



The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds: Part 1 of a 2-Part Study ()

Pages
531

Size
8.5 x 10

ISBN
030903230X

Committee on Nitrite and Alternative Curing Agents in Food; Assembly of Life Sciences; National Research Council

 [Find Similar Titles](#)

 [More Information](#)

Visit the National Academies Press online and register for...

✓ Instant access to free PDF downloads of titles from the

- NATIONAL ACADEMY OF SCIENCES
- NATIONAL ACADEMY OF ENGINEERING
- INSTITUTE OF MEDICINE
- NATIONAL RESEARCH COUNCIL

✓ 10% off print titles

✓ Custom notification of new releases in your field of interest

✓ Special offers and discounts

Distribution, posting, or copying of this PDF is strictly prohibited without written permission of the National Academies Press. Unless otherwise indicated, all materials in this PDF are copyrighted by the National Academy of Sciences.

To request permission to reprint or otherwise distribute portions of this publication contact our Customer Service Department at 800-624-6242.

Copyright © National Academy of Sciences. All rights reserved.

REFERENCE COPY
FOR LIBRARY USE ONLY

The Health Effects of Nitrate, Nitrite, and *N*-Nitroso Compounds

Part 1 of a 2-Part Study by the Committee on
Nitrite and Alternative Curing Agents in Food
Assembly of Life Sciences

NATIONAL ACADEMY PRESS
Washington, D.C. 1981

NAS-NAE
DEC 11 1981
LIBRARY

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The National Research Council was established by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and of advising the federal government. The Council operates in accordance with general policies determined by the Academy under the authority of its congressional charter of 1863, which establishes the Academy as a private, nonprofit, self-governing membership corporation. The Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in the conduct of their services to the government, the public, and the scientific and engineering communities. It is administered jointly by both Academies and the Institute of Medicine. The National Academy of Engineering and the Institute of Medicine were established in 1964 and 1970, respectively, under the charter of the National Academy of Sciences.

The work on which this publication is based was funded by the U.S. Department of Agriculture and the U.S. Food and Drug Administration under USDA Contract No. 53-3A94-0-18.

Copyright © 1981 by the National Academy of Sciences

No part of this publication may be reproduced without the permission of the National Academy of Sciences except for official use by the United States Government.

Library of Congress Catalog Card Number 81-85542
International Standard Book Number 0-309-03230-X

Available from
NATIONAL ACADEMY PRESS
2101 Constitution Ave., N.W.
Washington, D.C. 20418

Printed in the United States of America

COMMITTEE ON NITRITE AND ALTERNATIVE CURING AGENTS IN FOOD

MACLYN McCARTY, The Rockefeller University, New York, New York, Chairman

LINDA M. B. BARTOSHUK, John B. Pierce Foundation Laboratory,
New Haven, Connecticut

FRANCIS F. BUSTA, Department of Food Science and Nutrition,
University of Minnesota, St. Paul, Minnesota

DAVID CLAYSON, Toxicology Research Division, Health and Welfare
Canada, Tunney's Pasture, Ottawa, Ontario, Canada

DAVID FINE, New England Institute for Life Sciences, Waltham,
Massachusetts

STANLEY GOLDFARB, Department of Pathology, University of Wisconsin
School of Medicine, Madison, Wisconsin

J. IAN GRAY, Department of Food Science and Human Nutrition,
Michigan State University, Lansing, Michigan

PETER GREENWALD, Division of Resources, Centers, and Community
Activities, National Cancer Institute, Bethesda, Maryland

ROSS H. HALL, Department of Biochemistry, McMaster University
Health Sciences Center, Hamilton, Ontario, Canada

MORRIS I. KELSEY, Division of Cancer Cause and Prevention,
National Cancer Institute, Bethesda, Maryland

BERT N. LA DU, Department of Pharmacology, University of Michigan,
Ann Arbor, Michigan

FREDERICK W. OEHME, Comparative Toxicology Laboratories, Kansas
State University, Manhattan, Kansas

DURWOOD R. ROWLEY, Food Sciences Laboratory, Natick, Massachusetts

JOHN VAN RYZIN, Division of Biostatistics, Columbia University School
of Public Health, New York, New York

SEYMOUR COHEN, Department of Pharmacological Sciences, State University
of New York, Stony Brook, New York, Advisor

PHILIP E. HARTMAN, Department of Biology, Johns Hopkins University,
Baltimore, Maryland, Advisor

PETER N. MAGEE, Fels Research Institute, Temple University School of
Medicine, Philadelphia, Pennsylvania, Advisor

SIDNEY S. MIRVISH, Eppley Institute for Research in Cancer, University
of Nebraska Medical Center, Omaha, Nebraska, Advisor

ROLF PREUSSMANN, Deutsches Krebsforschungszentrum, Institut für
Toxikologie und Chemie, Heidelberg, Federal Republic of Germany,
Advisor

National Research Council Staff:

SUSHMA PALMER, Project Director

ROY WIDDUS, Staff Officer

CATHERINE ST. HILAIRE, Staff Officer

ROBERT MATHEWS, Staff Officer

LESLIE GRAYBILL, Research Associate

FRANCES M. PETER, Editor

PREFACE

In September 1980 the Department of Agriculture and the Food and Drug Administration entered into a contract with the National Academy of Sciences to examine the health effects of dietary nitrate and nitrite and to evaluate possible alternatives to nitrite added to food as a preservative. Accordingly, the Committee on Nitrite and Alternative Curing Agents in Food was established within the Assembly of Life Sciences of the National Academy of Sciences/National Research Council. The committee was charged with the task of reviewing scientific literature pertaining to these subjects and preparing two reports. In this volume, its first report, the committee has attempted to assess the health risks associated with overall exposure to nitrate, nitrite, and N-nitroso compounds, placing emphasis on the risks resulting from natural and added nitrate and nitrite in food and the utility of nitrite added to food. In the second report, it will review the status of research and future prospects for developing feasible alternatives to the use of nitrite as a preservative.

A special effort was made to ensure that the collective knowledge of the committee encompassed all the types of expertise needed to conduct a study of this scope. The resultant multidisciplinary committee includes the biomedical expertise that was needed to evaluate the toxicological and carcinogenic significance of exposures to food additives and environmental chemicals, the metabolism and pharmacokinetics of xenobiotic compounds, and the practicality, antimicrobial efficacy, and utility of food additives.

A broad search was conducted to gather the information needed by the committee during its study. This effort went beyond a review of the vast scientific literature to include requests for information from scientists not on the committee, federal agency officials, and consultants from the food industry and trade associations. Consultants were also invited to make oral presentations or to prepare papers for consideration by the committee. In January 1981, a widely advertised public meeting was held in an attempt to ensure that all those wishing to contribute material to the committee had the opportunity to do so.

The committee recognizes that the subject of its study is of great interest to the public, which is concerned about the safety of the food supply; to the food industry, which must provide a safe and economical product while earning a fair commercial return; and to the regulatory agencies, which are responsible for monitoring production and distribution of food in the interest of public health. Having recognized these interests, the committee has directed its attention and efforts toward scientific matters requiring elucidation

to facilitate the development of a public policy concerning the addition of nitrite to food. In this context, the committee realized that it would be helpful to view the contribution of nitrite as one component of the overall risk that might be posed by exposure to N-nitroso compounds.

The scientific questions addressed by the committee were complex. Among the most important were the following: Of what relevance to public health is the addition of nitrite when nitrate and nitrite are present naturally in foods? In what manner and to what degree do naturally occurring and added nitrate and nitrite contribute to the formation of N-nitroso compounds? What is the impact on human health resulting from the overall exposure to these compounds? For what purposes are nitrate and nitrite added to foods? How precisely can one define the resulting effects? What are the relative risks of added nitrite compared to the risks of not adding it? Are there suitable alternatives? If so, are their effects on health understood?

To address these complex, but discrete questions, the committee formed from among its members two closely interacting subgroups. Each was given the primary responsibility for the initial analysis of the evidence pertaining to one of the two major subject areas--the risks and utility of nitrate and nitrite in food or the status of research on alternative curing agents. However, each subgroup contributed to the work of its counterpart through discussions and shared writing efforts. The committee as a whole reviewed this report, and resulting comments have been incorporated into the text.

Judgments balancing economic considerations and health effects were deliberately omitted from this report because the committee believed that such considerations are not solely scientific in nature but, rather, overlap into the public policy arena.

The committee is grateful to all who contributed to this study. It wishes especially to acknowledge the contribution of the following individuals who provided valuable information and, at the request of the committee, drafted manuscripts for review and use by the committee:

Dr. Robert Cassens, Muscle Biology Laboratory, University of Wisconsin, Madison, Wisconsin

Dr. Brian Challis, Department of Chemistry, Imperial College of Science and Technology, South Kensington, London, United Kingdom

Dr. Melvin Eklund, Northwest and Alaska Fisheries Center/Utilization Research Division, U.S. Department of Commerce, National Oceanic and Atmospheric Administration/National Marine Fisheries Service, Seattle, Washington

Dr. Harold L. Newmark, Hoffman-La Roche, Incorporated, Nutley, New Jersey

Dr. Glen R. Schmidt, Department of Animal Science, Colorado State University, Fort Collins, Colorado

Dr. Bruce Tompkin, Research and Development Center, Swift and Company, Oak Brook, Illinois

The committee is especially indebted to Dr. Brian Challis, who served as a consultant throughout the study, assumed major responsibility for drafting Chapter 4 of this report, and devoted innumerable hours in discussions with the committee.

Thanks are also due to the Board on Toxicology and Environmental Health Hazards and the Food and Nutrition Board, both units of the National Research Council; to Drs. James Kirk, Philip Landrigan, Robert Neal, and Steven Tannenbaum, who provided advice and assistance in planning the study; and to the Department of Applied Mathematics at the Brookhaven National Laboratory for assistance in programming and data analysis. Other organizations or individuals who supplied information requested by the committee include: the American Meat Institute; Dr. Terry Roberts of the Meat Research Institute in Bristol, United Kingdom; Dr. Lothar Leistner from the Institute for Microbiology, Toxicology and Histology, Bundesanstalt für Fleischforschung, Kulmbach, the Federal Republic of Germany; and Dr. John Sofos from Colorado State University. The committee is also grateful to the following individuals who presented useful data and participated in the public meeting held on January 22, 1981:

Dr. John J. Birdall, Scientific Activities, American Meat Institute, Arlington, Virginia

Mr. Ronald Briggs, Wasilla, Alaska

Dr. Mary P. Clarke, Nutrition Education, Extension Quality of Living, Manhattan, Kansas

Dr. H. Ned Draudt, Peter Echrich and Sons, Inc., Fort Wayne, Indiana

Dr. Hugh Gardner, Foundation for Urban and Neighborhood Development, Denver, Colorado

Dr. R. V. Lechowich, Department of Food Science and Technology,
Virginia Polytechnic Institute and State University,
Blacksburg, Virginia

Dr. E. J. LeRoux, Research Branch, Agriculture Canada, Ottawa,
Ontario, Canada

Mrs. Edward B. Poinsett, Atlanta, Georgia

Dr. Marvin A. Sackner, Medical Sciences, Mount Sinai Medical
Center, Miami, Florida

Mr. C. A. Santare, Western States Meat Packers Association, San
Francisco, California

Mr. Thomas Smith, Consumer Division, Community Nutrition Insti-
tute, Washington, D.C.

Mr. L. Strumskis, Georgia Oil and Gas Co., Ocala, Florida

The committee also wishes to thank Ronald E. Engel, Thomas P. Grumbly, and Donald L. Houston of the U.S. Department of Agriculture and Albert C. Kolbye of the U.S. Food and Drug Administration and their colleagues, who provided access to their data sources and shared their insights.

The staff of the Assembly of Life Sciences--Sushma Palmer, Roy Widdus, Catherine St. Hilaire, Robert A. Mathews, Leslie Graybill, Frances M. Peter, and Norman Grossblatt--provided the extensive support and assistance that was essential to the completion of the report. The committee is deeply indebted to them for their work. The dedicated secretarial services of Susan G. Barron, Eileen G. Brown, Chipella L. Reap, Dena Banks, Celia D. Smith, and Roberta A. Pirosko also contributed to the timely completion of this report.

MACLYN McCARTY
Chairman
Committee on Nitrite and
Alternative Curing Agents in Food

CONTENTS

| | <u>Page</u> |
|--|-------------|
| CHAPTER 1 - EXECUTIVE SUMMARY..... | 1-1 |
| CHAPTER 2 - HISTORICAL PERSPECTIVE..... | 2-1 |
| CHAPTER 3 - THE UTILITY OF NITRATE AND NITRITE ADDED TO FOODS.. | 3-1 |
| CHAPTER 4 - THE CHEMISTRY OF NITRATE, NITRITE, AND NITROSATION..... | 4-1 |
| CHAPTER 5 - NITRATE, NITRITE, AND NITROGEN OXIDES: ENVIRONMENTAL DISTRIBUTION AND EXPOSURE OF HUMANS..... | 5-1 |
| CHAPTER 6 - ENVIRONMENTAL DISTRIBUTION AND EXPOSURE OF HUMANS TO NITROSATABLE SUBSTRATES AND MODIFIERS OF NITROSATION REACTIONS..... | 6-1 |
| CHAPTER 7 - N-NITROSO COMPOUNDS: ENVIRONMENTAL DISTRIBUTION AND EXPOSURE OF HUMANS..... | 7-1 |
| CHAPTER 8 - METABOLISM AND PHARMACOKINETICS OF NITRATE, NITRITE, AND N-NITROSO COMPOUNDS..... | 8-1 |
| CHAPTER 9 - ADVERSE EFFECTS OF NITRATE, NITRITE, AND N-NITROSO COMPOUNDS..... | 9-1 |
| CHAPTER 10 - ESTIMATION OF RISK TO HUMAN HEALTH..... | 10-1 |

CHAPTER 1

EXECUTIVE SUMMARY

HISTORICAL PERSPECTIVE

Curing salts, some of which contain nitrate and nitrite, have been used for many centuries to preserve meat. However, the intentional use of nitrate and nitrite salts to cure meat is a relatively new practice.

Since the 1900s, the U.S. Department of Agriculture (USDA) has regulated and monitored the addition of nitrate and nitrite to red-meat and poultry products. The intentional use of these compounds was originally motivated by their ability to produce a reddish-pink color in meat. Subsequently, it was discovered that nitrite inhibited the growth of certain bacteria such as putrefactive anaerobes, which cause the spoilage of meats, and Clostridium botulinum, which causes a foodborne intoxication -- botulism.

In recent years, a number of observations have led to concern about potential risks to human health resulting from the use of nitrate and nitrite. At present, primary concern is focused on the possibility of carcinogenic effects, especially since nitrite can interact with substrates such as amines or amides to produce N-nitroso compounds, which can contaminate the nitrite-preserved foods. Many of these N-nitroso compounds (which include nitrosamines) are known to cause cancer in many animal species. In mid-1978, this concern was exacerbated by the results of a 2-year feeding study in animals, which suggested that nitrite per se causes cancer. These results would have necessitated the banning of nitrite in order to comply with the Food Safety Provisions and, for some uses of nitrite, the Delaney Clause of the Food, Drug, and Cosmetics Act, which proscribes the addition of known carcinogens to foods. However, further evaluation of these data suggested that the initial conclusion may not have been justified. Not only did the effects of nitrate and nitrite on human health need to be fully assessed, their contribution to the total body burden of nitrosamines had also to be determined. Thus, in 1980 the USDA and the Food and Drug Administration (FDA) asked the National Academy of Sciences to examine the current state of knowledge concerning these issues and to assess the status of research on curing agents that can be used as alternatives to nitrite.

THE UTILITY OF NITRATE AND NITRITE

Nitrate and nitrite are used as preservatives in food products, especially cured meats. In the United States, the nitrite content of cured meats and bacon has been reduced substantially in recent decades.

At present, sodium nitrite is added to most bacon at 120 mg/kg, to other pickle-cured products at 200 mg/kg, and to most comminuted (e.g., chopped or ground) cured products at 156 mg/kg.

The specific contribution of nitrite to the inhibition of potential pathogens and microorganisms that are responsible for spoilage varies with the products in which it is used and with variations in their processing, handling, and abuse (such as poor refrigeration of perishable foods).

Nitrite retards microbial spoilage of cured meats by inhibiting the growth of a variety of microorganisms, especially anaerobic and aerobic spore-forming bacteria. In association with other components in the curing salt mix, it exerts a concentration-dependent antimicrobial effect in cured products including, but not limited to, inhibition of the outgrowth of spores from Clostridium botulinum and other clostridia. However, under conditions of excessive contamination or prolonged temperature abuse, it does not indefinitely prevent such outgrowth. Thus, toxin production or spoilage may ultimately ensue.

Depending on a number of factors, including the concentration of nitrite, environmental conditions, and the type of food product, nitrite may also contribute to the control of other pathogens, including Bacillus cereus, Staphylococcus aureus, and Clostridium perfringens. However, nitrite is not generally considered to be a key factor in the control of these bacteria.

Other properties of nitrite are its ability to inhibit lipid oxidation (rancidity) in cured meats and its chemical reaction with myoglobin to produce a reddish-pink color in the muscle tissue of cured meats.

The contribution of nitrite to the flavor of cured meats varies among products, but has not been fully determined for all cured meats. It appears to contribute significantly to the flavor of pickle-cured hams and ham-based food items. However, sodium chloride is largely responsible for the "cured" flavor of some foods, especially bacon. "Flavor" characteristics may not be attributable to a specific chemical component of cured meats since the olfactory system can "fuse" individual aromas into one that is different from those of its components.

Detailed information on the utility of nitrite is provided in Chapter 3.

CHEMICAL REACTIONS

Nitrate can be reduced to nitrite in vitro and, at certain sites (e.g., in the saliva), in vivo. Enzymatic reduction of nitrate salts and both enzymatic oxidation and enzymatic reduction of nitrite

salts are readily accomplished in many bacterial, fungal, and plant systems. These oxidation-reduction reactions permit the interconversion of nitrate and nitrite salts and the generation of reactive nitrogen oxides. Nitrate and nitrite salts are also transformed to reactive nitrogen oxides by gamma radiation and by photolysis.

Chemical experiments have demonstrated that nitrate, nitrite, and some oxides of nitrogen can interact with substances such as secondary or tertiary amines or amides, resulting in the formation of N-nitroso compounds. These compounds may also be produced endogenously from the same precursors. In addition to nitrosamines, classes of N-nitroso compounds include nitrosamides (such as nitrosoureas, nitrosoguanidines, and nitrosocarbamates).

Certain agents or conditions have been shown to enhance or inhibit the nitrosation reactions that lead to the formation of N-nitroso compounds. For example, nitrosation of secondary amines by aqueous nitrous acid can be catalyzed by thiocyanate and iodide and by some phenols, thiols, and alkenes. On the other hand, nitrosation of secondary amines, amides, ureas, and guanidines by aqueous nitrous acid can be inhibited by ascorbic acid (vitamin C), α -tocopherol (vitamin E), and certain naturally occurring phenols and thiols.

Transnitrosation (the transfer of a nitroso group from a nitroso compound to an amino compound) of organic nitroso and nitro compounds can take place in vitro in simple chemical systems, but there is no information on its occurrence in foods.

The extent to which N-nitroso compounds are formed in foods is affected by the type of matrix (hydrophilic or hydrophobic), the presence of natural antioxidants, and methods of processing and cooking. Nitrogen oxides generated during the processing of foods may also produce N-nitroso compounds.

The chemistry of nitrate, nitrite, and N-nitroso compounds is discussed further in Chapter 4.

ENVIRONMENTAL DISTRIBUTION AND EXPOSURE OF HUMANS

Nitrate, Nitrite, and Nitrogen Oxides

Levels of nitrate and nitrite vary widely among foods and in the environment. In foods, they depend on a number of factors, including agricultural practices and storage conditions. These factors, combined with the limitations in analytical techniques for measuring nitrate and nitrite and in methods for the accurate determination of food consumption, make it difficult to estimate precisely the exposure of humans to these compounds. Nevertheless, the committee has made estimates of such exposures. These estimates should not be taken at face value; rather, they should be used as a guide to gain an understanding of the relative contribution of different sources to the exposure of humans to nitrate and nitrite.

Differing lifestyles and dietary habits can lead to wide variations in the amount of nitrate ingested by different population groups. In the average diet, vegetables contribute most of the nitrate ingested (~87% of daily intake). Therefore, vegetarians may consume substantially higher amounts of nitrate than does the general population. Milk generally contains very low levels of nitrate. Under certain circumstances, however, its nitrate content may be considerably higher. For example, high concentrations may be found in milk produced by cows grazed on forage with a high nitrate content. Thus, some milk may be an important source of nitrate for infants. Other sources of exposure include nitrate-rich drinking water and fruit juices.

Of the average daily intake of dietary nitrite, ~ 39% is contributed by ingestion of cured meats, ~ 34% by baked goods and cereals, and ~ 16% by vegetables. The concentration of nitrite in these foods, especially in cured meats, varies widely, and, depending on lifestyle and dietary habits, the daily exposure to nitrite from any one source can vary from 0 to 90%. Thus, there may be considerable variation in the total daily intake of nitrite. (The total gastric nitrite load, which includes nitrite resulting from the reduction of nitrate in vivo, is discussed below in the section entitled Metabolism and Pharmacokinetics.)

Three additional factors are important for determining the significance of exposure to nitrite. First, vegetables contain inhibitors of nitrosation, such as ascorbic acid and polyphenols, and possibly catalysts that can enhance nitrosation. These tend to affect the extent of in vivo nitrosation and, thus, the synthesis of N-nitroso compounds. Second, at the time of consumption, the amount of residual nitrite remaining in cured meat products (about 10 mg/kg) may be substantially lower than the amount estimated to be added during processing. This level, and thus the exposure, will vary, depending on the product. Finally, assays for the residual nitrite content of processed meats may not necessarily indicate the amount of nitrite that is available to participate in nitrosation reactions in vivo (i.e., some forms of bound nitrite may not be measured).

Although the intake of nitrogen oxides may contribute to the daily exposure to nitrate and nitrite, the contribution from this source is relatively small for the average U.S. citizen. However, peak levels of nitrogen oxides in smog-laden cities may result in more substantial exposure. These compounds are unlikely to be of major importance in converting ingested amines via nitrite to nitrosamines in the stomach. However, much remains to be learned about the role of nitrogen oxides in in vivo nitrosation.

For further information on exogenous exposure to nitrate, nitrite, and nitrogen oxides, see Chapter 5.

Amines

A number of substrates in the diet, including secondary or tertiary amines or amides, may interact with nitrite to form N-nitroso compounds. Foods, drugs, cosmetics, agricultural chemicals (e.g., pesticides), tobacco, and certain occupational settings are all significant sources of nitrosatable amines and amides. There is an enormous variation in the rate at which these compounds are nitrosated and the extent to which humans are exposed to them. In addition to exogenous sources of amines, nitrosatable amines such as dimethylamine and pyrrolidine are synthesized endogenously. There are only limited data on exogenous exposure to amines and on their endogenous production. However, evidence indicates that sufficient quantities of amines are present to participate in endogenous nitrosation reactions and that nitrosation does occur when amines and/or amides and nitrate and/or nitrite are ingested simultaneously. The key factors that determine the extent of these reactions in the stomach are the pH; the concentrations of nitrate, nitrite, nitrosatable amines and/or amides; and the rate of nitrosation of the amino compound. In addition, the presence of modifiers (catalysts and inhibitors) of nitrosation reactions will influence the extent of in vivo nitrosation.

