been ingesting antibiotics—unknown antibiotics, to be sure—and probably in concentrations not too far removed from those we are discussing.

Dr. Braude: "When you fed copper sulfate and got a growth response with pigs, did you do bacteriological studies to determine if feeding copper sulfate has the same effects on intestinal flora as antibiotic feeding?"

DR. BRAUDE: We have not gone that far. The whole copper story is extremely interesting. By feeding very high levels of copper, levels which according to many textbooks are toxic in pigs, we found that they certainly are not toxic but that they stimulate growth, and consequently we got responses very similar to those we get from feeding antibiotics.

An interesting thing, too, in relation to what was said earlier today, is that we found no synergistic effect between copper and antibiotics. We have not followed it up yet with bacteriological studies. I don't believe in them, anyhow, with our present methods.

We are trying to investigate the effect on thickness of the intestinal walls, which was referred to the other day, which we can confirm in pigs. That is in progress now but I have no results yet to offer.

DR. SPINK: I understand someone in the audience would like to comment upon the naturally occurring antibiotics in food and plants. Dr. Deatherage?

DR. FRED E. DEATHERAGE: I think that Dutch workers have shown that one reason why some cheese makers have trouble with Clostridia developing in the cheese and spoiling it is that those particular cheese makers are using bacteria which produce little niacin, and in fact the British a few months ago in *Food Manufacturing* had an editorial that niacin-producing bacteria are used in certain cheeses and niacin is even artificially added to cheese in Britain to prevent clostridial spoilage. Is that correct?

DR. BRAUDE: Yes, that is correct.

DR. SPINK: Thank you.

Dr. Eagle: "What is the relationship between the dosage of antibiotic administered and the induction of resistant strains? Are you able to confirm the suggestion of Gould, *et al.*, that the use of very low doses of antibiotic is less likely to induce antibiotic resistance?"

DR. EAGLE: This question gets to the very heart of the problem as to what effect ingested antibiotics might have on the development of resistance to bacteria. I can only repeat that a given concentration of antibiotic will affect only those organisms which are sensitive to that concentration. A concentration of one part in ten million will have no effect on bacteria which are killed only by concentrations of one million. A concentration of one million will not promote resistance in an organism which is totally killed by one part in a million. The only bacteria which will be affected in the direction of increased resistance will be those strains in which the concentration of antibiotic falls directly in the middle of the spectrum of resistance of that organism. Further, the antibiotic will have an effect only for the time for which it remains at that specific level.

I can't help but remind you that all the reservations which have been expressed over these past years with respect to the possible effect of antibiotics in food on the

development of resistance—precisely those same reservations were made over an extended period of time in relation to prophylactic studies in man.

If you care to, you can go back to the *Journal of the American Medical Association* and find very lucid expositions of the possible dangers of continuous, prolonged ingestion of antibiotics. A priori those make sense. In actual fact, however, none of those dangers has developed.

There have been many large-scale studies in man, and they don't develop resistance to antibiotics. The fact is that the normal flora may disappear on the ingestion of large doses, and when the antibiotic is removed the normal flora reestablishes itself. There is no evidence whatever that small concentrations of antibiotics which do not kill bacteria have any effect on the resistance of the strain.

DR. SPINK: Thank you.

Dr. Randall: "Your slide showed more antibiotic in the tissues of chickens fed 50 parts per million than fed 200 parts per million. Is that correct?"

DR. RANDALL: That is correct. The only explanation that I can give of that is that these are comparatively high level feedings, that is, high compared to the ones usually used for nutritional purposes. This was done on a large scale. There were hundreds of chickens involved in this particular study.

We have no way of knowing. But in our own studies, which were done on a small scale, as the concentration of the antibiotic in the feed rose up to a certain point, we noticed a failure to ingest as much of the ration. I offer that as a tentative explanation of the higher concentration with 50 parts per million as contrasted with the 200 parts per million.

DR. SPINK: I have one more question here, and then Dr. Keefer is going to take over and summarize what has been presented both from the floor and from the platform.

I would like to ask if any of the panel have any comment to make at this point. If not, I will give Dr. Randall this last question or two, knowing that he didn't write the bill.

"1. Do you object to the use of antibiotics in foods as preservatives if no active drug is present when consumed?"

DR. RANDALL: No.

DR. SPINK: "2. If no active drug is present, there is inactive drug. What are you going to do about it?"

DR. RANDALL: That is concerned with the degradation products which may occur following the use of the antibiotic in whatever form it is used, for that matter, even in chemotherapy. A tolerance has not been granted yet, but before a tolerance would be granted very extensive studies would have to be submitted on the use of the drug at various levels, and if the chronic toxicity data on a variety of animals indicates no effect, then we are bound to conclude that at least under the experimental conditions no bad effect has resulted from the degradation products.