Chapter 6 contains discussions of the environmental distribution of amines and certain modifiers of nitrosation reactions.

N-Nitroso Compounds

Analytical methods are sufficiently sensitive to measure volatile nitrosamines. Consequently, the levels of many of these compounds in various environmental sources have been determined. By comparison, very little is known about the occurrence and exposure of humans to unstable and/or nonvolatile N-nitroso compounds.

Humans may be exposed to preformed nitrosamines in the environment via inhalation, ingestion, and dermal contact. Moreover, nitrosamines and nitrosamides may be formed in the body from various precursors.

Because large quantities of nitrosamines are formed in certain occupational settings and are present in tobacco and tobacco smoke, humans may be exposed to high concentrations of these compounds from these sources. For example, maximum exposure to N-nitrosodimethylamine (NDMA) at 440 $\mu\text{g}/\text{day}$ was estimated to occur in a leather-tanning facility. In one study of airborne concentrations in the rubber industry, a maximum N-nitrosomorpholine (NMOR) level of 250 $\mu\text{g}/\text{m}^3$ was found. At this concentration, daily exposure would be 2,500 $\mu\text{g}/\text{day}$. Maximum exposure of humans to NDMA in a rocket fuel factory was calculated to be 260 $\mu\text{g}/\text{day}$. These figures are maximum possible exposures estimated from area air samples; exposure of workers could be approximately 10% of these amounts over an 8-hour day, depending on their activities.

The levels of nitrosamines in tobacco products range from an average of 0.87 μg per U.S. filter cigarette to an average of 1.4 μg per French filter cigarette and approximately 11 μg for one small cigar. If these data are typical of an average cigarette, a pack of 20 U.S. filter cigarettes would provide an intake of ~ 17 μg . By comparison, the intake of nitrosamines from all dietary sources, including beer, has been estimated to be only 1.1 μg per day.

Assays of foodstuffs in the Netherlands and the Federal Republic of Germany have indicated that, until this year, the largest single dietary source of nitrosamines was beer. However, the concentrations of these compounds in beer have been decreased by recent modifications in the malting process. Currently, the most important sources of nitrosamines in the diet are cured meat products, especially bacon, which may contribute approximately 0.17 μg of N-nitrosopyrrolidine to the total daily intake of nitrosamines. Other important exogenous sources of nitrosamines are cosmetics, pharmaceuticals, pesticides, water, and air.

In summary, with the exception of occupational exposures, which were not considered in the above calculations, cigarette smoking contributes the greatest amount to total nitrosamine intake.

Exogenous exposure to nitrosamines is discussed further in Chapter 7.

METABOLISM AND PHARMACOKINETICS

Endogenous Exposure to Nitrate and Nitrite

The metabolism and pharmacokinetics of nitrate and nitrite vary among individuals because of differences in physiology, age, and general health. The conversion of nitrate to nitrite by bacterial reduction in the saliva is an important metabolic reaction. Certain clinical conditions, such as gastric achlorhydria (abnormally low acidity in the stomach) and urinary tract infections, can also greatly enhance the opportunity for bacterial reduction of nitrate to nitrite. However, in persons with normal gastric acidity, nitrate is converted to nitrite mainly by microflora in the saliva. Approximately 25% of ingested nitrate is reported to be recirculated into the saliva, and approximately 20% of salivary nitrate is reduced to nitrite.

The contribution of various sources to exogenous exposure to nitrate and nitrite is discussed in the section on environmental distribution and exposure of humans. However, chemical changes that occur in the saliva and upper gastrointestinal tract affect the endogenous exposure to these compounds. Reduction of nitrate by oral bacteria accounts for most of the nitrite that is present in the stomach -- the most likely site for in vivo nitrosation reactions. Assuming

that approximately 5% of ingested nitrate is converted to nitrite in saliva, the committee has estimated that the main contributors to the gastric nitrite load are vegetables (~72%), cured meats (~9%), baked goods and cereals (~7%), and fruits and fruit juices (~5%). These estimates do not take into consideration the contribution of drinking water, which may be the major source of nitrate intake in some areas.

Endogenous Synthesis of Nitrate

The formation of nitrate by bacteria in the large intestine (heterotrophic nitrification) had been postulated as one mechanism to account for differences in ingestion and urinary excretion of nitrate in humans. However, this conclusion appears to be erroneous since studies in germ-free rodents indicate that such reactions are not important. Moreover, the nitrate content of ingested food, water, and air may have been underestimated in the earlier studies. Recent studies suggest that mammalian tissues synthesize nitrate and that this may partially explain excess urinary nitrate excretion.

In-Vivo Nitrosation

The formation of N-nitroso compounds in vivo has been well-documented in laboratory animals. In humans, the evidence is sparse. However, one recent study showed that a noncarcinogenic nitrosamine was synthesized in a human subject following the ingestion of an amine (proline) and nitrate. In that experiment, the ingestion of a large excess of ascorbic acid or α -tocopherol effectively reduced the endogenous formation of nitrosamines. Based on the methodology used in this experiment, the committee has estimated that the amount of preformed nitrosamines in the diet of the average person is roughly equivalent to the amount formed in vivo from the intake of nitrate and nitrite. However, for special population groups, such as those ingesting high-nitrate water, the increased intake of nitrate could lead to a corresponding increase in the amount of nitrosamines formed in vivo.

In addition to chemicals such as thiocyanate, ascorbic acid, and α -tocopherol, which are capable of modifying nitrosation reactions in vivo, it has been suggested that biological factors may also play an important role in the formation of nitrosamines. For example, bacteria may modify these reactions in such organs as the achlorhydric stomach and the infected bladder, where they colonize.

Mechanisms of the Carcinogenicity of N-Nitroso Compounds

Studies have demonstrated that nitrosamines require metabolic activation in order to become carcinogenic; whereas nitrosamides, including nitrosoureas, nitrosoguanidines, and nitrosocarbamates, are direct-acting carcinogens. The active chemical species alkylates deoxyribonucleic acid (DNA). The organotropic, toxic, and carcinogenic effects of nitrosamines probably result from preferential metabolism by specific organs. The primary reaction required for activation may involve an enzyme-mediated α -hydroxylation. Oxidative dealkylation appears to be necessary for the toxic and carcinogenic action of nitrosamines. The balance among adduct formation, repair mechanisms, and cellular replication may be the most important determinant of the carcinogenic process. Mechanisms for repairing the DNA alkylation damage have been demonstrated in a number of tissues, including the liver and kidney.

See Chapter 8 for additional information on the metabolism and pharmacokinetics of nitrate, nitrite, and N-nitroso compounds.

ADVERSE EFFECTS ON HEALTH

Acute Toxicity in Humans

Ingestion of sufficiently large amounts of nitrate has been shown to cause methemoglobinemia -- excessive production of abnormal hemoglobin -- primarily in infants. Information on the distribution of nitrate in foods and the exposure of the general population to nitrate indicates that such acute toxicity is likely to occur rarely in the United States and generally only in people who have consumed well water contaminated with high levels of nitrate. Nitrite-induced methemoglobinemia is even less common, but has been observed to result from ingestion of home-processed vegetables. N-Nitroso compounds are acutely toxic to humans only at levels that are much higher than those normally encountered in the environment.

Chronic Toxicity in Humans

Evidence implicating nitrate, nitrite, and N-nitroso compounds in the development of cancer in humans is largely circumstantial. Epidemiological studies have suggested a possible association between exposure to high levels of nitrate and nitrite and a high incidence of stomach and esophageal cancer. For example, a high incidence of stomach cancer was found in regions of Colombia where well water contained high concentrations of nitrate. In the Henan Province in China, there is a high incidence of esophageal cancer. The formation of N-nitroso compounds in some commonly consumed foods is a plausible explanation for these observations in China; however, in none of these studies was the exposure to nitrate, nitrite, or N-nitroso compounds actually measured in the individuals who developed cancer.

Moreover, in many studies, other plausible causative agents were also identified. Thus, it is not yet known whether nitrate or nitrite play any role in the causation of these cancers.

Chronic Toxicity in Other Species

Studies conducted to determine the carcinogenicity of nitrate and nitrite have not provided sufficient evidence to conclude that these agents are carcinogenic. Nitrite is known to be mutagenic in microbial tests, and under certain conditions it interacts with nitrosatable substances to produce N-nitroso compounds. Tests for carcinogenicity in animals provide evidence that N-nitroso compounds are likely to be carcinogenic in humans. For example, most of the approximately 300 N-nitroso compounds tested have been shown to be carcinogenic in one or more species of animals. There is a wide range in the carcinogenic potency of these compounds; the potency of some is relatively high.

Tests have also indicated the importance of enhancers and inhibitors of carcinogenicity. For example, agents that promote cell proliferation in the liver enhance hepatocarcinogenicity of certain nitrosamines. Compounds such as ascorbic acid, α -tocopherol, and other antioxidants can inhibit carcinogenicity by blocking the formation of N-nitroso compounds from nitrite and nitrosatable substances.

Many N-nitroso compounds have been shown to be mutagenic in microbial tests, either directly or with metabolic activation. Mammalian-cell-mediated mutagenicity assays may ultimately provide a quantitative indication of carcinogenic activity in animals and in humans.

The adverse effects on health are discussed further in Chapter 9.

RISK ESTIMATION

Evidence of carcinogenicity provided by well-conducted experiments in animals should be regarded as indicating a potential for carcinogenicity in humans. This is especially true when results of investigations have demonstrated carcinogenicity in more than one species -- as they have for N-nitroso compounds, which have been shown to be carcinogenic in numerous species of animals.

However, there is no completely reliable method for using data obtained from animal experiments to derive the magnitude of tissue- or organ-specific carcinogenic potency of a chemical in humans. Furthermore, reliable estimates cannot be made because of the lack of definitive information on nitrate, nitrite, and N-nitroso compounds.

For example: There are insufficient data concerning the average and extreme levels of exposure to nitrate, nitrite, and N-nitroso compounds. There are many inadequately characterized variables that determine the extent of endogenous nitrosation. There is uncertainty about the molecular mechanisms leading to the carcinogenic effect of N-nitroso compounds and their precursors and uncertainty about the comparable ability of humans and laboratory animals to repair genotoxic damage.

There is a possibility that individuals and subgroups of the population vary in their susceptibility to the carcinogenic effects of N-nitroso compounds. Additional uncertainty is introduced by the selection of a set assumptions and mathematical models to develop risk estimates. Therefore, the committee suggests that the numerical estimates in this report be used solely as rough indicators of the relative risk to each of these population groups.

Chapter 10 contains a framework for estimating risk and some first approximations of risk estimates for exposure to nitrate, nitrite, and N-nitroso compounds. The committee wishes to emphasize that although the estimates may be valuable in providing insights into the relative risks for various population groups from exposure to these compounds, their principal value is to provide a point of departure for scientists who may later, on the basis of better data, refine the risk estimates. Therefore, although the numbers and the ranges presented in Chapter 10 provide useful information on the relativity of risks in population subgroups, they are not intended as a guide for policy formation, nor should they be perceived by the public as conclusive.

Although a reduction in exposure to nitrite is likely to reduce the risk of cancer, there is insufficient evidence to support the plausible assumption that a reduced exposure to nitrate and nitrite will lead to a directly proportional reduction in the risk to human health. There is better evidence for N-nitroso compounds: Studies of N-nitrosodimethylamine in animals indicate that a directly proportional reduction in risk could result from the reduction of exposure to N-nitroso compounds.

Nitrosamines formed endogenously from nitrite ingested in cured meats provide only a small proportion of the total exposure of the general population to nitrosamines from all sources. Thus, it does not appear that the reduction of nitrite in cured meats will lead to a major decrease in risk to humans arising from total nitrosamine exposure. However, if only dietary contributors to exposure to N-nitroso compounds are considered, the diminution in risk will be proportionally greater if nitrite were removed from cured meats. These conclusions are based on estimates of average exposure to N-nitroso compounds and do not fully consider the effects of exposure to peak levels, which may be a critical factor.

The committee examined various approaches to estimating the risk of botulism and the increment in its incidence that might arise from omitting nitrite from cured meats. It found that previous attempts to derive such estimates were based on speculation with which it did not wholly concur. It concluded that a more adequate data base must be developed before one can predict the likelihood of a product becoming toxic and, from this, the incidence of botulism.

The committee believes that the degree of protection against botulism is likely to decrease if the essential preservative uses of nitrite are substantially reduced without introducing an efficacious, but safer alternative.

RECOMMENDATIONS

1. Results of limited experiments suggest that nitrate is neither carcinogenic nor mutagenic. However, evidence from several epidemiological studies in human populations is consistent with the hypothesis that exposure to high levels of nitrate may be associated with an increased incidence of cancer of the stomach and the esophagus. Thus, the committee recommends that to confirm these preliminary findings, future epidemiological studies focus on correlating the incidence of cancer and established precursor lesions with actual exposure to nitrate, nitrite, N-nitroso compounds, nitrosatable substances, and inhibitors or enhancers of nitrosation. Where possible, exposure should also be correlated with levels of nitrate, nitrite, and N-nitroso compounds in biological fluids such as blood, saliva, or urine.

2. Evidence does not indicate that nitrite acts directly as a carcinogen in animals. However, because it is mutagenic in microbial systems and because of its implied role in the induction of esophageal and stomach cancer in humans, further testing in animals may be warranted. If such tests provide any indication of carcinogenicity, then the committee recommends that attempts be made to distinguish between the types of carcinogenic activity, i.e., activity as a complete carcinogen, cocarcinogen, or promoter.

3. Most N-nitroso compounds are carcinogenic in laboratory animals, mutagenic in microbial and mammalian test systems, and some are teratogenic in laboratory animals. Although these tests are indicative of potential carcinogenicity in humans, they are of limited value for predicting the quantitative risk to humans. The committee recommends that future carcinogenicity assays emphasize quantitative assessment of potency as well as the qualitative outcome. It also recognizes the need to characterize premalignant lesions induced by N-nitroso compounds and to develop short-term in vivo bioassays to determine their carcinogenicity.

4. Because nitrate and nitrite can exert acute toxic effects such as methemoglobinemia and probably contribute significantly to the total body burden of N-nitroso compounds, which are carcinogenic

in laboratory animals and may be carcinogenic in humans, the committee recommends that exposure of humans to these agents be reduced. Exposure to nitrite should be reduced to the extent that protection against botulism is not compromised. Additionally, the committee recommends that, with the exception of dry-cured products and fermented sausage products in which the presence of nitrate may be necessary, the use of nitrate salts in the curing process be discontinued in all meat and poultry products. Furthermore, the committee suggests that attention should be given to the feasibility of reducing the nitrate content of vegetables and drinking water and that further studies should be conducted to develop methods to reduce nitrate in vegetables while maintaining the content of ascorbic acid and other inhibitors of nitrosation.

5. The committee suggests that the sources of exposure to N-nitroso compounds in various environmental media be determined so that methods to reduce the exposure to these contaminants can be developed. Standardized analytical methods are needed to assess the total body burden of nonvolatile N-nitroso compounds. In addition, it is necessary to obtain accurate estimates of exposure to nitrate and nitrite by improving the assay procedures, especially to distinguish between free and bound nitrite, and to determine whether the residual nitrite is a true measure of nitrosating capacity.

6. The exposure of humans to amines and nitrosamines can be reduced in certain circumstances by modifying manufacturing practices that result in high levels of exposure. For example, pesticides produced as secondary and tertiary amine salts could be replaced by other formulations and certain readily nitrosated drugs could be replaced by drugs that have the same therapeutic effect but are not nitrosated. Further research should be conducted to identify amino compounds that could be nitrosated in vivo, especially those that are readily nitrosated or to which humans are extensively exposed.

7. The committee believes that additional studies are needed to increase understanding of the metabolism and pharmacokinetics of nitrate in humans. Also requiring clarification is the role of bacteria in the reduction of nitrate to nitrite and the formation of N-nitroso compounds, especially in certain clinical conditions such as gastric achlorhydria and bladder infection.

8. The nitrosation-inhibiting effects of ascorbate and other substances have been established, and this knowledge has been put to use commercially to inhibit the formation of nitrosamines in bacon. Normal dietary constituents that enhance or inhibit nitrosation should be studied further to determine the extent of their effects in the diet and in vivo. Specifically, further research is needed to determine the amount of nitrite that is destroyed in the human stomach and the extent to which nitrosation reactions are modified by the various inhibitors. Attention should also be paid to interactions among inhibitors, catalysts, and other components of the diet (e.g., lipids).

The nature and extent of in vivo nitrosation by nitrite, by nitrogen oxides, and by transnitrosation also require further evaluation.

9. Further studies are required to determine the mechanisms whereby nitrite controls the outgrowth of C. botulinum spores. Research is also needed to determine its mechanism of action in cured meats, especially its antioxidant activity and its effect against microorganisms that are responsible for spoilage and against pathogens other than C. botulinum. Since the effect of nitrite varies considerably among products, it should be examined on a product-by-product basis.

10. Although it is not possible to estimate the potential morbidity or mortality from C. botulinum in the absence of nitrite as a curing agent in certain products, the prudent approach to protecting public health requires consideration of the possibility that certain preserved food items may be contaminated and may be abused.

11. In view of the possible but unquantified risk resulting from the use of nitrite as a curing agent, the committee recommends that the search for alternatives and alternative approaches to the use of nitrite be continued. However, no new agent or combination of agents should be substituted for nitrite until adequate testing has ensured that it does not present a hazard to human health.

CHAPTER 2

HISTORICAL PERSPECTIVE

| | <u>Page</u> |
|--|-------------|
| EARLY CURING PRACTICES..... | 2-3 |
| RECOGNITION OF THE ANTIMICROBIAL ACTIVITY OF NITRITE..... | 2-4 |
| THE DEVELOPMENT OF REGULATIONS FOR ADDITION OF NITRATE AND NITRITE TO CURED MEATS AND FISH..... | 2-5 |
| TRENDS IN THE PRODUCTION OF CURED MEATS..... | 2-7 |
| THE ORIGINS OF RECENT CONCERN ABOUT NITRATE AND NITRITE IN FOODS..... | 2-8 |
| BOTULISM..... | 2-12 |
| REFERENCES..... | 2-15 |

CHAPTER 2

HISTORICAL PERSPECTIVE

Salting has been used to preserve the aesthetic and healthful qualities of meat and fish in most civilizations for more than 3,000 years. In their review of curing practices, Binkerd and Kolari (1975) speculated that curing with salt was first practiced in saline desert or coastal regions, e.g., near the Dead Sea. They noted that desert salt contains nitrate as an impurity and that saltpeter, or nitre (potassium nitrate), or "wall saltpeter" (calcium nitrate) from cave walls were used by ancient people for their preservative quality. The earliest specific mention of the characteristic pink color of cured meat did not appear until late Roman literature (Binkerd and Kolari, 1975). References to the flavor conferred by saltpeter as part of the curing mixture were made as early as 1835 (Binkerd and Kolari, 1975), but it was not until much later that this effect was scientifically investigated (Brooks *et al.*, 1940). By the late 1800s, saltpeter was specifically recommended as an ingredient in curing recipes to promote the development of "cured color." For example, Edward Smith in 1873 observed that "Meat, when prepared by salt alone, loses its colour, but when saltpetre is added the flesh becomes a reddish color throughout, provided the action be sufficiently prolonged." (Smith, 1873, p. 35).

Failures to achieve consistent results in curing, particularly in the attainment of safety of cured products, were well documented by Kerner in Germany (1817, 1820, 1822). He studied many outbreaks of often-fatal "sausage poisonings" or "botulism" (a term derived from the Latin "botulus" for sausage) and identified the omission of nitrate from the salt mixture used to cure incriminated sausage as a common feature of the outbreaks. Van Ermengen (1897) demonstrated that the cause of botulism was a neurotoxin produced in the food by a bacterium. He identified this microorganism as an anaerobic spore-forming bacillus, which he named *Bacillus botulinus*. It is now known as *Clostridium botulinum*. The first recorded case of botulism in the United States occurred in 1899 (Center for Disease Control, 1979). Botulism is traditionally regarded as the most serious, life-threatening foodborne disease of microbial origin. It is discussed in more detail in the last section of this chapter.

During the second half of the 19th century, various investigators were implicating microorganisms in the putrefaction of foods and in the causation of some diseases (Brock, 1961). Despite this and the fact that curing was effective in preventing putrefaction and health hazards, it was not until many decades after Kerner's and Van Ermengen's observations that the specific antimicrobial contribution of the various ingredients in curing mixtures was investigated.

The first significant scientific investigations of curing began in the late 19th century. Polenske (1891) reported the presence of nitrite in cured meat and in pickling solutions used for curing. He attributed this to the reduction of nitrate to nitrite by bacterial action.

Lehmann (1899) and Kisskalt (1899) independently reported that nitrite rather than nitrate conferred the typical color to cured products. Subsequently, Haldane (1901), on the basis of experiments with blood and hemoglobin, proposed that the reaction of hemoglobin with nitric oxide derived from nitrite was the chemical basis for the cured meat color. This reaction mechanism was confirmed in uncooked cured meats and sausages by Hoagland (1910).

Although many old curing mixes contained nitrate and possibly nitrite impurities, it is not known when nitrite itself was first purposefully used in curing salt. But as early as 1917, Doran received a patent in the United States for the use of nitrite in curing (Doran, 1917).

RECOGNITION OF THE ANTIMICROBIAL ACTIVITY OF NITRITE

The specific contribution of nitrite (and indirectly nitrate) to the antimicrobial effects of the curing salt mixture was not recognized until the late 1920s. Kerr et al. (1926) stated that neither nitrate nor nitrite had any preservative value, but 2 years later Lewis and Moran (1928) suggested that nitrite had antimicrobial effects. This was later confirmed by other investigators (Evans and Tanner, 1934; Tarr, 1941, 1942, 1944).

A review by Tanner (1944) indicates some of the uncertainties at that time regarding the antimicrobial actions of nitrite and the magnitude of its role in the inhibition of C. botulinum in cured meats. Steinke and Foster (1951) appear to have been the first investigators to provide definitive evidence of sodium nitrite's antibotulinal efficacy in a meat product when it is added at the levels commonly used by commercial producers today.

In the two subsequent decades, many reports on the antibotulinal activity of nitrite were published, and it became generally accepted that nitrite exerted such activity in most cured products (Foster and Duncan, 1974; Sofos et al., 1979). However, definitive evidence of the relative contribution of nitrite in controlling C. botulinum in various foods has only been obtained for certain products during the last decade (Christiansen et al., 1973, 1974, 1975; Hustad et al., 1973).

THE DEVELOPMENT OF REGULATIONS FOR ADDITION OF NITRATE AND NITRITE TO CURED MEATS AND FISH

Of the nitrate and nitrite added to foods in the United States, most has traditionally been used in the production of red-meat products. The responsibility for assuring the safety of such products resides with the U.S. Department of Agriculture (USDA) as it has since 1907 when its Meat Inspection Act (P.L. 242) was passed.

Shortly thereafter, the use of potassium nitrate in curing salt mixes was sanctioned (U.S. Department of Agriculture, 1908), and sodium nitrate was permitted in 1922 (U.S. Department of Agriculture, 1922). However, the demonstration that the active "color-fixing" agent was, in fact, nitrite offered the possibility that the substitution of nitrite for nitrate could provide more consistent results in the curing process, especially in the production of cured color.

In 1923, pilot studies on the use of sodium nitrite, without nitrate(s), in the curing salt mix began under the supervision of the USDA. These studies focused predominantly on the development of cured color. The investigators concluded that sodium or potassium nitrate could be successfully replaced by sodium nitrite in the curing of meat. The quantity of sodium nitrite needed ranged from 0.25 to 1 oz per 100 lb of meat (156-625 mg/kg), depending on the type of meat and the process used. They also observed that the curing period could be shortened by the use of sodium nitrite (Kerr et al., 1926).

On the basis of these results, the USDA in 1925 authorized the use of sodium nitrite as a substitute for sodium or potassium nitrate in the curing of meat (U.S. Department of Agriculture, 1925). The agency suggested that nitrite could be substituted for nitrate at approximately one-tenth the weight of nitrate. At that time, the USDA established 200 mg/kg as a maximum allowable residual level of sodium nitrite in the meat after processing (Kerr et al., 1926; U.S. Department of Agriculture, 1925). This level was lower than the residual level resulting from the use of nitrate(s) at that time. Since no acute toxic effects had been observed to result from these residual concentrations, it was presumed that the 200 mg/kg level was safe.

Thus, these regulations permitted a "mixed" cure, i.e., one containing both nitrate and nitrite, but no residual limit was imposed on nitrate. In 1926, observations that meat processors were using more nitrate than needed led to the issuance of a regulation limiting the level of nitrate salt to 1% in the pickling solution in which meat was immersed (U.S. Department of Agriculture, 1926).

Tanner (1944) has described the various trends and debates that took place during this period regarding the use of nitrate, nitrite, and "mixed cures." Shortly after the establishment of the first regulations permitting the use of nitrite, the USDA recommended a

nitrite cure for sausages and comminuted meats. However, some processors continued to favor the use of a mixed cure and, in 1931, the USDA approved a mixed cure containing 0.25 oz of sodium nitrite and 2.75 oz of nitrate salt per 100 lb of meat, i.e., 156 mg/kg and 1,716 mg/kg, respectively (Tanner, 1944).

Binkerd and Kolari (1975), Cervený (1980), and Sebranek (1979) have reviewed studies that indicate a slow trend over the ensuing decades toward the use of nitrite cures. However, the levels of nitrite and nitrate, which formed the basis of the early regulations (Tanner, 1944; U.S. Department of Agriculture, 1925), were reiterated in the 1970 USDA Meat Inspection Regulations (U.S. Department of Agriculture, 1970, p. 15590). These regulations permit the addition of sodium or potassium nitrite at 2 lb per 100 gallons of pickling solution, which results in an initial concentration of approximately 200 mg/kg meat, 1 oz per 100 lb of meat in dry cure (625 mg/kg), or 0.25 oz per 100 lb in chopped meat products (156 mg/kg). Sodium or potassium nitrate were permitted at 7 lb per 100 gallons of pickle (~700 mg/kg), 3.5 oz to 100 lb of meat in dry cure (~2,200 mg/kg), or 2.75 oz per 100 lb of chopped meat (~1,700 mg/kg). The use of nitrate, nitrite, or combinations could not result in a residual of more than 200 mg/kg nitrite, calculated as sodium nitrite, in the finished product.

These regulations are in force today (Code of Federal Regulations, 1981a) for all cured meats except bacon. Subsequent regulations limiting the amount of nitrite and nitrate in bacon are discussed later in this chapter.

In the early 1960s, there were several outbreaks of botulism attributed to temperature-abused,¹ smoked fish processed without the use of nitrate or nitrite in the Great Lakes region of the United States (Center for Disease Control, 1979). Subsequently, research was conducted to establish guidelines for the use of nitrite to produce safe smoked fish products.

The addition of nitrate and nitrite to fish products is regulated by the Food and Drug Administration (FDA). These regulations currently permit up to 500 mg residual sodium nitrate per kilogram or up to 200 mg residual sodium nitrite per kilogram in smoked cured sable fish, shad, and salmon as a preservative and color fixative (Code of Federal Regulations, 1981d,e); a residual of between 100 and 200 mg sodium nitrite per kilogram in hot smoked chub to inhibit outgrowth and toxin formation by C. botulinum, type E (Code of

¹Temperature abuse is exposure of a product to a temperature more favorable to the growth of microbial contaminants than is the recommended storage temperature. For example, exposure to room temperature of a product that should be refrigerated.

Federal Regulations, 1981f); up to 10 mg residual sodium nitrite per kilogram in smoked cured tuna as a color fixative (Code of Federal Regulations, 1981e); and up to 200 mg residual potassium nitrate per kilogram in cod roe as a curing agent (Code of Federal Regulations, 1981c).

TRENDS IN THE PRODUCTION OF CURED MEATS

Reviews of the trends in the production and distribution of cured meat products have been published by Binkerd and Kolari (1975), Cerveny (1980), and Sebranek (1979). Some of these trends, especially those pertaining to refrigeration, are pertinent to the safety or spoilage of cured meats.

During the first two decades of this century, mechanical refrigeration was practiced in processing plants and warehouses, but during distribution, which was largely accomplished by horsedrawn wagons, ice or ice and salt was used for cooling. Local distribution and retail display were often unaccompanied by refrigeration, and home use of ice boxes was limited. Meat curing processes relied predominantly on heavy salting or smoking for preservation without refrigeration. Thermal processing (canning) was just beginning to be used (Anonymous, 1952; Cerveny, 1980).

In the 1920s, hygiene in processing plants was improved by the adoption of solvent extraction of spices for flavorings and the use of aqueous chlorine as a sanitizing agent for food and equipment. Both served to reduce microbial contamination of products (Anonymous, 1952; Reddish, 1957; White, 1972). During this decade, motorized transportation became more widespread, but the mechanically refrigerated trucks used by ice-cream manufacturers were adopted only slowly by meat packers, most of whom continued to use ice or ice and salt for chilling. Retail outlets began to use display cases chilled by ice or ice and salt, and the use of iceboxes in private homes increased (Anonymous, 1952; Cerveny, 1980).

Between 1930 and 1940, meat product distributors began to use mechanically refrigerated delivery trucks (Fowler, 1952), and mechanical refrigerators were installed in many retail stores and homes. This increased use of refrigeration had a substantial impact on the meat product industry. Milder cures could be used since refrigeration complemented preservation by curing, and meat packers began to package processed meats in consumer-sized packages. Moreover, raw materials and finished products could be collected from and distributed to larger geographical areas. Thus, many operations expanded considerably, and the number of individual producers started to decline. Canned luncheon meats, sausages, and hams were also introduced during this period (Cerveny, 1980).

These trends continued into the 1940s along with the introduction of refrigerated self-service display cases which, after 1945, led to increased in-plant packaging of sausages and sliced meats. During the war years, certain cured products, e.g., more highly salted bacon, were developed specifically for military operations when refrigeration was not readily available (Cervený, 1980).

Shortly after 1950, oxygen-impermeable films were developed for the packaging of meat products, replacing the oxygen-permeable packaging that had been in use up to that time. The new packaging delayed color fading and spoilage of products by aerobic microorganisms. The subsequent introduction of vacuum packaging increased the expected shelf life of many perishable cured meats to 30 and up to 60 days (Cervený, 1980), when these products were properly refrigerated. Such packaging also inhibits spoilage at higher temperatures to the extent that certain products are acceptable from a sensory viewpoint after 6 or more days of storage at 20°C to 30°C (Pivnick and Bird, 1965; Pivnick *et al.*, 1967) -- temperatures at which the growth of C. botulinum is possible.

THE ORIGINS OF RECENT CONCERN ABOUT NITRATE AND NITRITE IN FOODS

Nitrite can react with nitrosatable substrates such as amines to produce potentially carcinogenic N-nitroso compounds such as N-nitrosamines² (see Chapters 4, 8, and 9). Thus, current concern over the use of nitrate and nitrite in foods stems not from the potential for acute toxic sequelae, but mostly from possible chronic, carcinogenic effects. The occurrence of nitrosation reactions has been known for more than 100 years (Hein, 1963; Ridd, 1961); however, simple and reliable methods to detect the reaction products at the levels present in foods exist only for the volatile nitrosamines, and these methods have only been developed recently (see Chapter 7).

Following indications that N-nitrosodimethylamine might be the cause of cirrhosis and other toxic effects in industrial workers, Barnes and Magee (1954) examined the toxicity of this compound in rats, dogs, rabbits, mice, and guinea pigs. Their results indicated that N-nitrosodimethylamine was hepatotoxic in the animals listed and that the lesions resembled those induced by liver carcinogens. In subsequent investigations, they demonstrated that it was carcinogenic in the rat (Magee and Barnes, 1956).

²Except where specified otherwise, the term nitrosamine is used to imply N-nitrosamines.

Since then, approximately 300 other N-nitroso compounds administered by a variety of routes have been shown to induce tumors in laboratory animals. All species tested, including primates, rats, and hamsters, were susceptible to at least one agent (Chapter 9).

Possible nitrosamine contamination of foods preserved with nitrite was first indicated in the early 1960's when outbreaks of hepatotoxicosis in mink and sheep were reported from Norway (Boehler, 1960, 1962). These outbreaks of liver toxicity were traced to N-nitrosodimethylamine arising from the addition of nitrite as a preservative to herring meal, the drying of the meal at high temperatures, and its use in feeds (Ender et al., 1964, 1967; Hansen, 1964; Koppang, 1964; Sakshaug et al., 1965).

Outbreaks of botulism caused by smoked fish in the early 1960s prompted research on processes using nitrite to inhibit C. botulinum in fish products. Concern about the possible production of nitrosamines through this use led to an investigation of their distribution in nitrite-treated fish by Fazio et al. (1971), who demonstrated that N-nitrosodimethylamine was present in products from marine species, such as salmon and shad, that had been treated with nitrate, nitrite, or both, at the levels then permitted.

Results of analyses performed in the late 1960s and early 1970s indicated that nitrosamines were present in a number of foods, including cured meats and cheeses. These studies were reviewed by Sebranek and Cassens (1973), Scanlan (1975), Crosby (1976), and Crosby and Sawyer (1976), who noted that several volatile nitrosamines occurred sporadically in the lower $\mu\text{g}/\text{kg}$ range in cured meat and fish products, but that N-nitrosopyrrolidine was found rather consistently in cooked bacon at concentrations ranging from 1 to 100 $\mu\text{g}/\text{kg}$.

While the use of nitrite in smoked fish and the implications of food contamination by preformed nitrosamines were being debated, the possibility that nitrosamines could be formed endogenously from the reaction of nitrite with amines in the human stomach was indicated by the experiments of Sander and coworkers (Sander, 1967, 1968; Sander and Seif, 1969; Sander et al., 1968) and by Sen et al. (1969). (See review by Mirvish, 1975.) The regulatory dilemma thus became more complex.

Responding to their mandate to ensure the wholesomeness of the food supply, the USDA and the FDA in 1970 formed a group to coordinate the activities of the two agencies and to define research needs in collaboration with the meat-curing industry and with academia. In 1971, concern about the use of nitrite was expressed to the House Intergovernmental Relations Subcommittee at hearings on nitrosamines (U.S. Department of Agriculture, 1975). In 1972, the USDA was petitioned by consumer representatives to ban or greatly reduce the amount of nitrite used in the curing process. The petition was

denied by the USDA, which ruled that further information was needed on the formation of nitrosamines. When the petitioners took the issue to court, the case was dismissed on procedural grounds. Thus, the denial remained in effect. In the early 1970's, the USDA, the FDA, and representatives of the meat-processing industry agreed upon and commenced research to define more precisely the need for nitrite in cured products (U.S. Department of Agriculture, 1975). This work, reported by Christiansen *et al.* (1973, 1974, 1975) and Hustad *et al.* (1973), clarified the inhibitory effect of nitrite against C. botulinum.

In Canada, Sen *et al.* (1973) demonstrated that nitrosamines, especially N-nitrosopiperidine, were formed in sausage-curing premixes containing nitrite and spices. This resulted from the reaction of nitrite with amino compounds in the spices. In the United States, rapid action to verify this finding resulted in the banning of such premixes (Code of Federal Regulations, 1981b).

In the early 1970s, reports of nitrosamine contamination of cured meats, especially cooked bacon, continued to accumulate (Gray, 1976; Scanlan, 1975; Sebranek and Cassens, 1973), and in 1973, the Secretary of Agriculture appointed an advisory Expert Panel on Nitrites and Nitrosamines. The panel was charged with the task of reviewing information concerning the presence of nitrosamines in foods, evaluating the significance to public health and specific problems associated with the use of nitrite in foods, and determining if there were alternative methods of processing.

In September 1974, the Expert Panel submitted a preliminary report on its review and evaluation of the literature and other pertinent information. In response, the USDA published proposals for regulations incorporating the following recommendations made by the panel (U.S. Department of Agriculture, 1975, p. 52614):

1. That use of nitrate salts in the curing process be discontinued in all meat and poultry products with two exceptions, dry-cured products and fermented sausage products....
2. That the level of nitrite salt permitted to be added for curing of meat and poultry be limited to 156 parts per million (ppm) in all processed products, with the exception of bacon and dry-cured products....
3. That the current permitted 200 ppm residual nitrite salt level be reduced in various product categories to reflect what is achievable with current technology. The Panel believes that 100 ppm in cooked sausage products, 125 ppm in canned cured and pickle cured products, and 50 ppm in canned cured sterile product[s] would be sufficient to maintain product safety.

Action on bacon, fermented sausage products, and dry-cured products is deferred until additional research data being developed become available.

The USDA also proposed a prohibition of the addition of nitrate and nitrite to baby foods and foods for toddlers, a maximum concentration of 125 ppm (mg/kg) for sodium nitrite added to bacon, a requirement for the addition of 500 ppm (mg/kg) sodium ascorbate or erythorbate (isoascorbate) to bacon to inhibit nitrosamine formation, and a minimum level of sodium chloride when food preservation was intended. During 1976, the USDA reviewed comments received in response to its proposals, and in 1977, with the FDA, it attempted to clarify questions pertaining to the status of nitrite as a "prior sanctioned" substance used as a preservative in poultry products and as a color fixative in red meat. Concurrently, research was being conducted to find ways to reduce the nitrosamine contamination of cured products. As techniques became more sensitive and selective, they indicated that levels of nitrosamines were highest in cooked bacon (Scanlan, 1975; U.S. Department of Agriculture, 1978b).

In October 1977, the USDA published a notice in the Federal Register asking interested parties to provide data demonstrating that bacon could be produced with low levels of nitrosamines through other processing or manufacturing procedures. The original submission date of January 16, 1978 was later extended. While the Expert Panel was deliberating, endogenous synthesis of nitrate and nitrite was reported by Tannenbaum et al. (1978). Although this paper was later challenged (Archer et al., in press; Witter et al., 1979), it brought into the debate the question of the relative contribution to exposure of humans from nitrite added to foods as compared to the total exposure from nitrite, nitrate, and nitrosamines from all sources.

In its final report, issued in February 1978, the Expert Panel recommended ingoing and residual levels of nitrite and ascorbate for a variety of products and proposed several research programs the USDA might undertake to clarify the unresolved questions (U.S. Department of Agriculture, 1978a). Acting on the panel's recommendations, the USDA's Food Safety and Quality Service in May 1978 published a final regulation on the use of nitrite in bacon (U.S. Department of Agriculture, 1978b). It specified that sodium nitrite (120 mg/kg) or potassium nitrite (148 mg/kg) be added to bacon along with sodium ascorbate or erythorbate (550 mg/kg) to inhibit nitrosamine formation. It also prohibited the addition of nitrate to bacon. In the same action, it required routine monitoring with a thermal energy analyzer to determine the nitrosamine levels in bacon at its production site. For levels exceeding 10 µg/kg after cooking, the USDA required confirmation by gas liquid chromatography and mass spectrometry and subsequent monitoring on a lot-by-lot basis until the contamination is reduced to a level lower than 10 µg/kg.

In 1980, the FDA specified that malt beverages, which had been shown in some cases to contain nitrosamines, should not contain more than 5 µg of N-nitrosodimethylamine per liter (Food and Drug Administration, 1980).

Further action on the rules proposed by the USDA in 1975 and the Expert Panel's recommendations for products other than bacon were halted in mid-1978 by results of an animal feeding study funded by the FDA, which were interpreted as indicating that nitrite per se caused cancer in rats (Newberne, 1978). The existing legislation, i.e., the Food Safety Provisions [Sec. 402(a)(2)(c)] and, for some uses of nitrite, the "Delaney Clause" [Sec. 409(c)(1)(A)] of the Food, Drug, and Cosmetic Act (U.S. Congress, 1980), required that the USDA and FDA proscribe the addition of known carcinogens to foods. Thus, the USDA and FDA made plans for banning nitrite contingent upon further evaluation of the results of the FDA-sponsored study. Subsequently, a group of pathologists established by the Universities Associated for Research and Education in Pathology (UAREP) reviewed the 50,000 histological slides from the animal feeding study and concluded that the initial interpretation was unjustified (Universities Associated for Research and Education in Pathology, 1980). As a result, the USDA and FDA took no action to ban nitrite. However, the potential of nitrite in cured meat products for contributing to the total body burden of nitrosamines remained to be determined.

Thus, in 1980, the USDA and FDA requested that the National Research Council of the National Academy of Sciences examine the current state of knowledge regarding the health effects of nitrate and nitrite in foods and the status of research on alternative curing agents. This report responds to the first part of that request. A second report of the committee will focus on alternative approaches to the current use of nitrate and nitrite.

BOTULISM

A brief review of this disease and its causative organism may be helpful in developing a perspective on the current use of nitrite and nitrate.

Clostridium botulinum is an anaerobic, gram-positive bacterium. The strains of C. botulinum are divided into seven types (A-G), on the basis of their production of antigenically specific neurotoxins (Sugiyama, 1980), and into four groups (I-IV), based on their proteolytic ability and other characteristics (Smith, 1977). Outbreaks of botulism in humans are generally caused by strains of types A, B, E, or occasionally F, in groups I and II. Botulism also occurs in other mammals, birds, and fish (Smith, 1977). The bacterial production of toxin appears to be determined by the presence of a specific bacteriophage, at least in some strains of types C and D C. botulinum (Sugiyama, 1980). Groups I and II have different minimum growth temperatures, heat resistance, and salt tolerance (Genigeorgis and Riemann, 1979).

The symptoms of foodborne botulism usually develop 18 to 36 hours after ingestion of a food in which the toxin has been produced by C. botulinum, although incubation periods can be as short as 2 hours or as long as 14 days. Typical symptoms are gastrointestinal

disturbances such as nausea, diarrhea, or vomiting, followed by neurological symptoms such as dizziness, blurred vision, respiratory impairment, and progressive muscular paralysis (Center for Disease Control, 1979; Sakaguchi, 1979).

Food is not the only source of botulism. Toxin synthesis during the multiplication of *C. botulinum* in a wound or in the gastrointestinal tract of an infant usually less than 6 months of age can also produce the disease (Center for Disease Control, 1979). The symptoms of neurotoxicity in these instances are similar to those of foodborne botulism; gastrointestinal disturbance occurs in infant botulism, but not in wound botulism. The multiplication of *C. botulinum* accompanied by toxin production in the gastrointestinal tract of adult chickens and rats has been demonstrated by Smart and Roberts (1977), Miyazaki and Sakaguchi (1978), and Sugiyama (1981). Russian investigators have suggested that this process of "toxico-infection" also occurs in humans (Minervin, 1967), but little evidence on this possibility has been collected in the United States. The probability of toxico-infection might be enhanced in humans whose normal gastrointestinal microflora has been disturbed by antibiotic treatment or surgery (Sugiyama, 1981).

The occurrence of the various types of botulism in the United States, their geographic distribution, and their causes have been well documented by the Center for Disease Control (1979). From 1899 to 1977, there were a total of 766 outbreaks of foodborne botulism involving a 1,961 cases, of which 999 were fatal. The average number of outbreaks per year from 1899 to 1949 was 9.7, and from 1950 to 1970 it was 10.3. There were 2.6 cases per outbreak during the first period, and 2.4 during the second period. The proportion of outbreaks in which the toxin type was determined has been increasing over the last few decades. From 1970 to 1977, outbreaks were most often caused by type A toxin (51%), followed by type B toxin (21%) and type E toxin (12%). The toxin type was undetermined in 16% of the outbreaks (Center for Disease Control, 1979).

Since 1950, there has been a gradual decrease in the case-fatality ratio, probably due primarily to improvements in supportive and respiratory intensive care and the prompt administration of antitoxin (Morris and Hatheway, 1980). The case-fatality ratio was significantly higher for individuals 20 years of age or older than for individuals less than 20 (Center for Disease Control, 1979). The ratios were higher for intoxications caused by type E (31%) and type A (24%) than for type B (8.8%) (Feldman et al., 1981).

The information on cases of botulism reported between 1950 and 1979 has been classified into four separate categories. During this period, 215 outbreaks (566 cases) of foodborne botulism occurred, and there were 119 outbreaks (210 cases) in which no food vehicle or wound could be identified. There were 19 cases of wound botulism. One hundred and twenty-one cases of infant botulism, the most recently recognized form of the disease, were reported from 1975 to 1979 (Feldman et al., 1981).

Of foodborne botulism outbreaks occurring between 1899 and 1977, 72% (548) have been traced to home-processed foods, and 9% (60) to commercially processed foods. In 19% of the outbreaks, the type of food processing was unknown (Center for Disease Control, 1979). From 1950 to 1979, the most commonly implicated foods were vegetables (44%), fish and fish products (16%), and condiments (14%) (Feldman et al., 1981). Beef, milk products, pork, poultry, and other vehicles caused fewer outbreaks (Center for Disease Control, 1979).

Tompkin (1980) has reviewed reported outbreaks of botulism attributed to commercially processed or home-processed meat and poultry products in the United States, Canada, and other countries. Since 1899, 15 outbreaks (39 cases, 16 deaths) of botulism in the United States and Canada were attributed to commercially processed meat and poultry products. Seven outbreaks (21 cases, 6 deaths) involved products that are normally cured; the remainder were associated with products that are not normally cured. When information was available on the history of the implicated products, it generally indicated that there had been faulty processing or temperature abuse by the retail outlet or by the consumer (Tompkin, 1980).

Because of similarities to the symptoms of other diseases, botulism may be incorrectly diagnosed as cerebral vascular accident, acute poliomyelitis, myasthenia gravis, chemical poisoning, food poisoning, or acute Guillain-Barré syndrome. Conversely, diseases such as Guillain-Barré syndrome, staphylococcal food poisoning, and carbon monoxide poisoning may be incorrectly diagnosed as botulism (Sakaguchi, 1979).