I think everyone will realize that you can ask, "What will happen in man? What will happen in this animal and that animal?" I don't think we have the answer to that yet. I think the policy is not to be unreasonable about it and, to conclude, I think you can say that the commonly used antibiotics, that is, those which are used

for therapeutic purposes in man, in those cases at least the degradation products following ingestion are not of very great importance.

DR. SPINK: Now we will have a summary of this symposium by Dr. Chester Keefer.

SUMMARY OF THE FIFTH SESSION

DR. KEEFER: In attempting to summarize what has been said here this afternoon, there will inevitably be some repetition. It is apparent that one of the first problems that was encountered in modern therapy was the occurrence of infection which failed to respond to treatment. With the passage of time this problem has come into sharper focus, and we are learning more about it every day. It is one of the more important features in this area of infectious diseases, and the emergence of resistant organisms certainly is related to the problem.

Dr. Finland has reviewed in an exhaustive and masterful manner the important problem of the emergence of antibiotic-resistant strains of bacteria. This is of course a growing and increasingly important problem in infectious disease in man, and Dr. Finland has identified the areas in which we need to concentrate our research efforts of the future.

There is general agreement that the greatest number of antibiotic-resistant staphylococci are encountered in hospital patients and in hospital personnel. It is further agreed from what he has said that the incidence of resistant staphylococci is highest among the population receiving antibiotics for prophylaxis or treatment.

This problem can be attacked on two fronts. First, by seeking methods of reducing the total number of cases of cross-infections in hospitals and by using antibiotics with more discriminating judgment in hospitalized patients. Secondly, by searching for new antibiotics which will destroy staphylococci which are resistant to those which exist at the moment.

The other area of agreement appears to be that resistant strains of bacteria emerge or thrive in an environment which has been made favorable for their growth and survival by suppressing or eliminating certain sensitive species or strains of bacteria. This is especially true in urinary tract infections, respiratory tract infections, and where oral therapy is used, particularly following the employment of the broad spectrum antibiotics.

It should be stressed, as Dr. Finland has already remarked, that the shift in the bacterial flora following antibiotic therapy is not always associated with a continuing infection or a super-infection. Indeed, the incidence of super-infection is relatively small. The problems of the shift of bacterial population and their significance needs continuing examination and study. From the animal feeding experiments we learn that the suppression of the growth of sensitive bacteria and the increase of the growth of non-sensitive organisms is more often beneficial than detrimental, at least insofar as nutrition is concerned. Here is an exciting and stimulating observation, and the question might be posed: What is the proper biological balance for optimum nutrition?

Mr. Randall has run up the flag of caution and turned on the yellow light with respect to the addition of antibiotics to food supply, and has implied that it may be well to restrict the use of antibiotics to prevention and treatment of diseases. He has

explained the Miller Amendment to the Food, Drug, and Cosmetic Act, and likewise the role of the Secretaries on 4th and 14th Streets with respect to certification of policy statements and the role of the Commissioner of the Food and Drug Administration concerning the problem of safety. He has done this in a clear manner.

What is really needed in this area is a continuing study of the problem by the experimental as well as the statistical method. These are not matters which can be settled by vote, by opinion polls, or by legislation. They must be settled by development of the facts, based upon the experimental and scientific method.

It is important to assess the risk of sensitization or of sensitive reactions from antibiotic residues, and also to assess the significance of altering the oral or gastrointestinal flora, and the emergence of resistant microorganisms as it is related to the public health. Certainly the evidence to date would not lead one to believe that the risks outweigh the great benefits which have flowed from the use of antibiotics in animals, to improve nutrition or to prevent or treat diseases, or for that matter from their use as crop sprays in combatting plant diseases or in the preservation of food. In any event, this area of antibiotic residues needs continuing study and research, and a fixed and rigid posture should not be taken without a sound basis of evidence. One must weigh the great advantages against the slight risks or the disadvantages.

When one attempts to add anything to our food or drink he is immediately in trouble with the public. This is very well illustrated time and time again, and it has been brought into very sharp focus in the last few years with respect to fluoridation of water. So one must acquaint the public, when he adds anything to the food or drink, that it is not harmful and that it is beneficial. This requires, of course, the experimental method.

Dr. Braude reminds us once again about British conservatism, and especially in the field of antibiotic residues he has informed us that they take a different and somewhat more conservative approach. This is one of the reasons the British get lemonade out of the lemon.

Finally, I would like to say that the symposium this afternoon has been most instructive to me, and I know that many important questions have been raised and questions that can be answered only by continuing study and work in this most important field. Perhaps when another conference of similar nature is held, we will have more accurate information as to how to inject the whale with antibiotics in a satisfactory manner.

DR. SPINK: Thank you, Dr. Keefer. Dr. Byerly, I don't presume that you or anyone else would object if I adjourned this meeting ahead of time. The meeting is ended.

. . . The Conference ended at five o'clock.