The dose of toxin causing botulism is too small to stimulate antitoxin production. Thus, it appears unlikely that immunity would develop from repeated low-dose exposures. However, resistance has been observed in certain individuals, such as those with the toxin in their circulatory systems as a result of exposure during outbreaks but with no clinical symptoms. The mechanism of this resistance is unknown (Sakaguchi, 1979).

Factors contributing to the risk of botulism are discussed in Chapters 3 and 10.

REFERENCES

- Anonymous. 1952. The "Significant Sixty." A Historical Report on the Progress and Development of the Meat Packing Industry: 1891-1951. National Provisioner, January 26, Section 2.
- Archer, M. C. In press. Hazards of nitrate, nitrite, and N-nitroso compounds and human nutrition. In J. N. Hatchcock, ed. Nutritional Toxicology, Vol. 1. Academic Press, New York.
- Barnes, J. M., and P. N. Magee. 1954. Some toxic properties of dimethylnitrosamine. Br. J. Ind. Med. 11:167-174.
- Binkerd, E. F., and O. E. Kolari. 1975. The history and use of nitrate and nitrite in the curing of meat. Food Cosmet. Toxicol. 13:655-661.
- Boehler, N. 1960. En ondartet leversykdom hos mink og rev. Nor Pelsdyrbl. 34:104-106.
- Boehler, N. 1962. Ondartet leversykdom hos pelsdyr i Norge. Pp. 774-776 in Vol. II, Proc. IX Nord. Vet. Congr., Copenhagen.
- Brock, T. D. 1961. Milestones in Microbiology. American Society for Microbiology, Washington, D.C. 273 pp.
- Brooks, J., R. B. Haines, T. Moran, and J. Pace. 1940. The function of nitrate, nitrite and bacteria in curing bacon and hams. Department of Scientific and Industrial Research. Food Investigation Board Special Report No. 49. His Majesty's Stationery, London, United Kingdom.
- Center for Disease Control. 1979. Botulism in the United States, 1879-1977. Handbook for Epidemiologists, Clinicians, and Laboratory Workers. Center for Disease Control, U.S. Department of Health, Education, and Welfare, Public Health Service, Atlanta, Georgia. 41 pp.
- Cervený, J. G. 1980. Effects of changes in the production and marketing of cured meats on the risk of botulism. Food Technol. 34:240-243.
- Christiansen, L. N., R. W. Johnston, D. A. Kautter, J. W. Howard, and W. J. Aunan. 1973. Effect of nitrite and nitrate on toxin production by Clostridium botulinum and on nitrosamine formation in perishable canned comminuted cured meats. Appl. Microbiol. 25:357-362. (Erratum 26:653).
- Christiansen, L. N., R. B. Tompkin, A. B. Shaparis, T. V. Kueper, R. W. Johnston, D. A. Kautter, and O. J. Kolari. 1974. Effect of sodium nitrite on toxin production by Clostridium botulinum in bacon. Appl. Microbiol. 27:733-737.

- Christiansen, L. N., R. B. Tompkin, A. B. Shaparis, R. W. Johnston, and D. A. Kautter. 1975. Effect of sodium nitrite and nitrate on Clostridium botulinum growth and toxin production in a Summer style sausage. J. Food Sci. 40:488-490.
- Code of Federal Regulations. 1981a. Title 9, Section 318.7. Office of the Federal Register, Washington, D.C.
- Code of Federal Regulations. 1981b. Title 21, Section 170.60. Office of the Federal Register, Washington, D.C.
- Code of Federal Regulations. 1981c. Title 21, Section 172.160. Office of the Federal Register, Washington, D.C.
- Code of Federal Regulations. 1981d. Title 21, Section 172.170. Office of the Federal Register, Washington, D.C.
- Code of Federal Regulations. 1981e. Title 21, Section 172.175. Office of the Federal Register, Washington, D.C.
- Code of Federal Regulations. 1981f. Title 21, Section 172.177. Office of the Federal Register, Washington, D.C.
- Crosby, N. T. 1976. Nitrosamines in foodstuffs. Residue Rev. 64: 77-135.
- Crosby, N. T., and R. Sawyer. 1976. Nitrosamines: A review of chemical and biological properties and their estimation in foodstuffs. Adv. Food Res. 22:1-71.
- Doran, G. F. 1917. Art of curing meats. U.S. Patent 1,212,614.
- Ender, F., G. Havre, A. Helgebostad, N. Koppang, R. Masden, and L. Ceh. 1964. Isolation and identification of a hepatotoxic factor in herring meat produced from sodium nitrite preserved herring. Naturwissenschaften 51:637-638.
- Ender, F., G. Havre, R. Masden, L. Ceh, and A. Holgebostad. 1967. Studies on conditions under which N-nitrosodimethylamine is formed in herring meat produced from nitrite-preserved herring: The risk of using nitrite uncritically as a preservative agent. Tierphysiol. Tierernaht. Futtermittelk. 22:181-189.
- Evans, F. L., and F. W. Tanner. 1934. The effect of meat curing solutions on anaerobic bacteria. IV. The effect of mixed curing solutions. Zentralbl. Bakteriol. Parasitenkd. 91:135-147.
- Fazio, T., J. N. Damico, J. W. Howard, R. H. White, and J. O. Watts. 1971. Gas chromatographic determination and mass spectrometric confirmation on N-nitrosodimethylamine in smoke-processed marine fish. J. Agr. Food Chem. 19:250-253.

- Feldman, R. A., J. G. Morris, and R. A. Pollard. 1981. Epidemiological characteristics of botulism in the United States, 1950-1979. Pp. 271-284 in G. E. Lewis, Jr., ed. Biomedical Aspects of Botulism. Proceedings of Symposium held on March 16-18, 1981, at the U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland. Academic Press, New York.
- Food and Drug Administration. 1980. Dimethylnitrosamine in malt beverages. Availability of guide. Fed. Regist. 45:39341-39342.
- Foster, E. M., and C. L. Duncan. 1974. The interaction between nitrite and the clostridia. Presented to the Expert Panel on Nitrites and Nitrosamines, April 25, 1974. U.S. Department of Agriculture, Washington, D.C. 12 pp.
- Fowler, B. B. 1952. Men, Meat and Miracles. Julian Messner, Inc., New York.
- Genigeorgis, C., and H. Riemann. 1979. Food processing and hygiene. Pp. 613-713 in Foodborne Infections and Intoxications. H. Riemann and F. L. Bryan, eds. Academic Press, New York. 748 pp.
- Gray, J. I. 1976. N-Nitrosamines and their precursors in bacon: A review. J. Milk Food Technol. 39:686-692.
- Haldane, J. 1901. The red colour of salted meat. J. Hyg., Camb. 1: 115-122.
- Hansen, M. A. 1964. An outbreak of liver toxic injury in ruminants: Clinical observations and results of some hepatic tests in cattle and sheep. Nord. Vet. Med. 16:323-342.
- Hein, G. E. 1963. The reaction of tertiary amines with nitrous acid. J. Chem. Educ. 40:181-184.
- Hoagland, R. 1910. The action of saltpeter upon color of meat. Pp. 301-314 in Bureau of Animal Industry 25th Annual Report: 1908. U.S. Department of Agriculture, Washington, D.C.
- Hustad, G. O., J. G. Cervený, H. Trenk, R. H. Deibel, D. A. Kautter, T. Fazio, R. W. Johnston, and O. E. Kolari. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in weiners. Appl. Microbiol. 26:22-26.
- Kerner, J. 1817. Vergiftung durch verdorbene Würste. Tübinger Blätter für Naturwissenschaften und Arzneikunde 3:1-25.
- Kerner, J. 1820. Neue Beobachtungen über die in Würtemberg so häufig vorkommenden tödlichen Vergiftungen durch den Genuss geräucherter Würste. C. F. Oslander, Tübingen, Germany.

- Kerner, J. 1822. Dass Fettgift über die Fettsäure und ihre Wirkungen auf den thierischen Organismus. Ein Beitrag zur Untersuchung des in verdorbenen Würsten giftig wirkenden Stoffes. Stuttgart und Tübingen, Germany.
- Kerr, R. H., C. T. Marsh, W. F. Schroeder, and E. A. Boyer. 1926. The use of sodium nitrite in the curing of meat. J. Agric. Res. 33:541-551.
- Kisskalt, K. 1899. Beiträge zur Kenntris der Ursachen des Rotwerdens de Fleisches beim Kochen nebst, einigen Versuchen über die Wirkung der Schwefligen. Säure auf der Fleischforbe. Arch. Hyg. Bakt. 35:11.
- Koppang, N. 1964. An outbreak of toxic liver injury in ruminants: Case reports, pathological-anatomical investigations and feeding experiments. Nord. Vet. Med. 16:305-322.
- Lehmann, K. B. 1899. Über das Haemorrhodin. Ein neues weit verbreitetes Blutfarbstoffderivat. Sber. Phys.-Med. Ges. Wurzb. 4:57.
- Lewis, W. L., and J. A. Moran. 1928. The present status of our knowledge of ham souring. Pp. 1-141 in Bull. No. 4, American Institute of Meat Packers, Chicago, Illinois.
- Magee, P. N., and J. M. Barnes. 1956. The production of malignant primary hepatic tumors in the rat by feeding dimethylnitrosamine. Br. J. Cancer 10:114-122.
- Minervin, S. M. 1967. On the parenteral-enteral method of administering serum in cases of botulism. Pp. 336-345 in M. Ingram and T. A. Roberts, eds. Botulism, 1966. Chapman and Hall, London, United Kingdom.
- Mirvish, S. S. 1975. Formation of N-nitroso compounds: Chemistry, kinetics, and in vivo occurrence. Toxicol. Appl. Pharmacol. 31: 325-351.
- Miyazaki, S., and G. Sakaguchi. 1978. Experimental botulism in chickens: The cecum as the site of production and absorption of botulinal toxin. Japan J. Med. Sci. Biol. 31:1-15.
- Morris, J. G., and C. L. Hatheway. 1980. Botulism in the United States, 1979. J. Infect. Dis. 142:302-305.
- Newberne, P. M. 1978. Final Report on Contract FDA 74-2181. Dietary nitrite in the rat. Food and Drug Administration, Department of Health and Human Services, Washington, D.C. 187 pp.
- Pivnick, H., and H. Bird. 1965. Toxigenesis by Clostridium botulinum types A and E in perishable cooked meats vacuum-packed in plastic pouches. Food Technol. 19:132-140.

- Pivnick, H., L. J. Rubin, H. W. Barnett, H. R. Nordin, P. A. Ferguson, and C. H. Perrin. 1967. Effect of sodium nitrite and temperature on toxigenesis by Clostridium botulinum in perishable cooked meats vacuum-packed in air impermeable plastic pouches. *Food Technol.* 21: 100-102.
- Polenske, H. 1891. Über den Verlust, welchen Rindfleisch und Nährwert durch das Pökeln erleidet, sowie über die Veränderungen salzpeterhaltiger Pökellaken. *Arbeiten aus dem Kaiserlichen Gesundheitsamt* 7:471-747.
- Reddish, G. F. 1957. *Antiseptics, Disinfectants, Fungicides, and Chemical and Physical Sterilization*, 2nd Ed. Lea and Fabiger, Philadelphia, Pennsylvania.
- Ridd, J. H. 1961. Nitrosation, diazotisation and deamination. *Quarterly Review* 15:418-441.
- Sakaguchi, G. 1979. Botulism. Pp. 389-442 in *Foodborne Infections and Intoxications*. H. Riemann and F. L. Bryan, eds. Academic Press, New York. 748 pp.
- Sakshaug, J., E. Sognen, M. A. Hansen, and N. Koppang. 1965. Dimethylnitrosamine: Its hepatotoxic effect in sheep and its occurrence in toxic batches of herring meal. *Nature* 206:1261-1262.
- Sander, J. 1967. (In German; English summary.) Kann Nitrit in der menschlichen Nahrung Ursache einer Krebsentstehung durch Nitrosaminbildung sein. *Arch. Hyg. Bakt.* 151:22-28.
- Sander, J. 1968. (In German; English summary.) Nitrosaminsynthese durch Bakterien. *Hoppe. Seylers Z. Physiol. Chem.* 349:429-432.
- Sander, J., and F. Seif. 1969. (In German; English summary.) [Bacterial reduction of nitrate in the human stomach as a cause for nitrosamine formation.] *Arzneim. Forsch.* 20:1091-1093.
- Sander, J., F. Schweinsberger, and H. P. Menz. 1968. (In German; English summary.) Untersuchungen über die Entstehung cancerogener Nitrosamine im Magen. *Hoppe. Seylers Z. Physiol. Chem.* 349: 1691-1697.
- Scanlan, R. A. 1975. N-Nitrosamines in foods. *Crit. Rev. Food Technol.* 5:357-402.
- Sebranek, J. G. 1979. Advances in the technology of nitrite use and consideration of alternatives. *Food Technol.* 33:58-62, 93.
- Sebranek, J. G., and R. G. Cassens. 1973. N-Nitrosamines: A review. *J. Milk Food Technol.* 36:76-91.

- Sen, N., D. C. Smith, and L. Schwinghamer. 1969. Formation of N-nitrosamines from secondary amines and nitrite in human and animal gastric juice. *Food Cosmet. Toxicol.* 7:301-307.
- Sen, N. P., W. F. Miles, B. Donaldson, T. Panalakas, and J. R. Iyengar. 1973. Formation of nitrosamines in a meat curing mixture. *Nature* 245:104-105.
- Smart, J. L., and T. A. Roberts. 1977. An outbreak of type C botulism in broiler chickens. *Vet. Rev.* 100:378-380.
- Smith, E. 1873. *Foods*. D. Appleton and Company, New York.
- Smith, L. DS. 1977. *Botulism: The Organism, Its Toxins, The Disease*. Charles C Thomas, Springfield, Illinois. 236 pp.
- Sofos, J. N., F. F. Busta, and C. E. Allen. 1979. Botulism control by nitrite and sorbate in cured meats: A review. *J. Food Protect.* 42:739-770.
- Steinke, P. K. W., and E. M. Foster. 1951. Botulinum toxin formation in liver sausage. *Food Res.* 16:477-484.
- Sugiyama, H. 1980. Clostridium botulinum neurotoxin. *Microbiol. Rev.* 44:419-448.
- Sugiyama, H. 1981. Production of botulinum toxin in the gut. Pp. 151-163 in G. E. Lewis, Jr., ed. *Biomedical Aspects of Botulism*. Proceedings of Symposium held on March 16-18, 1981, at the U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland. Academic Press, New York.
- Tannenbaum, S. R., D. Fett, V. R. Young, P. D. Land, and W. R. Bruce. 1978. Nitrite and nitrate are formed by endogenous synthesis in the human intestine. *Science* 200:1487-1489.
- Tanner, F. W. 1944. *The Microbiology of Foods*, 2nd Edition. Gerrard Press, Champaign, Illinois. 910 pp.
- Tarr, H. L. A. 1941. The action of nitrites on bacteria. *J. Fish. Res. Board Can.* 5:265-275.
- Tarr, H. L. A. 1942. The action of nitrites on bacteria: Further experiments. *J. Fish. Res. Board Can.* 6:74-89.
- Tarr, H. L. A. 1944. Action of nitrites on bacteria. *J. Fish. Res. Board Can.* 6:233-342.
- Tompkin, R. B. 1980. Botulism from meat and poultry products -- A historical perspective. *Food Technol.* 34:229-236, 257.

- Universities Associated for Research and Education in Pathology. 1980. Re-evaluation of the Pathology Findings of Studies on Nitrite and Cancer: Histologic Lesions in Sprague-Dawley Rats. Final Report, Department of Health and Human Services, Washington, D.C. 231 pp.
- U.S. Congress. 1980. Federal Food, Drug, and Cosmetic Act, as amended, January 1980. Pp. 1-140 in Food and Drug Administration Acts, Superintendent of Documents, U.S. Government Printing Office, Washington, D.C.
- U.S. Department of Agriculture. 1907. Federal Meat Inspection Act: Public Law 242. 34 Stat 1258 (21 USC 601 et seq.).
- U.S. Department of Agriculture. 1908. Regulations Governing the Meat Inspection of the U.S. Department of Agriculture, Order 150, Regulation 22, Dyes, Chemicals, and Preservatives (as amended, May 1st). P. 31, Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C.
- U.S. Department of Agriculture. 1922. Regulations Governing the Meat Inspection of the U.S. Department of Agriculture, Regulation 18: Section 6, Para. 2, Order 211 (issued December 2, 1922, effective November 1, 1922). Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C.
- U.S. Department of Agriculture. 1925. Service and Regulatory Announcements, November (issued December 1925), pp. 102-103. Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C.
- U.S. Department of Agriculture. 1926. Service and Regulatory Announcement, January (issued March 1926), pp. 2-3. Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C.
- U.S. Department of Agriculture, 1970. Meat Inspection Regulations: Revisions Pursuant to Wholesome Meat Act. Fed. Regist. 35: 15552-15617.
- U.S. Department of Agriculture. 1975. Nitrates, nitrites and salt: Notice of proposed rulemaking. Fed. Regist. 40:52614-52616.
- U.S. Department of Agriculture. 1978a. Final Report on Nitrites and Nitrosamines. Report to the Secretary of Agriculture by the Expert Panel on Nitrites and Nitrosamines, Food Safety and Quality Service, U.S. Department of Agriculture, Washington, D.C. 127 pp.
- U.S. Department of Agriculture. 1978b. Nitrates, nitrites and ascorbates (or isoascorbates) in bacon. Fed. Regist. 43:20992-20995.

Van Ermengen, E. 1897. Über einen neuen anäroben Bacillus und seine Beziehungen zum Botulismus. Z. Hyg. Infektionskr. 26:1-56.

White, G. C. 1972. Handbook of Chlorination. Van Nostrand Reinhold Company, New York. 744 pp.

Witter, J. P., S. J. Gatley, and E. Balish. 1979. Letter to the Editor: Nitrate and nitrite: Origin in humans. Science 205: 1335-1337.

CHAPTER 3

THE UTILITY OF NITRATE AND NITRITE ADDED TO FOODS

| | <u>Page</u> |
|---|-------------|
| THE USE OF NITRATE AND NITRITE..... | 3-5 |
| Categories of Meat Products..... | 3-6 |
| Annual Production of Cured Meat Products..... | 3-7 |
| Use of Nitrite in Fish Products..... | 3-11 |
| Use of Nitrate in Dairy Products..... | 3-11 |
| Methods of Adding Nitrite to Meat Products..... | 3-12 |
| The Fate of Nitrite in Meat..... | 3-13 |
| Postprocessing Handling of Cured Products: Factors Influencing the Requirement for Preservatives..... | 3-15 |
| MICROBIOLOGICAL EFFECTS OF NITRITE..... | 3-16 |
| Preservation..... | 3-16 |
| The Effect of Microbial Growth Patterns on Spoilage or Hazard..... | 3-19 |
| Contributions to the Control of <u>C. botulinum</u> and Other Clostridia..... | 3-21 |
| Efficacy of Nitrite as an Antibotulinal Agent..... | 3-30 |
| The Contribution of Nitrite to the Control of Microbial Pathogens Other Than <u>C. botulinum</u> in Cured Meats..... | 3-33 |
| The Contribution of Nitrite to the Control of Microbial Spoilage of Meats..... | 3-34 |
| The Mechanism of Action of Nitrite..... | 3-36 |
| Interactions Affecting the Antimicrobial Activity of Nitrite.. | 3-37 |
| Other Factors Affecting Microorganisms in Cured Products..... | 3-39 |
| Discussion: Antimicrobial Effects of Nitrite..... | 3-40 |
| ANTIOXIDANT EFFECTS OF NITRITE..... | 3-41 |
| Effects of Lipid Oxidation on Flavor..... | 3-41 |
| Chemistry of Lipid Oxidation..... | 3-42 |
| Catalysis and Mechanism of Lipid Oxidation..... | 3-43 |
| Antioxidant Role of Nitrite in Cured Meats..... | 3-44 |
| Health Implications of Lipid Oxidation..... | 3-45 |
| Discussions and Conclusions..... | 3-46 |
| EFFECTS OF NITRITE ON SENSORY PROPERTIES OF MEAT PRODUCTS: FLAVOR, COLOR, TEXTURE..... | 3-46 |
| Chemical Aspects of the Formation of Cured Meat Flavor..... | 3-46 |
| Chemical Aspects of the Formation of Cured Meat Color..... | 3-49 |

| | |
|--|-------------|
| Chemical Aspects of Cured Meat Texture..... | 3-53 |
| Sensory Evaluation of Cured Meat Flavor..... | 3-53 |
| Hedonic Evaluation of Cured Meats..... | 3-57 |
| Product-by-Product Evaluation of Nitrite and Cured Meat Flavor..... | 3-57 |
| Sensory Evaluation of Nitrite Levels Needed for Adequate Cured Color Formation..... | 3-59 |
| EFFECTS OF NITRATE IN CURED PRODUCTS..... | 3-60 |
| SUMMARY: FINDINGS AND CONCLUSIONS..... | 3-61 |
| Effects on Pathogenic and Spoilage Microorganisms..... | 3-64 |
| Effects of Nitrite on Lipid Oxidation..... | 3-65 |
| Effects of Nitrite on Sensory Characteristics..... | 3-65 |
| RECOMMENDATIONS..... | 3-66 |
| Chemistry..... | 3-66 |
| Antimicrobial Action..... | 3-66 |
| Antioxidant Effects..... | 3-67 |
| Sensory Studies..... | 3-67 |
| Use of Nitrate..... | 3-67 |
| REFERENCES..... | 3-68 |

CHAPTER 3

THE UTILITY OF NITRATE AND NITRITE ADDED TO FOODS

Nitrate, nitrite, or both are added with salt (sodium chloride) to a number of foods. This chapter focuses mostly on the utility of nitrite in commercial products of U.S. origin. If nitrate is added, it serves mainly as a reservoir from which nitrite is derived as a result of bacterial reduction. In the United States, nitrite (generally as sodium nitrite) and nitrate are used predominantly in curing red meats and poultry. To a lesser extent, both nitrate and nitrite are used in some species of smoked fish (Chapter 2). Therefore, discussions in this chapter focus on red meats and poultry, and deal only with special considerations pertaining to fish products. The addition of nitrate to some types of cheese -- as practiced in some countries, especially in Europe -- is not permitted in the United States.

Nitrate and nitrite are added to cured products for a variety of purposes. Around 1900, they were shown to be responsible for the "fixation" of color in cured meats (Chapter 2). However, only during the last two or three decades has there been any concerted or extensive attempt to define the specific contributions of nitrate and nitrite to the other sensory and antimicrobial effects of curing.

Many factors complicate evaluations of the utility of nitrite in cured products. For example, there are several reasons for using nitrite to inhibit microbial growth (such as adding nitrite to protect against some foodborne pathogens and to extend shelf life, thereby lengthening the time available for distribution), and their relative importance depends on the assessor's viewpoint. But in practice, the variation in or unpredictability of such factors as time to product consumption and time before the product is subjected to temperature abuse makes it impossible to know the extent to which nitrite must inhibit microbial growth in a product to ensure protection against pathogens or spoilage.

Nitrite is only one of many factors that interact and contribute to a product's sensory properties, such as color, texture, and flavor (i.e., taste and aroma) or its wholesomeness. For example, the composition of a product, including added spices, and smoking contribute to its flavor. Furthermore, the development of spores¹ or growth of

¹Certain microorganisms can exist as spores as well as vegetative cells. Dormant spores are resistant to adverse conditions, such as heat, which affect vegetative cells. Under favorable conditions, spores will germinate, undergo outgrowth, and produce multiplying vegetative cells.

microorganisms may also be inhibited by other components of the curing salt mixture (e.g., sodium chloride), by other characteristics of the product (e.g., pH and water activity²), or by a production process (e.g., heating). Maintenance of hygienic practices during production reduces the possibility of contamination by spores or microorganisms and may also affect the proportions of the contaminating microorganisms and their interactions in the developing microflora. Refrigeration may selectively inhibit the growth of microorganisms and reduce the rate of chemical changes, such as lipid oxidation (which is related to rancidity). The characteristics of packaging -- e.g., whether it is oxygen-permeable or impermeable -- may selectively affect the growth of microorganisms or the stability of the color produced by nitrite. Because of these complex interactions, it is difficult to isolate nitrite and assess its contribution to overall product safety or sensory characteristics.

Ideally, experimentation to determine the efficacy of nitrite would include examination of its contribution to the inhibition of pathogenic and spoilage microorganisms, chemical changes, and sensory characteristics in the framework of the variables listed above. Investigators have often focused on a specific action of nitrite in a model system or in a particular product. Caution must be used in extrapolating information from such model systems to commercial products, or from one type of product to another, because differences in conditions, such as water activity and pH, may affect the results.

This chapter briefly describes the current use of nitrate and nitrite in foods and evaluates the scientific evidence of their antimicrobial, antioxidant, and sensory effects. It includes an evaluation of the influence of nitrite and other factors, such as salt and pH, on the germination and outgrowth of Clostridium botulinum spores.

C. botulinum spores are distributed ubiquitously in the environment, e.g., in soil and dust (Smith, 1977). Thus, although it is possible to reduce contamination of carcasses, it is not practicable to produce raw meat with a guarantee that it will never contain spores. Similarly, because of the widespread distribution of C. botulinum in marine and freshwater environments (e.g., Eklund and Poysky, 1970; see also the monograph by Smith, 1977), the committee believes that it is unrealistic to assume that contamination of fish products with C. botulinum can be avoided. Surveys reviewed by Sakaguchi (1979) indicate that fish

²The water activity, a_w , of a food is defined as the ratio of the vapor pressure of water in the food (p) to that of pure water (p_0) at the same temperature, i.e., $a_w = p/p_0$. The growth of some organisms can be inhibited by lowering the water activity of a product (see p. 3-27).

are contaminated with C. botulinum more frequently than are meats (e.g., Hayes et al., 1970). If unchecked, botulinal spore outgrowth is followed by cell multiplication and may lead to the production of botulinum toxin in foods. If a food containing toxin is ingested without having been thoroughly cooked at temperatures of 80°C or higher, it may cause botulism. Chapters 2 and 10 contain additional discussion of botulism and the events in the sequence that may lead to this disease.

Economic considerations may influence decisions that have an impact on the safety and acceptability of certain products; however, the committee was not asked to assess the possible economic benefits to consumers or producers from the use of nitrite or nitrate nor was it asked to relate scientific evaluation of flavor or color to consumer demand for cured products. The necessity for the current levels of nitrite used to achieve the desired effects in various products will be discussed more fully in the second report of the committee.

THE USE OF NITRATE AND NITRITE

It is difficult to describe the current use of nitrate and nitrite in cured red meats and poultry in the United States because processing, packing, and distribution techniques are constantly changing (Cervený, 1980). Moreover, diverse procedures are used to produce and distribute the wide range of products to which nitrate and nitrite have been added. The size of meat-processing facilities and the sophistication of their quality-control procedures also vary. Many common industrial practices are not described in textbooks nor are they examined in the open literature.

Given these circumstances, any attempt at concise description of the current use of nitrite and nitrate runs the risk of oversimplifying a complex, varied, and ever-changing situation. It is especially difficult now, when new procedures are being sought to reduce residual concentrations of nitrite and, thus, the possibility of food contamination by nitrosamines resulting from the reaction of nitrite and nitrosatable substrates.

It is also difficult to generalize about product classes. Although one can define general categories into which most products will fit, some products may have certain characteristics, such as intermediate water activity, that render them difficult to categorize. The final characteristics of products (such as water activity and pH) may result from traditional manufacturing practices rather than from procedures deliberately designed to produce specific target values for these characteristics. The adoption of "least-cost" formulations may mean that the composition of a product can vary, even over relatively short periods. For example, chicken meat is increasingly being incorporated into traditionally red-meat products as a source

of protein. Raw materials and methods of production, such as fermentation, may also vary among batches. Unfortunately, specific information on these variations is often not available.

The following description of the use of nitrite and nitrate in U.S. cured meats and poultry should therefore be regarded as general; in practice, there is much variation in the way the compounds are used. The reader is cautioned that processing procedures for some types of product also vary among countries. For example, English (Wiltshire) bacon, Canadian bacon, and U.S. bacon are taken from different cuts of meat and are processed differently.

Categories of Meat Products

Meat products can be divided into categories based on the extent to which they are heated (if at all) during production, on whether they are cured, and on their water activity. They may also be subdivided into comminuted and noncomminuted (primal) products. The general categories of meat products are briefly described below to facilitate discussion of the role of nitrite in the product and of product susceptibility to effects of microorganisms, which can cause spoilage of the product or make it hazardous to health. The term "processing" is used to indicate manipulation, such as curing or comminution. "Thermal processing" encompasses heating processes that control *C. botulinum*, but excludes milder heating, such as pasteurization and smoking. "Perishable" products are those that require refrigeration. They may be either raw or pasteurized.

Raw, Uncured Products. This category includes whole or comminuted fresh meats distributed in the uncured state -- products that are not examined in this report.

Raw, Cured Products with High Water Activity (i.e., > 0.92). This category includes raw corned beef packaged with free pickling solution (Price and Schweigert, 1971, p. 465). Other raw, cured products, such as bacon, are subjected to some smoking and mild heating during production (Kramlich et al., 1973, p. 228).

Raw, Cured Products with Low Water Activity (< 0.92). Scotch, prosciutto, Westphalian, and country hams; dry-cured bacon; and dried sausages may be cold-smoked and not heated appreciably during processing. Other products in this category, such as some sausages, may be mildly heated if smoked (Kramlich et al., 1973, p. 228; Nitrite Safety Council, 1980). These products are sold as raw, cured products with a low water activity due to drying and the addition of salt. Many dried meat products such as dried beef, jerky, dry-cured bacon, dry-cured ham, and many dried sausages are produced with added nitrite. Many of these products depend on relatively high salt content and, consequently, low water activity for preservation at room temperature (Komarik et al., 1974).

Cooked, Uncured Products. This category includes some meat loaves, some loaves containing meat and other ingredients, poultry rolls, bratwurst, and ring liver sausage (Kramlich *et al.*, 1973, p. 98). During processing, most of these products are subjected to temperatures of 65°C or higher in order to pasteurize them. They are not cooked sufficiently to destroy C. botulinum spores.

Cooked, Cured Products. This is by far the largest category of products processed with added nitrite (Price and Schweigert, 1971, p. 485). Pasteurized products, heated to 65-75°C center temperature, include hams in casings and in cans, frankfurters, bologna, liver sausage, meat loaves, some loaves containing meat and other ingredients, and some roll products. Bacon is classified as a raw, cured product because it is generally not heated sufficiently for pasteurization, but its microbial profile more closely resembles that of cooked, cured products. A second class of cooked, cured products includes the so-called "shelf-stable" products, such as canned meats and prefried canned bacon (Cervený, 1980). These products are normally heated to a center temperature of 95-112°C. This alone is not sufficient to kill all spores of C. botulinum or other organisms, but in conjunction with other factors, such as the presence of nitrite, heating to this extent can delay spore outgrowth. Many of these products contain not only meat but also many other ingredients. Other products in this class are luncheon meat, hams, and pork shoulders.

There are only a few "commercially sterile" cured products. Among these are corned beef hash, deviled ham, meat spreads, and Vienna sausages. These "commercially sterile" products are defined as products free of pathogens as well as of microorganisms capable of growing under normal nonrefrigerated storage conditions. They are thermally processed to the extent that the slowest heating portion of the can receives a treatment that is at least as destructive to the most resistant C. botulinum spore as treatment at 2.78 min at 121°C (page 3-22).

Annual Production of Cured Meat Products

The major cured meat products to which nitrite is added and the general methods of addition are shown in Table 3-1. As the table illustrates, nearly 4 billion kilograms of these products were processed with added nitrite in the United States during 1979. Table 3-2 lists the amounts of meat products processed without added nitrite. Although the use of nitrate in products has been declining (Binkerd and Kolari, 1975; Cervený, 1980; Sofos and Busta, 1980), it is still added to a variety of products (Table 3-3). The committee believes that the use of nitrate in U. S. products other than fermented sausages and dry-cured cuts is normally limited to small-scale producers.

TABLE 3-1

Meat Products Processed with Added Nitrite Under Federal
Inspection in the United States During 1979^a

| <u>Product</u> | <u>U.S. Production, billions of kilograms</u> | <u>Sodium or Potassium Nitrite Added,^b mg/kg</u> | <u>Most Probable Method of Adding Nitrite</u> |
|---|---|---|---|
| Cured beef | 0.13 | 200 | Multineedle injection |
| Ham, not canned | 0.83 | 200 ^c | Multineedle injection |
| Bacon | 0.76 | 120 | Multineedle injection |
| Sausage: | | | |
| Semidry and dry | 0.15 | 156 | Direct addition as cure salt |
| Frankfurters | 0.68 | 156 | Direct addition as cure salt |
| Bologna | 0.37 | 156 | Direct addition as cure salt |
| Loaves, cured meat | 0.05 | 156 | Direct addition as cure salt |
| Loaves, mixed meat and nonmeat ingredients | 0.09 | 156 | Direct addition as cure salt |
| Liver | 0.06 | 156 | Direct addition as cure salt |
| Other cooked items | 0.38 | 156 | Direct addition as cure salt |
| Canned Meats: | | | |
| Hams | 0.13 | 200 | Multineedle injection |
| Luncheon meats | 0.13 | 156 | Direct addition as cure salt |
| Hash | 0.04 | 156 | Direct addition as cure salt |
| Vienna sausage | 0.05 | 156 | Direct addition as cure salt |
| Miscellaneous | 0.05 | 156 | Direct addition as cure salt |

^aData from American Meat Institute, 1980, and U.S. Department of Agriculture, 1980.

These numbers are subject to adjustment for the weight of nonmeat ingredients, weight loss during cooking, and production in other than federally inspected plants.

^bAmount of nitrite added to meat products is based on amount of meat in the formulation. Thus, as extenders and other nonmeat ingredients are increased, nitrite added to total product is decreased. Most commonly, sodium nitrite is the nitrite salt used.

^cThis degree of nitrite addition does not apply to country style or dry-cured hams (see text and Chapter 2).

TABLE 3-2

Meat Products Processed without Added Nitrite Under Federal
Inspection in the United States During 1979^a

| <u>Product</u> | <u>U.S. Production, billions of kilograms</u> |
|---------------------------------------|---|
| Beef, cooked | 0.11 |
| Steaks and chops (chopped and formed) | 0.13 |
| Meat patties | 0.21 |
| Hamburger, ground beef | 1.29 |
| Other meat products | 0.42 |
| Pizza | 0.21 |
| Pies | 0.08 |
| Dinners | 0.12 |
| Entrees | 0.17 |
| Other products containing meat | 0.13 |
| Sausage: | |
| Fresh beef | 0.01 |
| Fresh pork | 0.37 |
| Other fresh sausage | 0.10 |
| Canned: | |
| Chile con carne | 0.15 |
| Meat stew | 0.07 |
| Pasta meat products | 0.20 |
| Other | 0.23 |

^aData from American Meat Institute, 1980, and U.S. Department of Agriculture, 1980. These numbers are subject to adjustment for weight of nonmeat ingredients, weight loss during cooking, and production in other than federally inspected plants.

TABLE 3-3

PORK

Bacon (brown-and-serve, Canadian-style, country-cured, country-style, jowl, pancetta rolled, pork shoulder); ears; fatback; hocks; jowls; loin; roll; shoulder butt; shoulder picnic; hams (canned, chopped, chopped loaf, boneless, country-style, cured, prosciutto, roll, semi-boneless, shanks, sliced, sliced boneless, Westphalian); smoked, chopped; spareribs

POULTRY

Cured parts, carcasses, products; sandwiched; smoked; uncooked and stuffed; cured turkey ham

SAUSAGES

Cooked; cooked and smoked; dry; for pizza; Italian (cooked); Italian (cooked, cured); liver; New England brand; Polish (bratwurst); pork; semidry; smoked; smoked, country; summer; summer (cooked); summer (dry)

BEEF

Bologna (beef, garlic, Lebanon); breakfast beef; corned beef; corned beef brisket; corned beef hash; creamed, chipped; cured; frankfurters; in brine; jerky, meat bar; patties; roll, cooked, corned; smoked; sticks; tenderloin steaks; tongue, cured and smoked

SALAMI

Cooked; cotto; dry, hard; Genoa; German (dry); Italian (dry, hard)

OTHER PRODUCTS

Beerwurst; braunschweiger; bratwurst; cannelloni or tortellini; cappicola; cervelat; chorizo, dry; freizzes; galentini; head-cheese; sailcicca with cheese; Holsteinen; kielbasa; knockwurst; landjager; linguisa; cured meat loaves (beef, ham and cheese, minced, olive, pepper, pepperoni, pickle and pimento); longaniza; meats (e.g., poultry in sauces, and soy protein product, casseroles, cured mixes, cured patties, dehydrated, dressing with meat and poultry, in a blanket, luncheon, macaroni with meat, meat and gravy, omelets with meat or poultry or other components, or poultry, vegetables in gravy, pickled, salads, soup with meat, spreads); Milano; mortadella (cooked); pastels; pastrami; special items (burritos, corn dogs, crepes, enchiladas, hors d'oeuvres, jellied products, lima beans smoked--pork, ham, bacon in sauce, pate, pizza, quiche product, sauerkraut products, veal cordon bleu)

^aProducts were identified from labels, approved by the U.S. Department of Agriculture from 1979 to 1981, on which nitrate was listed as an ingredient. Data from U.S. Department of Agriculture, 1981, personal communication.

Canadian manufacturing and handling practices are generally similar to those in the United States. Also, the climate in certain regions of those countries is nearly identical. However, the use of nitrate in Canadian meat products has been forbidden, with some exceptions, for several years (Health and Welfare Canada, 1975).

Use of Nitrite in Fish Products

Total production of commercially smoked fish products in the United States in 1979 was 9,564,900 kg (National Oceanic and Atmospheric Administration, 1980). Products in which nitrite was allowed constituted 5,750,000 kg of this total. Thus, in comparison with the volume of cured red meats and poultry, the amount of fish in which nitrite was used was relatively small. On the basis of a total population of 218 million, the 1979 consumption of smoked fish products was 43.9 g per person. For products in which nitrite is allowed, the per capita consumption was no more than 26 g. Despite the relative productions of smoked fish and cured meat products in the United States, there have been more recent outbreaks of botulism attributed to the former (Center for Disease Control, 1979).

Use of Nitrate in Dairy Products

Cheese of the Gouda and Edam types, as produced in some European countries, are very susceptible to late blowing (swelling) as a result of the proliferation of clostridia (Galesloot, 1961, 1964; Gray et al., 1979). One of the most successful methods of preventing late blowing of cheese is the addition of potassium or sodium nitrate (Galesloot et al., 1975). The addition of nitrate has been shown to inhibit the development of clostridia shortly after the cheese is immersed in brine when the water activity of the cheese is high. During this period, the low salt content permits the most active spores to germinate. The germinating spores are very susceptible to nitrite, which is produced from the added nitrate primarily by action of the milk enzyme, xanthine oxidase (Galesloot, 1961). So few spores remain that their germination can be controlled by the salt concentration, which gradually increases as the brine penetrates the cheese.

Dutch cheesemakers are permitted to add 15 g of sodium nitrate per 100 liters of milk. In Canada, a recent change in regulations permits the addition of 20 g of sodium nitrate per 100 liters of milk in the manufacture of some cheeses. The nitrate contents of different varieties of cheeses are well documented in the literature (Brathen and Svensen, 1973; McKay, 1974; Rammel and Joerin, 1972); however, there have been few reports related to the course of nitrate degradation and formation of nitrite during ripening of these cheeses. Goodhead et al. (1976) studied the fate of nitrate in Gouda cheese prepared from milk containing sodium nitrate at 15 g/100 liters of milk and reported that the nitrate ion content of the cheese decreased from 56 mg/kg immediately after preparation to approximately 30 mg/kg

after 6 weeks. After this period, only a slight further decrease was observed. The nitrite ion content of these cheeses was very low -- a maximum of approximately 1 mg/kg after 2 to 3 weeks. Even cheese prepared from milk containing sodium nitrate at 60 g sodium nitrate per 100 liters contained nitrite ion at only 1.5 mg/kg when analyzed 14 weeks after manufacture.

The practice of adding nitrate to cheese milk has been criticized for constituting a health hazard on the grounds that it may lead to the formation of nitrosamines. Most data indicate that the concentrations of nitrosamines in cheeses made with added nitrate range from 1 to 5 $\mu\text{g}/\text{kg}$. However, nitrosamines are also found in cheeses without added nitrate (Gray et al., 1979). In only a few studies have the concentrations exceeded 10 $\mu\text{g}/\text{kg}$. In those cases, the methods of analysis were not specific, nor were they sensitive enough for the purpose (Cantafora et al., 1974; Cerutti et al., 1975).

The potential that exists in Europe for clostridial spoilage of cheese does not appear to constitute a major problem for products made in the United States, where the addition of nitrate (or nitrite) to cheese is not permitted. However, the nitrate added to imported cheese is relevant to the intake of nitrite and nitrate by the U.S. population (Gray et al., 1979). This is discussed in Chapter 5.

Methods of Adding Nitrite to Meat Products

Nitrite is added to meat products either as a nitrite salt, usually sodium nitrite, in a nitrite-containing curing salt mixture, or in a solution of nitrite and other ingredients, which is referred to as "pickle." The method of addition may affect the uniformity of the distribution of nitrite and, thus, the minimal concentration needed to achieve consistent results, e.g., in color development.

For most intact or "primal" (chunk) products that are made from portions of meat that weigh from 100 to 200 g or more, the pickling solution containing nitrite is injected into the product. Multineedle injectors are most commonly used for boneless products and sometimes for bone-in products (Kramlich et al., 1973, p. 58). In general, according to good manufacturing practices, the pickling solution is made up daily (Komarik et al., 1974, p. 2). It typically contains water, salt, sugar, phosphate, ascorbate (or isoascorbate), and nitrite (Kramlich et al., 1973, p. 40) and is produced by using a sodium chloride carrier that contains approximately 6.25% sodium nitrite or by adding sodium nitrite to the solution to be used for the pickle. It is often advantageous for the processor to use sodium nitrite, if quality control is adequately supervised. By using sodium nitrite, the processor can avoid paying a considerable premium for the sodium chloride included in an amalgam of sodium nitrite and common salt (Price and Schweigert, 1971, p. 459).

With properly adjusted multineedle injection, it is possible to obtain a reasonably uniform distribution of the nitrite-containing pickle in the processed product. Some boneless hams and cured beef products are subjected to mechanical treatment -- tumbling or massaging -- after injection of the pickling solution in order to distribute the solution more uniformly and to enhance protein functionality in the processed product, thereby promoting product uniformity and tenderness and facilitating slicing and other portioning of the product.

For comminuted products, the nitrite or nitrite-containing curing salt is generally added directly during blending. Often, the meat is ground and blended with salt, nitrite, or curing salt and water. This process results in a very uniform distribution of the nitrite. During the manufacture of sausage, the meat is ground again and blended or chopped to the desired consistency. The coarsely comminuted products are used for dry and semidry sausages and for some loaf products. Very finely comminuted emulsions are used for a variety of skinless frankfurters, bologna, and loaves.

Nitrite or nitrate (or both) may be applied to some products as part of a dry rub (Kramlich et al., 1973, p. 52; Price and Schweigert, 1971, p. 463). This rub usually contains salt, sugar, and nitrite or nitrate (or both), which are blended and then mechanically or manually applied to hams, pork bellies, or beef. Much time is needed for the diffusion of the cure throughout the product and its subsequent drying. In the United States, at least 45 million kilograms of such products are produced each year. Dry-cured products are often traditionally consumed by populations in specific geographic areas, where they are usually manufactured by small- and medium-sized processors.

The Fate of Nitrite in Meat

Typical fresh muscle consists of approximately 70% water, 20% protein, 9% fat, and 1% analyzable ash. The composition may vary widely, depending on the particular muscle or cut of meat, the species, the nutritional state of the animal, and other factors.

From a structural viewpoint, meat consists primarily of myofiber, but it also contains fat cells, fibroblasts, endothelial cells, and neural cells. All cells are held in place by extensive connective tissue. In living muscle, the components are compartmentalized by the structure, but postmortem conversions may allow more freedom (not necessarily unrestricted) for the movement of chemicals. The presence of fat cells in meat entails lipid-polar interfaces that may facilitate certain chemical reactions (Cassens et al., 1979b).

Because of the interest in the fate of nitrite in cured meat, "bookkeeping" or "balance" experiments have been attempted to determine, at least generally, how much of the nitrite remains in the meat and how it is distributed. As long ago as 1940, there was interest in the decomposition or loss of nitrite, especially as a result of heating. Greenwood (1940) suggested that the aliphatic diazo reaction could be important in the loss upon heating.

Nitrogen has been selected as the marker in such experiments. Two problems have impeded progress. First, radioisotopes of nitrogen have such a short half-life that they cannot be used, so the stable nitrogen-15 has been used. Second, meat is an extremely complex system and, because many kinds of techniques are used for processing cured meat, simple all-encompassing answers have been difficult to obtain.

Two groups of investigators have expended considerable effort using [^{15}N]-nitrite in attempts to learn about its fate. Japanese workers have published a series of papers on the topic. Using a model system of myoglobin, nitrite, and ascorbate, Fujimaki *et al.* (1975) recovered all [^{15}N]-labeled nitrite-nitrogen added to the system in the forms of residual nitrite, nitrate, the nitrosyl group of denatured nitrosomyoglobin, and gaseous nitrogen. However, when they used a meat system, recovery fell to a range of 66% to 90% (Emi-Miwa *et al.*, 1976). When sodium ascorbate was added to the system, recovery was even lower. The authors pointed out that much of the nitrogen-15 was found in the water-soluble and salt-soluble protein fractions and that a substantial amount was converted to gaseous forms.

Sebranek *et al.* (1973) reported results of experiments in which they traced nitrogen-15 from $\text{Na}^{15}\text{NO}_2$ added to commercially cured products. They concluded that the added nitrite was changed rapidly to other compounds when it was added to meat and that little of it escaped from the product in volatile form. After processing, the change continued, but at a slow rate, until the concentration of residual nitrite was low. Subsequent studies were conducted by the same group to determine the percent of added label (from $\text{Na}^{15}\text{NO}_2$) that can be recovered in a given portion of meat shortly after processing (Goutefongea *et al.*, 1977; Woolford and Cassens, 1977; Woolford *et al.*, 1976). The results were: with myoglobin, 5-15%; as nitrite, 5-20%; as nitrate, 1-10%; as gas, 1-5%; with sulfhydryl groups, 5-15%; with lipid, 1-5%; and with non-heme protein, 20-30% (Cassens *et al.*, 1977). It is not known if these percentages change as the time after processing increases. Cassens *et al.* (1979a) have recently reviewed what is known about the reactions of nitrite in meat and some of the issues yet to be clarified.

On the general subject of the fate of nitrite, two other studies should not be overlooked. Frouin (1977) contended that most of the

added nitrite in cured meat remains as bound nitric oxide and that many compounds can fix nitric oxide, thereby greatly increasing its solubility. Walters et al. (1979) reported that nitric oxide can react with unsaturated fatty acids. They suggested that pseudonitrosites formed across the two double bonds in palmitodiolein. When the latter compound was heated with morpholine in lipid solvent, nitrosation of the secondary amine occurred. The authors suggested that a similar mechanism may lead to the formation of N-nitrosopyrrolidine in bacon.

When nitrite is added to meat immediately after processing, only about 50% is detectable as residual nitrite by the usual analytic methods. The chemical forms of this residual nitrite detected by those methods are not known, nor are the forms and reactivity of the added nitrite that is not detectable as residual, including the portion of the added nitrite that could not be accounted for in some balance studies. These issues are discussed in Chapter 5. The measured residual nitrite undergoes depletion as the product is stored. The depletion is faster at abuse temperatures -- e.g., room temperature -- than when refrigerated (Nordin, 1969) and in the presence of reductants (Fox and Nicholas, 1974). The importance of added and residual nitrite in the inhibition of C. botulinum is discussed below.

Postprocessing Handling of Cured Products: Factors Influencing the Requirement for Preservatives

Cured meat products are often distributed through complex distribution chains. A product from a national packer may have to pass through the packer's warehouse, a broker, a retail-chain warehouse, and a retail store. Cooked, cured, vacuum-packed, and refrigerated meat products must have a 30- to 60-day shelf life. In Europe, the distribution chain may be located in a relatively small geographic area, so the shelf life may not need to be that long. This may also apply in the United States, if local and regional processors are preparing products that will be consumed within 2 weeks.

Foods are frozen and refrigerated more extensively in the United States than elsewhere. In this country, most raw, uncured meats or meat products are distributed or stored either refrigerated or frozen, and many raw or pasteurized cured products are also refrigerated. Meats that are cured with nitrite and salt are generally not frozen because freezing longer than several months would result in the development of a rancid flavor (Kramlich et al., 1973). However, some cured meats may be stored frozen, e.g., by the Armed Forces, for limited periods. Consumers may buy large quantities of cured meat products and keep them in home freezers for long periods. Freezing increases the shelf life of a product.

The possibility always exists that a product may be subjected to temperature abuse. This can occur in the production plant (e.g., on the loading dock), in transit during distribution, in a warehouse, in a retail store (e.g., at the top of a display case), or in the possession of the consumers (e.g., in a car trunk or in an insufficiently cold refrigerator).

The time between production and consumption of nitrite-cured products may vary tremendously. Some pasteurized canned hams might not be consumed earlier than a year after manufacture. During this time, the residual nitrite will decline considerably, possibly to levels too low to be detected. Some products with added nitrite may be consumed shortly after they are removed from the heat-processing equipment in the manufacturer's plant. Thus, the time to consumption of cured products, which is not subject to regulation, may vary from less than 1 day to more than 1 year after production. Because of this great variation in the time to consumption and the unpredictability of the timing and duration of abuse, it is not possible to specify the degree to which a preservative should inhibit microbial growth to ensure product safety.

MICROBIOLOGICAL EFFECTS OF NITRITE

Preservation

The method used to preserve a meat product frequently determines the potential for microbially induced spoilage or hazard to human health (International Commission on Microbiological Specifications for Foods, 1980, pp. 333-409). Raw meats stored under chilled conditions exhibit spoilage patterns that are fundamentally different from those of frozen or dried meat products. Raw cured meat products support growth of various microorganisms, but the water activity largely determines the flora that develops. In heated products, the potential for microbial proliferation is determined by a complex interaction involving the degree of heating, the presence of curing salts, and the characteristics of the product.

Meat Spoilage. Table 3-4 lists some of the effects of microbial activity observed in red meat products in temperate climates. These spoilage patterns illustrate that the preservation method used determines the type of microbial spoilage that occurs.

Sources of Contamination. Meat products may be contaminated any time from the moment of slaughter through all processing and handling procedures. Equipment, personnel, additives, and other environmental contacts serve as possible reservoirs of contamination. There are also numerous opportunities for contamination by microorganisms from the intestinal tract, the lymphatic system, and the outer surfaces of the animals (Johnston and Elliot, 1976). The potentially hazardous organisms include those that cause foodborne infections and foodborne

TABLE 3-4

Microbial Defects of Red Meats and Their Products^a

| <u>Product Category</u> | <u>Description of Defect</u> | <u>Microorganism</u> |
|---------------------------------|-----------------------------------|---|
| <u>Fresh Meat</u> | | |
| Fresh, refrigerated (0°-5°C) | Off-odor, slime, discoloration | <u>Pseudomonas</u> , <u>Aeromonas</u> <u>Alcaligenes</u> , <u>Acinetobacter</u> , <u>Microbacterium</u> , <u>Moraxella</u> , <u>Proteus</u> , <u>Flavobacterium</u> , <u>Alteromonas</u> , <u>Saccharomyces</u> |
| | Lipolysis, pungent odor | <u>Pseudomonas</u> , yeasts |
| | Moldy | <u>Penicillium</u> |
| | Whiskery | <u>Thamnidium</u> |
| | Black spot White spot | <u>Cladosporium</u> <u>Sporotrichum</u> |
| Fresh (15°-40°C) | Bone taint | <u>Clostridium</u> |
| | Gassy | <u>C. perfringens</u> |
| | Foul odor | <u>C. bifermentans</u> , <u>C. histolyticum</u> , <u>C. sporogenes</u> |
| Vacuum packed | Acid, sweet, rancid | <u>Lactobacillus</u> , <u>Microbacterium</u> , <u>Enterobacter</u> , <u>Hafnia</u> |
| <u>Cured Meat:</u> | | |
| Bacon | Cheesy, sour, rancid | <u>Micrococcus</u> |
| | Discoloration | Molds |
| | Slight souring | <u>Lactobacillus</u> , <u>Micrococcus</u> , <u>Vibrio</u> , <u>Alcaligenes</u> , <u>Corynebacterium</u> |
| | Putrefaction | <u>Clostridium sporogenes</u> |
| Vacuum packed | Cabbage odor | <u>Proteus inconstans</u> |
| | Tainted | <u>Vibrio</u> |
| Brines | Turbid | <u>Debaryomyces</u> , <u>Kloeckera</u> |
| Ham | Surface slime | <u>Micrococcus</u> , <u>Microbacterium</u> , Yeasts |
| | Gassy or puffy | <u>Clostridium</u> |
| | Green discoloration | <u>Lactobacillus</u> , <u>Streptococcus</u> , <u>Leuconostoc</u> |
| | Bone and meat "sours" | <u>Clostridium</u> |
| | Surface | Molds |

(Table continued on next page)

TABLE 3-4 Continued

| <u>Product Category</u> | <u>Description of Defect</u> | <u>Microorganism</u> |
|----------------------------|--|---|
| <u>Cured Meat (Cont.):</u> | | |
| Sausages | Slime on surface Gas production (vacuum packed) Greenish discoloration | <u>Micrococcus</u> , yeasts <u>Lactobacillus</u> <u>Lactobacillus viridescens</u> , <u>Leuconostoc</u> |
| Fermented sausages | Slime Spots | Yeasts Molds |
| <u>Canned Meat:</u> | | |
| Commercially sterile | Gas, putrefaction | Spore-formers (e.g., <u>Bacillus</u> , <u>Clostridium</u>) |
| Semipreserved | Souring, discoloration Putrefaction, gas | <u>Streptococcus</u> <u>Bacillus</u> , <u>Clostridium</u> |

^aAdapted from Banwart, 1979, pp. 430-431.

intoxications as well as disease-producing microorganisms for which the meat serves solely as a vector. After processing, distribution to retail outlets, and subsequent sale to the consumer, microbial contamination may occur during storage, food preparation, and handling immediately before consumption. The source(s) of contamination frequently determine the range of microbial contaminants present and, thus, the potential spoilage or hazard.

Potential Health Hazards. Pathogenic microorganisms or the toxins they produce, or both, are potential hazards associated with meat products. The frequency with which meat products are implicated in foodborne illnesses has been reported in annual summaries produced by the Centers for Disease Control (1981). Bryan (1980) has also summarized and analyzed surveillance data on reported outbreaks of foodborne illnesses specifically associated with meat and poultry. These data indicate that commercially produced meats and meat products have the potential for producing infections and intoxications in consumers, although they are not considered to be extremely hazardous.

Some products are subjected to heating, e.g., pasteurization, which does not kill all pathogens. Subsequent inappropriate storage or handling of these products could result in foodborne illnesses. Recontamination, e.g., by salmonellae, coupled with mishandling can also lead to a foodborne infection, e.g., salmonellosis (Bryan, 1980).

Certain conditions can favor the survival and growth of anaerobic spore-formers. These conditions include the elimination of competition by the killing of vegetative cells, especially those of non-spore-formers, which can occur when products are thermally processed. In these circumstances, the most serious potential hazard might arise from C. botulinum, the toxin from which may cause botulism. However, C. perfringens, Bacillus cereus, and other spore-formers may also pose a hazard. In some situations, when competition from other organisms is minimized, contamination by Staphylococcus aureus followed by mishandling could permit the production of a heat-resistant enterotoxin that could result in a foodborne intoxication. Even if products are resistant to most bacterial invasions, mycotoxins could be produced during mold growth, and, consequently, mycotoxicosis could be a threat to the consumer. One could speculate that gastroenteritis might be caused by Campylobacter fetus subsp. jejuni, Yersinia enterocolitica, enteropathogenic Escherichia coli, and similar pathogens if these organisms contaminate meat and if the conditions are favorable for infection of the consumer. Red meats have not yet been implicated as a cause of such illness; however, poultry may be responsible for some outbreaks of illnesses caused by C. fetus (Bryan, 1979, p. 265.) Curing procedures that effectively control some of these hazardous microorganisms are discussed below and are listed in summary Table 3-7 at the end of this chapter.

The Effect of Microbial Growth Patterns on Spoilage or Hazard

Microbial growth patterns must be understood in order to evaluate food preservation methods and to assess potential health hazards. Most microorganisms in meats multiply by binary fission, i.e., they propagate by dividing into two daughter cells. Some microorganisms, such as yeasts and molds, multiply by budding daughter cells or by forming fruiting bodies with multiple propagules; however, these differences do not significantly modify the pattern of overall population growth.

Characteristic growth patterns are shown in Figure 3-1. An increase in cell numbers is usually preceded by a lag phase during which the microorganisms adjust to their new environment and prepare to divide. If the microorganism is initially present in its spore form, a germination step occurs during the lag phase. However, some spores may remain dormant for long periods. After the lag phase, the cells divide at a uniform rate. This is called the exponential

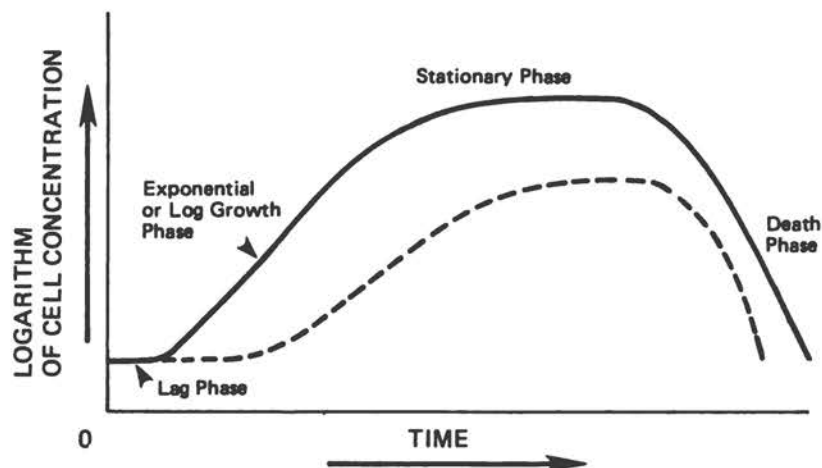


FIGURE 3-1. Microbial growth patterns. Solid line represents the hypothetical growth under ideal conditions. The broken line represents the hypothetical growth under unfavorable conditions.

or logarithmic phase. The growth rate and length of the lag phase are dependent upon many factors. When conditions are not optimal for growth (e.g., reduced or elevated temperatures, dehydration, inadequate nutrients, and the presence of chemical inhibitors of growth such as antibiotics, nitrite, etc.), the lag phase may be extended for lengths of time corresponding to the severity of the antagonistic environment. If growth commences under these unfavorable conditions, the growth rate during the exponential phase may be slower or the population increase smaller. Antagonistic conditions may also increase death rates in the final phase. These differences in growth patterns are illustrated in Figure 3-1.

The initial concentration of contaminants influences the length of time needed for the development of detectable or undesirably large populations. Large populations of specific microorganisms are required to induce spoilage of products. Infection of a host requires a minimum population of pathogenic microorganisms that can establish and colonize a specific organ or tissue. The size of this minimum population varies among pathogens. Response to a microbially produced toxin is dose dependent, and the concentration of the toxin is often directly related to the number of cells capable of producing it. Sometimes, these toxins are not produced until late in the growth phase of a microbial population. Successful competition of a specific microorganism depends upon its ability to outgrow the competitive flora by having a shorter lag phase or a more rapid exponential growth rate, or both.

Lag phases can be so short that they are experimentally undetectable; they can range from a few minutes to several days. During the exponential phases of growth, populations of some species can double in 8 minutes under ideal conditions or the process may take weeks under suboptimal conditions. Initial population levels can be as low as one cell per kilogram of product or as high as levels approaching those observed in the stationary phase, which frequently range from 10^6 to 10^{10} per gram of growth substrate.

In meat products, unless some effective or severe processing procedure selects for one kind of microorganism, a mixed flora usually predominates. This means that several species and strains of microorganisms may be competing for domination of the meat product. However, "starter" cultures are added to some products to establish a desired flora rapidly.

It is possible to have one or more of a variety of cultural interactions ranging from symbiosis to neutrality to synnecrosis. Consequently, there may be spoilage microorganisms that inhibit growth of pathogens, there may be lactic acid starter cultures that are effective protective agents, or there may be no competition permitting pathogens to proliferate.

Factors Affecting Growth. Many factors influence the growth of microorganisms, and various species of microorganisms have dramatically different nutritional requirements. The composition of the food or meat product determines the substrates available for growth. Temperature is critical in the regulation of growth rates and survival. Physical factors, such as the water activity (a_w), the pH, the oxidation-reduction (redox) potential (E_h), and the gaseous environment also have a substantial influence on growth. Closely linked to these factors are the species of solutes (e.g., acids, curing adjuncts, salts, and sugars) and the antimicrobial agents (both added preservatives and naturally occurring inhibitors) that are present in the microenvironment immediately surrounding the cells. Finally, the effects of processing procedures and the interactions of these many factors must be considered when examining the growth of microorganisms.

Contributions to the Control of *C. botulinum* and Other Clostridia

Many moderate heat-processing treatments or other factors that stress cells will inactivate most vegetative bacterial cells and eliminate competition, but allow viable spores, including those of *C. botulinum*, to remain in the meat system. Frequently, these treatments will also activate spores for subsequent initiation of germination, which, under adequate conditions, will lead to outgrowth, cellular replication, and, ultimately, toxin production, in the case of *C. botulinum*. Thus, because of its potential to cause botulism,

Clostridium botulinum in cured meat products has become the microorganism of key concern. In the laboratory, C. botulinum has some rather specific requirements for growth and toxin production. In a mixed microflora, it is moderately competitive, but less so as pH is lowered. Consequently, it benefits from processes that eliminate competition and reduce the E_n of the system favoring anaerobic growth. Cured meats, especially heat-processed cured meats, provide a near optimal balance of nutrients, growth environment, reduced competition, and many of the other factors that promote growth of C. botulinum (Sakaguchi, 1979).

A variety of physical and chemical factors determine whether C. botulinum proliferates and forms toxin in cured meats. Many of these factors may also control the proliferation of spoilage organisms or other pathogens. They include thermal processing; level of C. botulinum spores and vegetative cells at time of abuse; concentration of sodium chloride; pH of the meat; concentration of carbohydrate; storage temperature; water activity or brine level

% salt

(% brine = $\frac{\% \text{ salt}}{\% \text{ salt} + \% \text{ moisture}} \times 100$, also defined in some reports as % salt per unit volume); presence and concentration of ascorbate and isoascorbate (erythorbate), including their effect on the level of available iron in the product; input and/or residual level of nitrite; other curing adjuncts; and packaging. Certain of these factors interact in exerting control over microorganisms. Such interactions are described later in this chapter.

Thermal Processing. Spores of pathogens such as C. botulinum and putrefactive spoilage organisms can be inactivated by thermal processing. Spores of some putrefactive, but nonpathogenic microorganisms commonly found in foods are more heat-resistant than those of C. botulinum (International Commission on Microbial Specifications for Foods, 1980, pp. 1-37, 136-159). Thus, the latter will be controlled if the former are.

Thermal processing is used extensively in the production of a variety of food products. A "botulinum cook" is a 12D process,³ i.e., one that will inactivate 99.999999999% of the C. botulinum spores in any volume. To assign an appropriate thermal process for specific foods, a number of assumptions must be made based on empirical findings. Among these are the assumptions that the spores have a uniform level of heat resistance, that they are uniformly distributed throughout the product, and that inactivation proceeds with linear, first-order kinetics. If one were to assume a normal population of 10^2 spores per can, a 12D process would leave one surviving spore in a total of 10^{10} cans of products.

³D denotes a decimal reduction in microbial population, i.e., to 10% of the initial population.

To ensure that thermal processing is adequate, the temperature and duration of heating are measured and safety margins incorporated into the treatment. However, the reliability of thermal processing is compromised most frequently by failure to deliver the intended treatment rather than by faulty process design. The cause of the failure to deliver the intended treatment can be mechanical, e.g., defective materials and equipment, or human error, e.g., mismeasurement of temperatures. The Hazard Analysis and Critical Control Point Program currently recommended by the regulatory agencies has contributed greatly to increasing the reliability of thermal processing (Genigeorgis and Riemann, 1979).

To obtain a 12D inactivation of C. botulinum spores in phosphate buffer at pH 7.0, the thermal treatment must be administered for 2.78 minutes at 121°C. For C. botulinum, it is generally accepted that a decrease or increase of 10°C from this temperature will produce a tenfold decrease or increase in the number of spores killed (Genigeorgis and Riemann, 1979). Accordingly, 10 minutes at 111°C or 0.1 minute at 131°C are both equivalent to 1 minute at 121°C. By custom, a heat treatment of 1 minute at 121°C has been accorded a "lethal value" (F_0) of 1 (Hauschild, 1980).

Thus, the "botulinum cook" is designated as having a lethal value (F_0) of 2.78. If one knows the length and temperature of treatment and that lethality varies logarithmically with change in temperature, the lethal value of any thermal process can be calculated. For low acid foods, e.g., canned mushrooms, thermal processes of $F_0 > 2.78$ (or, often, double that value) are used to prevent spoilage losses from organisms whose spores are more resistant to heat than those of C. botulinum (International Commission on Microbial Specifications for Foods, 1980, pp. 1-37).

The sensory characteristics (e.g., color, flavor, and texture) and functionality of most cured meat products would be unacceptable after thermal processing to $F_0 = 2.78$ or even after a lower level of processing to $F_0 = 1.0$. Thermal processes with F_0 values ranging from 0.05 to 0.4 are frequently used for shelf-stable cured meat products. These lethal values are from 10 to 100 times lower than those apparently required for a minimal "botulinum cook" for low acid foods. Consequently, the process must be highly dependent upon other inhibitors such as nitrite and sodium chloride to ensure safety and freedom from spoilage (Lechowich et al., 1978; Pivnick et al., 1969).

Thermal processing of most cured meat products has been successful because of the supplementary effects of nitrite, salt, and the very low incidence of anaerobic bacterial spores in raw meat (Riemann, 1963; Silliker, 1959). Thermal processes delivering an $F_0 < 0.1$ can produce adequately preserved, shelf-stable meat products if accompanied by sodium chloride or nitrite concentrations higher than those commonly used or permitted in the United States or Canada (Pivnick et al., 1969).

Current thermal processing practices used by industry to control pathogens and spoilage reflect earlier comprehensive studies by Stumbo et al. (1945a,b,c), Gross et al. (1946a,b), Vinton et al. (1947a,b), Schack et al. (1959), and Pivnick et al. (1969, 1970).

In reviews of effective thermal processes for controlling bacterial spores in canned cured meats, Duncan (1970) and Riemann (1963) have reaffirmed earlier observations that the stability of cured meats is dependent upon a complex interaction between the heat treatment and the curing agents. Pivnick et al. (1969) described the interaction of salt, nitrite, the number of C. botulinum spores in the inoculum, and severity of heat treatment on subsequent toxin production. A thermal process with an F_0 of 0.15 did not control subsequent toxin production when there were 10^4 spores per gram of ground cured pork. A treatment of $F_0 = 0.3$ was adequate for 10^4 spores per gram, but was insufficient for 10^6 spores per gram, which could be controlled by an F_0 of 0.6. Viable botulinum spores were recovered after 18 months, even in a product that was unspoilied and nontoxic. Meat devoid of curing salts (i.e., sodium chloride and sodium nitrite) was inoculated with one spore per gram and processed to an F_0 of 0.62. Upon incubation it became toxic.

Heat-damaged spores of clostridia or Bacillus spp. are more sensitive to the effects of curing salts than are undamaged spores (Roberts and Ingram, 1966; Roberts et al., 1966). Spores of C. botulinum subjected to a sublethal heat process at 95°C were inhibited by unheated nitrite less than by a nitrite-containing medium that had been heated at 115°C for 15 minutes (Ingram and Roberts, 1971). These responses were not observed in a model meat system heated at lower temperatures (80°C) for longer times (4 hours) (Ashworth et al., 1973). Jarvis et al. (1976) suggested that although spores heated and damaged at higher temperatures of 90°C might be sensitized to nitrite and salt, those subjected to the lower temperatures used for pasteurization (63°C to 74°C) might not be sensitized. Furthermore, inhibitory effects produced upon heating were much more evident in some laboratory media than in meat systems (Johnston and Loynes, 1971; Perigo et al., 1967). Thus, precaution should be used when extrapolating laboratory data to commercial meat processing.

Thermal processing is generally regarded as encompassing only those heating procedures which have a preservative action against C. botulinum. Pasteurization processes with temperatures ranging from 63°C to 74°C have no direct effect on spores of C. botulinum in cured, perishable comminuted meats (Tompkin et al., 1978b); however, such temperatures will kill many types of germinated spores or vegetative cells. This will reduce subsequent spoilage of products and the competition that surviving spores will encounter after their germination and outgrowth.

Product Contamination. The degree to which a product is contaminated will affect the likelihood of its spoilage and the adequacy of the protection provided by nitrite and other factors.

Skovgaard (1980) and Holley (1978, 1981) reviewed various reports indicating that meats may occasionally contain low levels of C. botulinum. In the United Kingdom, investigators studied a series of samples from one processor and reported that an average of 1 to 2 C. botulinum cells were present per kilogram of bacon and that sporadic contamination much higher than this average also occurred (Roberts and Ingram, 1977; Roberts and Smart, 1976a, b). However, the authors of a survey of random samples of commercial bacon in Canada suggested that the most probable number of C. botulinum cells was 0.064 per kilogram (Hauschild and Hilsheimer, 1980). The apparently low incidence of C. botulinum cells may be partially responsible for the excellent public health record associated with the consumption of cured products; however, research on this subject has not been systematic.

It has been well documented that typical heat processing and curing agents used for perishable or shelf-stable cured meats may be inadequate when the meat is heavily contaminated with C. botulinum spores (Christiansen et al., 1973; Pivnick et al., 1969; Sofos et al., 1979a).

Sodium Chloride. The final concentration of sodium chloride in most cured meat products is approximately 2 to 3% of the total product weight, which corresponds to approximately 3 to 6% salt in the aqueous phase (i.e., a 3 to 6% brine concentration) for most products. The brine concentration will, however, vary with the moisture content of the product. In meat products, it is the concentration of salt in the aqueous phase, rather than the percentage of salt based on the total product weight, that is the critical factor in determining the likelihood of microbial growth, which only occurs in the aqueous phase.

In some products, the brine concentration can lie outside the 3 to 6% range. It is higher in some products, such as certain dry-cured cuts and dried sausages, which typically have a brine concentration of 13 to 16%. In some products, such as farmer salami, the concentrations can be as high as 30% (International Commission on Microbial Specifications for Foods, 1980, p. 388). In certain dry-cured cuts, the slow penetration of the curing agents may lead to extremes in concentrations within one product (International Commission on Microbial Specifications for Foods, 1980, p. 384).

At the 3 to 6% level in brine, sodium chloride alone would be inadequate to inhibit the production of C. botulinum toxin; however, in combination with other factors, sodium chloride is an important component of cured meats and does play a significant role in preventing the outgrowth of germinated spores (International Commission on Microbial Specifications for Foods, 1980, pp. 136-159). As the salt concentration is increased or the pH is reduced, greater inhibition

of C. botulinum occurs (Roberts and Ingram, 1973; Roberts et al., 1976). In certain products, such as some country hams and certain dried sausage products, C. botulinum can be completely inhibited by high brine concentrations. Cultures of Group I C. botulinum⁴ are able to grow and produce toxin in the presence of 8 to 10% brine, whereas cultures of Group II are inhibited by 5 to 6% brine (Genigeorgis and Riemann, 1979; International Commission on Microbial Specifications for Foods, 1980, pp. 136-159).

pH. A pH range of 4.6 to 5.0 may limit the outgrowth of C. botulinum spores in certain media; however, the pH of most meats cured in the United States ranges from 5.5 to 6.6. On rare occasions, it may be as high as 7.0 to 7.2 (International Commission on Microbial Specifications for Foods, 1980, pp. 333-409). Reducing the pH from 7.0 to 6.0 or 5.5 lowered the salt tolerance of C. botulinum vegetative cells (Baird-Parker and Freame, 1967), and the nitrite sensitivity of Staphylococcus aureus has been observed to increase as pH was reduced from 6.9 to 5.5 (Castellani and Niven, 1955). As discussed below, residual nitrite appears to be important in the inhibition of C. botulinum (Christiansen, 1980; Christiansen et al., 1978). Thus, when considering manipulation of pH as a means of enhancing antimicrobial activity, one must recognize that the rate at which residual nitrite is depleted increases as the pH decreases (Nordin, 1969).

Direct acidulation of meat emulsion to inhibit microbial growth may affect the stability of the emulsion during handling, e.g., when stuffed into casings. Therefore, glucono- δ -lactone, which slowly hydrolyzes to gluconic acid, is often used to produce delayed acidulation (International Commission on Microbial Specifications for Foods, 1980, pp. 136-159).

Carbohydrates. Under abuse conditions, e.g., temperatures of 15-25°C, naturally occurring lactic-acid-producing bacteria in raw and perishable cooked cured meats will metabolize added or naturally occurring carbohydrates, thereby reducing the pH of the product by acid production, if sufficient fermentable carbohydrate is present (Christiansen et al., 1975). Protection by this mechanism undoubtedly explains observations in a recent study of bacon from four processing plants inoculated with C. botulinum spores (U.S. Department of Agriculture, 1979). One set of bacon samples contained 0.57% added sucrose; other sets of samples contained low concentrations

⁴Group I contains proteolytic C. botulinum strains producing toxin types A, B, and F; Group II contains nonproteolytic C. botulinum strains producing toxin types E, B, and F. Group I strains have a minimum growth temperature of 10°C and are more resistant to heat. Group II strains have a minimum growth temperature of 3.3°C and are less resistant to heat.

of added sugars or none at all. Samples were subjected to simulated temperature abuse by incubating them at 27°C. Only 0.5% of the samples containing sucrose became toxic during the 56-day incubation, whereas 30 to 94.5% of the packages containing less sugar became toxic. Within the first 28 days of the incubation, the pH of the sucrose-containing bacon was reduced to pH 5.0 or lower. The pH of bacon containing the lower concentration of sugar remained high (pH 5.4 or above) throughout the entire 56 days.

Storage Temperature. Temperatures of less than 10°C will inhibit outgrowth of spores of proteolytic C. botulinum strains producing type A or B toxins (Ohye and Scott, 1953). For example, no toxic samples were detected in perishable canned comminuted cured meat or in bacon incubated at 7°C (Christiansen et al., 1973, 1974). At temperatures below the optimum for C. botulinum growth (i.e., ~37°C) cell multiplication decreases with drops in temperature. This may in part explain the observation of Roberts et al. (1976) that the number of toxic samples observed after incubation at 15°C was less than that observed at 17°C.

Unfortunately, appropriate storage temperatures during distribution, in retail stores, or in the home cannot be guaranteed, but the addition of curing salts can inhibit C. botulinum more readily as the storage temperature is reduced (Ingram, 1974; Roberts et al., 1976). Since the level of residual nitrite appears to be an important factor in promoting product safety (Christiansen, 1980; Christiansen et al., 1978), one should realize that nitrite depletion occurs more quickly when the product is subjected to temperature abuse (27°C) than when it is refrigerated (Christiansen et al., 1974). The implications of the depletion of these residuals are discussed later in this chapter.

Even if proper refrigeration could be assured, psychrotrophic strains of C. botulinum (capable of growth at 3.3 to 5.6°C) can multiply at usual refrigeration temperatures and could be a potential hazard (Eklund et al., 1967; Roberts and Hobbs, 1968). However, these strains are more sensitive to salt (Genigeorgis and Riemann, 1979) and are not common in red meats and poultry in the United States (Holley, 1978, 1981).

Water Activity. C. botulinum spore outgrowth, vegetative cell growth, and toxin production are inhibited at a water activity less than 0.93. If other environmental factors (e.g., pH and temperature) are not favorable, inhibition may occur at water activity levels higher than 0.93. The inhibitory effect of water activity is also influenced by the selection of solute. For example, strains of C. botulinum producing toxin types A, B, or E multiplied at lower levels of water activity when the media were adjusted with glycerol instead of salt (Baird-Parker and Freame, 1967). The water activity of most cured meats is higher than 0.95; however, certain raw, dry, fermented sausages have a water activity of 0.92 and a pH of 5.0 -- a combination

that prevents growth. In other products, the water activity may also prevent growth, e.g., when the brine concentration is approximately 10%, which is equivalent to a water activity of 0.92 (International Commission on Microbial Specifications for Foods, 1980, pp. 333-409).

Ascorbate or Isoascorbate (Erythorbate). In the United States, bacon is now formulated with 550 mg/kg sodium ascorbate or isoascorbate to accelerate the curing reactions and inhibit the formation of nitrosamines during cooking. Many other products are also formulated with ascorbate or isoascorbate (Tompkin *et al.*, 1978a), but amounts added are not standardized in cured meats other than bacon. At 200 mg/kg, sodium ascorbate or isoascorbate enhanced nitrite's inhibition of *C. botulinum* in perishable canned cured meats, when the product was abused at 27°C shortly after manufacture (Tompkin *et al.*, 1978a). However, higher concentrations (>500 mg/kg) in perishable canned cured meats decreased the effectiveness of nitrite (Tompkin *et al.*, 1979b). The enhancement may have been due to the ability of ascorbate to chelate iron (Tompkin *et al.*, 1978b,c, 1979a), because other sequestering agents, such as EDTA (ethylenediaminetetraacetic acid) or cysteine, also enhance inhibition by nitrite, and the addition of iron decreases inhibition. The levels of ascorbate or isoascorbate enhancing or reducing the effectiveness of nitrite in other types of products may not be the same as those for perishable canned cured meat. These effects need further investigation.

Ascorbate or isoascorbate should be used in cured meats only with great care because high levels may enhance the rate of nitrite depletion, thereby reducing protection against botulism (Tompkin *et al.*, 1979b).

Added and Residual Nitrite Levels. There has been some disagreement as to whether the protection against botulism conferred by nitrite in a cured product can be predicted most accurately from the level of nitrite added or from the residual level of nitrite present at the time of abuse. Predictions are further complicated by the reactions of added nitrite with thermally processed meat. Some investigators have suggested that these reactions may result in the formation of inhibitory "Perigotype factors" (International Commission on Microbial Specifications for Foods, 1980, pp. 136-159; Pivnick and Chang, 1973), but the evidence for such factors is not conclusive (Sofos *et al.*, 1979a). Moreover, temperatures exceeding 90°C sensitize spores to the inhibitory effects of nitrite and other curing salts, thereby further impeding a facile solution of these problems (Jarvis *et al.*, 1976). Thus, the relative importance of added versus residual nitrite may have to be addressed for each product category individually.

Greenberg (1972) reported that for pasteurized canned hams, in which the level of heating does not damage spores, the likelihood of botulinum toxin production during temperature abuse could be predicted most accurately from the initial nitrite addition rather than from residual nitrite concentrations. Christiansen et al. (1978) studied the residual nitrite depletion and the germination, death, and outgrowth of botulinal spores in a canned, perishable ham product subjected to temperature abuse at 27°C immediately after processing with various levels of nitrite. They concluded that the safety of such products was dependent upon the presence of sufficient residual nitrite to inhibit germinated spore outgrowth until the number of viable cells had decreased to a point where cell growth could no longer be initiated.

They thus introduced the concept that the degree of protection depends on the outcome of a race between the depletion of nitrite (which is greater at abuse temperatures than under refrigeration) (Nordin, 1969) and the death rate of germinated spores (whose outgrowth is blocked by nitrite). During extended refrigeration of a product, residual nitrite may be reduced to a noninhibitory level, and viable, ungerminated spores may remain because of extended dormancy. If this product is then subjected to temperature abuse, the hazard of botulism increases since the remaining nitrite concentration is too small to be effective in controlling C. botulinum. A number of studies (cited earlier) have indicated that the protection provided by nitrite and other curing salt ingredients can be overwhelmed by high levels of inocula or contamination.

Christiansen (1980) presented data on nitrite depletion and C. botulinum death in hams at 27°C. This study emphasized that information on the rate at which viable C. botulinum cells decreased in number (which accompanies but may not be parallel to the rate of nitrite depletion, depending on temperature) is essential to determining the importance of residual nitrite.

These studies have been conducted with commercial products. However, there is no way of predicting if the levels of spore contamination introduced into these products and, thus, the numbers present at the time of temperature abuse, approximate contamination levels that might occur on occasion under normal commercial operating conditions.

The committee believes that the residual nitrite present at the time of abuse is one of the important determinants of the safety of cured products. Thus, any process or product changes that result in a lower level of residual nitrite, e.g., adding less nitrite or increasing its rate of depletion, will increase the likelihood that the product will become toxic if contaminated and subjected to temperature abuse. The effectiveness of nitrite depends on the level of product contamination and the treatment to which the product is subjected, neither of which is predictable.

Contamination of products with C. botulinum is generally not extensive, but there are sporadic peaks (Roberts and Smart, 1976a,b). Because of the unpredictability of product contamination and temperature abuse, it is not possible to derive a quantitative relationship between the protection conferred by nitrite and the risk of botulism. (See Chapter 10 and the discussion later in this chapter.)

Packaging. Product packaging may also affect microbial growth. During the early 1950s, a change from packaging films providing a low oxygen barrier to films with a high oxygen barrier was not accompanied by an observed increased incidence of botulism from cured meats, as had been feared. During the 1960s, vacuum packaging became widely used, but it was shown that, if other conditions were favorable, C. botulinum toxin would be formed whether the product was vacuum-packed or not (Christiansen and Foster, 1965). Vacuum packaging may delay growth of spoilage organisms and the production of typical spoilage odors, which often precede a potential microbial hazard. However, the presence of such odors is not a reliable indicator of toxin in foods (Christiansen and Foster, 1965; Sofos et al., 1980).

Curing Adjuncts. The addition of sodium tripolyphosphate and other polyphosphates to bind water or lessen shrinkage during cooking could increase the pH, thereby increasing the tolerance to sodium chloride of normal outgrowing C. botulinum spores, especially those injured by heating, and decreasing the inhibitory effectiveness of sodium nitrite. However, this increase in pH may be overshadowed by the ability of the negatively charged phosphate ions to chelate certain metallic ions, e.g., those of iron. Thus, polyphosphates may enhance the inhibitory effect of nitrite (Crowther et al., 1977; Skovgaard, 1980).

Efficacy of Nitrite as an Antibotulinal Agent

Tables 3-5 and 3-6 present some results of studies of the protection provided by nitrite against toxin production or swelling (spoilage) induced by C. botulinum in various inoculated commercial products. The fewer the days to toxin production or swelling for any nitrite-free product, the lower is the control provided by inhibitory factors (e.g., pH, salt, and water activity) other than nitrite. The lower the ratio of days to toxin production for nitrite-containing versus nitrite-free products, the smaller is the degree of protection provided by that level of nitrite. Nitrite protection against toxin production in fish has been demonstrated in chub, salmon, whitefish, and carp (M. W. Eklund, National Marine Fisheries Service, Seattle, personal communication, 1981). Levels providing protection will be discussed in the second report of the committee. For all product types shown in Tables 3-5 and 3-6, it appears that any reduction in added nitrite to levels lower than those currently

TABLE 3-5

Delay by Nitrite of Toxin Production in Bacon Inoculated with *C. Botulinum* Spores at an Abuse Temperature of 27°C.^a

| Inoculum Spores/g ^c | Cure Ingredients ^b | | | Days to Comparable Toxin Production, Ratio No Nitrite to: | | | Samples Toxic At Time of Comparison, % | Days to First Toxin Production, Ratio No Nitrite to: | | | Reference |
|--------------------------------|-------------------------------|------------------|---------|---|----------|-----------|--|--|----------|---------------------|---|
| | Sugar, mg/kg | Phosphate, mg/kg | Salt, % | 30 mg/kg | 60 mg/kg | 120 mg/kg | | 30 mg/kg | 60 mg/kg | 120 mg/kg | |
| 1,000 | 600 | 3,060 | 1.47 | -- ^d | -- | 5:12 | 50 | -- | -- | 4:6 | U.S. Department of Agriculture, 1979 ^e |
| 1,000 | 20 | 750 | 1.69 | -- | -- | 10:15 | 40 | -- | -- | 2:3 | U.S. Department of Agriculture, 1979 ^e |
| 1,000 | 1,230 | 1,210 | 1.81 | -- | -- | 6:16 | 20 | -- | -- | 2:5 | U.S. Department of Agriculture, 1979 ^e |
| 1,000 | 4,975 | 4,640 | 1.40 | -- | -- | 15:>60 | 20 | -- | -- | 7:16 | U.S. Department of Agriculture, 1979 ^e |
| 1,000 | 8,000 | 5,000 | 1.70 | -- | -- | 13:>60 | 20 | -- | -- | 10:>60 ^f | Sofos <i>et al.</i> , 1980 |
| 1,000 | 8,000 | 5,000 | 1.70 | -- | -- | <10:>40 | 20 | -- | -- | <10:>40 | Sofos <i>et al.</i> , 1980 |
| 52 | 3,100 | 2,600 | 1.33 | ~10:~10 | ~10:~20 | ~10:56 | 5 | <7:<7 | <7:>7 | <7:>40 | Christiansen <i>et al.</i> , 1974 |

^aAll tests were conducted with bacon produced under commercial procedures, except that of Christiansen *et al.* (1974), who used bacon prepared in a pilot plant. The efficacy of various treatments is analyzed by comparing the number of days of incubation that were required for the first package in a treatment group or for a particular percentage of those packages to become toxic at the simulated abuse temperature (27°C).

^bConcentrations listed were target values. Actual values may deviate, sometimes considerably from target values, e.g., U.S. Department of Agriculture, 1979.

^cData in this column represent the lowest spore inoculum used in the cited studies in order to approximate most closely probable contamination.

^d-- Denotes not tested or not recorded.

^eData pertain to toxic swollen packages from Phase I of the four-plant study conducted by the U.S. Department of Agriculture (1979).

^fOne package became toxic at 16 days, none of the other 199 packages became toxic during the 60 days of the study.

TABLE 3-6

Delay of Nitrite of Toxin Production or Swelling in Various Commercial Cured Products Inoculated with C. Botulinum Spores at Abuse Temperatures^a

| Product | Inoculum Spores/g ^b | Processing ^c | Sodium Chloride % | Days to Comparable Toxin Production or Swelling, Ratio of No Nitrite to: | | | | | | Samples Toxic or Swollen at Time of Comparison, % | References |
|------------------------------------|--------------------------------|-------------------------|-------------------|--|----------|-----------|-----------|-----------|-----------|---|---|
| | | | | 40 mg/kg | 50 mg/kg | 100 mg/kg | 150 mg/kg | 156 mg/kg | 200 mg/kg | | |
| Frankfurters, meat | 325 | 71°C | 2.6 | — ^d | <14:>56 | -- | -- | <14:>56 | -- | 20 | Hustad <i>et al.</i> , 1973 |
| Frankfurters, chicken | 5 | -- | -- | -- | < 7:>6 | <7:>12 | -- | < 7:>12 | -- | 80 | Christiansen <i>et al.</i> , 1977 |
| Frankfurters, chicken | 500 | 68.5 | 2.5 | < 4:>4 | -- | -- | -- | < 4:>6 | -- | 50 | Sofos <i>et al.</i> , 1979b |
| Perishable, canned comminuted pork | 100 | 68.5 | 2.5 | -- | 7:43 | 7:102 | -- | 7:107 | -- | 50 | Tompkin, 1978; Tompkin <i>et al.</i> , 1977 |
| Pork luncheon meat | 100 | F ₀ = 0.4 | 2.3 | -- | -- | 10:14 | -- | -- | 10:17 | 50 | Pivnick and Chang, 1973 |
| Summer sausage ^e | 100 | 58.3°C | 2.5 | -- | <14:>14 | -- | <14:>21 | -- | -- | 33 | Christiansen <i>et al.</i> , 1975 |
| Turkey ham | 5 | 68.3°C | 1.07 | -- | < 3:>6 | <3:>10 | -- | < 3:>12 | -- | 80 | Christiansen <i>et al.</i> , 1977 |

^aThe efficacy of various treatments is analyzed by comparing the number of days of incubation that were required for a particular percentage of products in a treatment group to become toxic or swollen at the simulated abuse (incubation) temperatures. The incubation temperature was 27°C in all studies except that of Pivnick and Chang (1973), which was 35°C.

^bData in the column represent the lowest spore inoculum used in the cited studies, in order to most closely approximate probable contamination.

^cF₀ or center temperature.

^d-- Denotes not tested or not recorded.

^eData presented are for products formulated without glucose and starter culture. For products to which glucose and starter culture, but no nitrite, had been added, 10% of the samples were toxic within 112 days. For products to which glucose and sodium nitrite (50 mg/kg), but no starter culture, had been added, toxin production was completely inhibited as long as 112 days.

used would (barring unusual dose responses) reduce the length of time that the product could withstand temperature abuse without becoming toxic if contaminated, unless other inhibitory factors were appropriately modified. This will also be discussed further in the committee's second report.

The Contribution of Nitrite to the Control of Microbial Pathogens Other Than C. Botulinum in Cured Meats

Staphylococcus aureus is capable of growth and can produce its enterotoxin, under aerobic conditions, at salt concentrations greater than the 3% to 6% present in the aqueous phase of most cured meats (International Commission on Microbial Specifications for Foods, 1980, pp. 136-159, 333-409). Crowther *et al.* (1977) observed that it grew in bacon at 15-25°C, irrespective of the presence of sodium nitrite in concentrations up to 200 mg/kg, but the production of enterotoxin did not occur under anaerobic conditions.

Genigeorgis and Riemann (1979) reviewed information on the interaction of nitrite and other factors in controlling growth of S. aureus. They concluded that the levels of salt, nitrite, and the pH of most cured meats that are not dried extensively would not prevent growth or production of enterotoxin under aerobic conditions but that these factors may, in combination, become inhibitory under anaerobic conditions such as those that occur in vacuum-packed products. The committee concurs with this conclusion. However, the opportunity for the production of the heat-stable toxin still exists in products that are not vacuum-packed and in fermented and dried sausages during the fermentation and drying periods. This is apparent from the food-borne disease outbreaks described by Bryan (1980).

Salmonellae are not generally inhibited by the concentrations of salt and nitrite or by the pH of most cured meats. They are more resistant to nitrite than are C. botulinum cells, but, like S. aureus, they are much more sensitive to heat than are C. botulinum spores.

Gough and Alford (1965) have shown that sodium nitrite at 400 mg/liter or 6% sodium chloride were required to inhibit the growth of various strains of Clostridium perfringens in a thioglycollate medium. There appears to be little information on the effects of various combinations of nitrite, sodium chloride, pH, and water activity on the growth of C. perfringens in meat products.

Foodborne pathogens such as S. aureus, salmonellae, C. perfringens, and mesophilic strains of C. botulinum are unable to multiply at refrigeration temperatures lower than 5 to 6°C, but psychrotrophic strains of C. botulinum (Group II) may grow at lower temperatures. Thus, the continuous and proper refrigeration of meats greatly minimizes the probability of health hazards from most microbial pathogens.

Certain nonpathogenic microorganisms such as lactobacilli are resistant to nitrite. In many cured products stored at an abuse temperature, the ecosystem allows the selective growth of these bacteria, which lower the pH if sufficient fermentable carbohydrate is present, thereby minimizing the probability that pathogens such as C. botulinum will grow.

The Contribution of Nitrite to the Control of Microbial Spoilage of Meats

Table 3-4 lists some types of microbial spoilage that occur in certain raw and cured meat products. The spoilage pattern of a product will vary with the temperature at which it is stored (or abused). Spoilage and the factors involved in its control have also been reviewed by the International Commission on Microbial Specifications for Foods (1980, pp. 333-409). Since curing has successfully eliminated or greatly reduced many types of spoilage, there has been little motivation for conducting systematic investigations of the relative contributions to spoilage inhibition made by components of the curing salt, of the characteristics of the product (e.g., water activity and pH), or of the responsible organisms in various types of products. Many of the microbial strains involved in spoilage have not yet been satisfactorily categorized (International Commission on Microbial Specifications for Foods, 1980, p. 351).

In raw meats stored at temperatures below 10°C, Pseudomonas and the related Acinetobacter and Moraxella genera are particularly important spoilage agents (International Commission on Microbial Specifications for Foods, 1980, pp. 333-409) because they produce volatile amines, hydrogen sulfide, esters of organic acids, and slime. These organisms developed under anaerobic conditions, but they are succeeded, as the residual oxygen dwindles, by Microbacterium thermosphactum and Enterobacteriaceae. Ultimately, gram-positive organisms such as lactic-acid-producing bacteria predominate and eventually cause souring by acid production (International Commission on Microbial Specifications for Foods, 1980, pp. 333-409).

The addition of relatively low levels of salt (approximately 2%) to meat under aerobic conditions inhibits the growth of pseudomonads, resulting in the predominance of lactic-acid-producing bacteria. In raw cured meats, the initial flora is primarily gram-negative, but there is a subsequent shift to a predominantly gram-positive flora, e.g., micrococci, lactobacilli, streptococci, and Microbacterium thermosphactum (Girolitti et al., 1971; International Commission on Microbial Specifications for Foods, 1980, pp. 333-409). As noted above, salt contributes to this shift. At 200 mg/kg, sodium nitrite inhibits, to varying degrees, the growth of gram-negative organisms such as Pseudomonas, Achromobacter, Moraxella, Flavobacterium, Aerobacter, Escherichia, and some micrococci at pH 5.7 to 6.0 (Tarr, 1941a,b, 1942, 1944).

The initial flora of fermented sausage emulsions is mainly composed of gram-negative rods. Acidulants such as glucono- δ -lactone, fermentable carbohydrates, and starter cultures are used to establish or enhance growth of the desired fermentative flora; salt and possibly nitrite also exert a selective influence on undesirable organisms (International Commission on Microbial Specifications for Foods, 1980, pp. 333-409).

In dry-cured hams, spoilage is caused primarily by the growth of clostridia near the bone (Giolitti *et al.*, 1971; Mumdt and Kitchen, 1951). The data on *C. botulinum* control in other raw cured products presented in Table 3-5 and the work of Stumbo *et al.* (1945a,b,c) and Nordin *et al.* (1975) indicate that nitrite probably contributes to the control of the clostridia-induced spoilage in these products.

Spoilage of shelf-stable cured meat products and its control have been thoroughly studied by Stumbo *et al.* (1945a,b,c) and Nordin *et al.* (1975). The latter group of investigators derived from a model meat system a formula expressing the contribution of salt, pH, and nitrite to the control of spoilage. They studied cured meat heated to 115°C for 30 minutes within the pH range of 5.5 to 6.6. The salt content of the samples ranged from 1.5% to 3.0%, and concentrations of sodium nitrite ranged from 0 to 200 mg/kg. Within these ranges, an increase of 65 mg of sodium nitrite per kilogram of product provided inhibition equivalent to that produced by a decrease of 0.1 pH unit or a 0.3% increase in salt. The effects were additive. Thus, on a weight basis, additions of sodium nitrite were approximately 45-fold more inhibitory to spoilage than was salt. Caution must be exercised when using this model to make quantitative predictions of the effect of nitrite in commercial products or other types of cured meat because the model may not mirror their characteristics.

Various chemical tests have been suggested to quantitate spoilage, but none has gained widespread acceptance. Most of these tests provide only equivocal results at stages before the meat is obviously organoleptically spoiled (International Commission on Microbial Specifications for Foods, 1980, pp. 333-409).

Data on nitrite's contribution to the control of all spoilage organisms cannot be presented in a fashion similar to that shown in Tables 3-5 and 3-6 for *C. botulinum* in various products. Most evidence pertains to the inhibitory action of nitrite against spoilage clostridia (International Commission on Microbial Specifications for Foods, 1980, pp. 136-159, 333-409). This action is similar to the antibotulinal activity of nitrite. Many other types of organisms are also involved in spoilage, and it is not possible to define the precise point at which meat is spoiled based on numbers of any one species or on total numbers. Moreover, there are differences in the individual perception of spoilage (e.g.,

odor and appearance). Overall tests of acceptability have not attempted to isolate microbial spoilage from chemical changes (e.g., lipid oxidation and color fading).

The Mechanism of Action of Nitrite

Studies in laboratory media indicate that nitrite, at the levels used commercially, inhibits the outgrowth of spores rather than their germination (Duncan and Foster, 1968; Gould, 1964; Pivnick, 1970). However, it is difficult to determine the mechanism by which nitrite inhibits microorganisms or spore outgrowth in products since the nitrite ion is capable of a variety of reactions in a given meat system and because such products are complex and lack uniformity. Benedict (1980) reviewed several possible mechanisms, namely, that nitrite (1) reacts with other components during heating to form an inhibitory substance; (2) acts as either an oxidant or a reductant on such cellular sites as enzymes, enzyme cofactors, nucleic acids, and cellular membranes; (3) reacts with cellular iron, thereby interfering with metabolism and repair mechanisms; or (4) reacts with thiols to form nitrosothiols, which in turn react with a spore membrane component, thereby interfering with metabolic and/or transport steps. In a bacteriological medium (containing tryptone, thioglycollate, nitrite, and iron), a substance (the Perigo factor) that inhibits C. botulinum is formed during heating. This factor is of questionable significance in perishable cured meats since it is formed at temperatures of 105°C or higher, which exceed those normally used in the processing of cured meats, and its antibacterial activity is neutralized by meat particles. Sofos et al. (1979a) have reviewed studies on the possible role of Perigo-type factors in cured meats (Pivnick and Chang, 1973).

Various studies offer clues to nitrite's possible mechanism of action. When nitrite is added to previously sterilized laboratory media, the inhibitory property of nitrite is enhanced by decreasing the pH to 6 and below. Such results suggest that free nitrous acid either is or produces the effective inhibitor. Metal ion-sequestering agents such as sodium ascorbate, EDTA, and cysteine enhance the antibotulinal effect of nitrite. Tompkin et al. (1978c, 1979a) suggested that nitrite reacts with the iron in ferredoxin, thereby rendering the latter inactive. Nitrite could also react with thiols and unsaturated lipids to form inhibitory oxidants. Because C. botulinum cells lack catalase and superoxide dismutase, they are sensitive to molecular oxygen and oxygen radicals.

Rowe et al. (1979) demonstrated that nitrite inhibits active transport, oxygen uptake, and oxidative phosphorylation by Pseudomonas aeruginosa, possibly by oxidizing ferrous iron of an electron carrier, such as cytochrome oxidase, to the ferric form. They also reported that glucose transport in Streptococcus faecalis and S. lactis was

not inhibited by nitrite -- probably because glucose transport in these organisms is not dependent on active transport or cytochromes. Yarbrough et al. (1980) indicated that nitrite inhibited the active transport of proline in Escherichia coli but not group translocation by the phosphoenolpyruvate:phosphotransferase system.

Support for the suggestion by Tompkin et al. (1978c) that ferredoxin is inactivated by nitrite was presented by Woods et al. (1981). They reported that nitrite inhibited the growth of Clostridium sporogenes cells by inhibiting the microorganism's phosphoroclastic system. This inhibition resulted from the reaction of nitric oxide, derived from nitrite, with the non-heme iron of pyruvate:ferredoxin oxidoreductase. Evidence that this mechanism also occurs in growing C. botulinum cells has recently been reported by Woods and Wood (in press).

The reasons that certain microorganisms are not susceptible to inhibition by nitrite have, unfortunately, received little attention. Page and Solberg (1979) investigated the exclusion of nitrite by cell-wall lipopolysaccharide and the existence of nitrite-metabolizing systems as possible resistance mechanisms. They concluded that the latter was the more likely reason for the relative lack of effect of nitrite on salmonellae as compared to susceptible microorganisms. Staphylococcus aureus also appears to metabolize nitrite when grown aerobically but not under anaerobic conditions (Buchanan and Solberg, 1972).

In summary, nitrite apparently attacks a number of targets in different bacterial species, and interference with the action of iron-containing enzymes may be a feature common to these inhibiting effects. It remains to be determined whether these effects are the primary mechanisms of action in complex meat systems and whether they are the mechanisms inhibiting clostridial spore outgrowth. Knowledge of the precise mechanism(s) of action of nitrite against vegetative cells and spore outgrowth in meat would facilitate the search for alternatives.

Interactions Affecting the Antimicrobial Activity of Nitrite

The antimicrobial activity of nitrite can be influenced by a variety of factors as described above and by the International Commission on Microbial Specifications for Foods (1980, pp. 136-159), Roberts et al. (1981a,b,c), and by Sofos et al. (1979a).

The antimicrobial action of both nitrite and sodium chloride salt depends on pH as does resistance to heat, but the effect of pH on heat resistance is relatively small in the pH range of 6.0 to 8.0 (International Commission on Microbial Specifications for Foods, 1980, pp. 1-37). The efficacy of nitrite correlates well with the undissociated nitrous acid concentration; a decrease of one pH unit

between pH 7.0 and pH 5.0 reduces about 5- to 10-fold the concentration of nitrite needed for a given degree of inhibition. Differences in salt tolerances among bacterial species are magnified as the pH decreases from 7.0 to 5.5. Redox potential was shown by Castellani and Niven (1955) and Henry *et al.* (1954) to affect the antimicrobial activity of nitrite against some, but not all, species. Different bacterial species and strains, including strains of *C. botulinum*, vary in their resistance to the inhibitory effects of nitrite (Perigo and Roberts, 1968; Roberts and Garcia, 1973).

Incubation temperature and processing involving heat both affect the efficacy of nitrite. At abuse temperatures of 17.5°C, the inhibition of *C. botulinum* toxin production is less than at 15°C (Roberts *et al.*, 1976). This may result from the increased rate at which residual nitrite is depleted as storage temperature increases or, more probably, because the higher temperature is more favorable to growth of mesophilic *C. botulinum* (Christiansen *et al.*, 1974; Nordin, 1969). Pasteurization does not influence the susceptibility of spores to nitrite, but it eliminates certain potential competitors that germinating *C. botulinum* spores encounter in unheated products. Higher treatment temperatures damage spores, making them more susceptible to nitrite and sodium chloride (Pivnick, 1970; Roberts and Ingram, 1966; Roberts *et al.*, 1966). Furthermore, nitrite, when heated, may react with meat components to produce inhibitory (Perigo-type) factors (Pivnick and Chang, 1973), but the evidence pertaining to this hypothesis is inconclusive (Sofos *et al.*, 1979a).

Prediction of Control. Attempts have been made to develop models or equations to predict the interaction of factors contributing to microbial control and the effects on product safety to be expected if these factors were modified. These efforts have focused on protection against *C. botulinum* and other clostridia.

Pivnick and Petrasovits (1973) proposed the following formula for estimating the degree of protection against *C. botulinum* intoxication afforded by heat processing or curing salts in shelf-stable cured products:

$$Pr = Ds + In,$$

where Pr = protection, Ds is the destruction of spores due to heat processing, and In is the inhibition of spore outgrowth and cell multiplication by curing salts. In this equation, Pr, Ds, and In are measured in units of \log_{10} of the number of spores of *C. botulinum*. The formula can be also used for unheated products, in which case Ds = 0.

The interaction of salt, pH, and nitrite in preventing microbial spoilage of a model system for cured shelf-stable products has been described by Nordin et al. (1975) as follows:

$$\hat{y} = -322 + 73.9 (\text{pH}) - 0.115 [N] - 24.7 [S],$$

where \hat{y} is the estimated percent of spoiled packages within 150 days at 23°C, pH ranges from 5.5 to 6.6, N is the concentration of sodium nitrite in the range 0 to 200 mg/kg, and S is the concentration of salt in the range of 1.5% to 3% (2% to 4% in the aqueous phase).

Both of these models are useful in describing the influence on safety provided by some of the factors involved in processing procedures.

Roberts et al. (1981a,b) have studied variables such as nitrite, nitrate, sodium chloride, isoascorbate, heat treatment, pH, and abuse temperature in a heated meat slurry model system. From their results they developed a computerized model that can be used to predict the influence of changes in these variables on the likelihood of toxin production within the limits used in the test system (Roberts et al., 1981c).

The committee strongly endorses the view expressed by Roberts et al. (1981c) that such models can be very helpful in assessing the relative effects produced by changes in concentrations of additives or other factors, but it believes that they should not be regarded as a means of providing quantitative predictions of changes in microbial proliferation in commercial products. There are no methods for predicting contamination levels, the probability and duration of temperature abuse, or variations in meat or product composition.

Other Factors Affecting Microorganisms in Cured Products

Within the United States, calculations of the amount of nitrite added to cured products are based on the uncured meat portion of the formulation. When extenders (such as soy protein) and other ingredients (such as previously cured products, especially those with low residual nitrite) are included in a product, the level of nitrite in the complete formulation will be less than that added to the meat.

Skovgaard (1980) has suggested that improved hygiene might justify lowering the level of nitrite added to products, but concluded that it was not yet feasible to determine the reduction that might be possible. The committee acknowledges that improved hygiene might lead to a lower probability of contamination with C. botulinum spores; however, it cautions that this reduction would be accompanied by a reduction in such organisms as lactic-acid-producing bacteria, which may, in some products (e.g., bacon), provide some measure of protection

against proliferation of C. botulinum, if fermentable carbohydrate is present, by producing acid, thus lowering the pH of the product.

Discussion: Antimicrobial Effects of Nitrite

Nitrite is known to have antimicrobial effects against a broad range of bacterial species, but the mechanisms of this action are not fully understood. In some bacteria, they probably involve iron-containing enzymes. The most important antimicrobial effect of nitrite is its action against the putrefactive and pathogenic clostridia, including C. botulinum. At the levels currently used in cured meat products, nitrite has no effect on germination, but it delays the outgrowth of spores of these organisms, thereby preventing spoilage and prolonging the period of temperature abuse that a cured product contaminated with C. botulinum can withstand before it becomes toxic (Tables 3-5 and 3-6). In this way, nitrite provides protection against the risk to health posed by botulism.

Depending on a number of factors, including the concentration of nitrite, environmental conditions, and the type of food product, nitrite may also contribute to the control of pathogens other than C. botulinum, including staphylococci, Bacillus cereus, and C. perfringens, and other spoilage organisms, such as bacilli, corynebacteria, and psychrotrophs, predominantly pseudomonads. The antimicrobial effects of nitrite are summarized in Table 3-7 at the conclusion of this chapter.

Relative Microbial Risks from Different Products. Realization of any potential microbial hazard to health posed by a cured product is obviously dependent on contamination of the product with a pathogen. But more important is the extent to which the production process, characteristics, and handling of the product allow multiplication of the pathogen. Factors that control the growth of the pathogens of most concern in cured meat products (e.g., C. botulinum, S. aureus, and salmonellae) vary among product classes. For some of these classes, factors other than nitrite may provide substantial protection. During the production of some products there may be opportunities for the multiplication of pathogens, e.g., staphylococci in fermented sausages if manufacturing practices are not optimal (Bryan, 1980). Thermal processing, final water activity, pH, and brine concentration in some products exert considerable control over some pathogens, in certain cases totally restricting their growth. Refrigeration is an effective means of controlling pathogen growth, but some products, e.g., shelf-stable canned cured meats, are not normally refrigerated. Thus, if other controls fail, refrigeration does not play a part in ensuring their safety. Consumers show greater disregard for the need to refrigerate some perishable products, e.g., ham, than for others, e.g., frankfurters (Bryan, 1980).

Taking all these factors into account, it is possible to rank product types in terms of the extent to which nitrite is desirable to ensure their freedom from microbial hazard to health. The committee will present such an evaluation in its second report.

The extent to which inhibitory factors other than nitrite (e.g., brine concentrations) can be modified to provide protection against specific microorganisms is dependent on many considerations that may vary with different products. For example, increased salt might not be detectable in some meat products but may be unacceptable in fish. Additionally, the potential of such changes for producing adverse health effects, e.g., the influence of increased salt intake on hypertension, need evaluation. Such considerations will also be discussed in the second report.

ANTIOXIDANT EFFECTS OF NITRITE

Effects of Lipid Oxidation on Flavor

The fat (lipid) component of meat contributes substantially to its flavor, texture, and public acceptability. The basic meaty flavor resides in the water-soluble fraction of meat, but the flavor that distinguishes pork from beef or fish, for example, resides in the lipid fraction (Hornstein, 1967; Hornstein et al., 1960). Because lipids are sensitive to oxidative changes, it is not surprising that such changes can markedly affect flavor. For example, the rancidity that can develop in stored meat is due to lipid oxidation.

Tims and Watts (1958) discussed the rapid development of a rancid or stale flavor due to lipid oxidation that occurred in refrigerated cooked meats within 48 hours of storage at 4°C. They were the first to describe this as "warmed-over flavor" (WOF). In contrast to cooked meats, the onset of rancidity in raw meats, fatty tissues, rendered fat, or lard is usually much slower, not normally becoming apparent until these products have been stored for weeks or months (Pearson et al., 1977). However, Sato and Hegarty (1971) suggested that WOF also develops rapidly in raw meat that has been ground and exposed to the air, but the term has been most often applied to cooked meat.

To understand why and how meat becomes rancid, it will be helpful to discuss the two types of fat found in meat: storage (adipose) fat and structural fat. Storage fat is set aside in the animal for emergency energy needs and also for insulation of vital organs. This fat exists as globules within special fat cells of the adipose tissue, which is not part of the actual muscle structure.

The storage fat of animals consists mainly of saturated fatty acids, chemically stored as triglycerides. Saturated fatty acids are not oxidizable under the conditions of storage likely to be encountered during distribution and in retail stores. Storage fat also contains oxidizable monounsaturated fatty acids, mainly oleic (C₁₈:1), and polyunsaturated fatty acids such as the diunsaturated linoleic (C₁₈:2) and the triunsaturated linolenic (C₁₈:3) acids. The types of fatty acid present will depend on the species. The greater the percentage of unsaturated fatty acids, especially those that are polyunsaturated, the greater the degree of susceptibility to oxidation. The polyunsaturated fatty acid contents of various products are lamb < beef < pork < chicken < turkey < fish (Pearson *et al.*, 1977; Wilson *et al.*, 1976). For example, certain species of fish are very difficult to store, even in the frozen state, without rancidity developing within a few days (Pearson *et al.*, 1977).

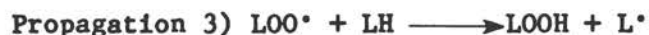
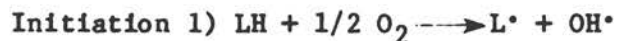
The fatty acid composition of the adipose tissue of nonruminants, i.e., swine and poultry, can be changed by altering the diet fed to the animals, because the fatty acids consumed by these animals are incorporated into the fat unaltered after ingestion. Current feeding practices generally favor the accumulation of a high percentage of unsaturated fatty acids in the adipose tissue of pork and poultry (Pearson *et al.*, 1977; Wilson *et al.*, 1976).

The second class of fat is structural. Each muscle cell of the meat is surrounded by a lipid-containing membrane. Membranous structures also criss-cross the interior of the cell. Membrane lipids are composed of the unsaturated fatty acids, linoleic and linolenic acids, and arachidonic and longer chain polyunsaturated fatty acids. They are all highly susceptible to oxidation. The fatty acids in membranes exist in phospholipid structures and are sometimes classified as such. The amount of membrane lipid is constant per unit muscle mass, whereas the amount of storage fat can vary enormously. Because of the high percentage of polyunsaturated fatty acids, membrane lipids are especially vulnerable to oxidation (Wilson *et al.*, 1976).

Chemistry of Lipid Oxidation

Oxidation disrupts the double bonds in lipids forming peroxides, polymers, and a variety of breakdown products, including aldehydes, ketones, and short-chain fatty acids. These small volatile molecules are the main contributors to rancid odor and flavor.

Oxidation appears to be a chain reaction involving a free radical mechanism:



According to Lundberg (1962), this autocatalytic reaction is initiated when a labile hydrogen (H) is detached from the lipid molecule (LH), resulting in the production of a lipid free radical (L[•]) and a hydroxyl radical (OH[•]). Reaction of the lipid free radical with oxygen (O₂) yields a peroxy radical (LOO[•]). This radical removes a hydrogen from another lipid molecule, thereby propagating the entire system. Decomposition of the lipid peroxide (LOOH) species forms more free radicals, giving rise to further chain reactions.

Catalysis and Mechanism of Lipid Oxidation

Muscle tissue contains a considerable amount of iron bound to proteins. Myoglobin, which resembles hemoglobin, is an oxygen storage protein within the muscle cells. Muscle tissues also probably contain residues of hemoglobin from blood. In addition, cells contain cytochromes. All these proteins contain the prosthetic group, heme, which has an iron atom at its center. The iron atom by itself promotes autoxidation of fats, but the entire iron-heme molecule appears to participate in the mechanism.

Tarladgis (1961) noted that in methemoglobin, for example, this iron has five unpaired electrons, which create a strong magnetic field that favors free radical formation. He suggested that decomposition of hydroperoxides could be mediated through donation of an electron from the cloud of the porphyrin ring.

A general scheme for the acceleration of lipid oxidation has been proposed by Tappel (1962). The principal mechanism seems to be catalysis of the decomposition of lipid peroxides (LOOH) by heme to form a lipid radical (L[•]) and a heme radical. The heme radical removes a hydrogen atom from another lipid molecule (LH), regenerating heme and, at the same time, generating a new lipid radical (L[•]).

Tappel also suggested that a heme molecule could attack a lipid directly:



These schemes and equations are those of chemical theory. In meat, the interactions are probably vastly more complex. Oxidation in meat occurs more rapidly than one would expect from consideration of the theoretical chemistry. There is also evidence that heme can also inhibit oxidation of lipids when it is present in an amount relatively higher than that of the fatty acids (Kendrick and Watts, 1969). In meat, however, the actual chemistry is difficult to ascertain.

Igene et al. (1979), Love and Pearson (1974), and Sato and Hegarty (1971) have suggested that non-heme iron is the major prooxidant in cooked meat. Igene et al. (1979) further demonstrated that non-heme iron is released from the heme pigments by cooking or by treatment with hydrogen peroxide, thereby accelerating lipid oxidation. These results agreed with the report by Haurowitz et al. (1941) that the prooxidation effect of hemin or hemoglobin on linoleic and linolenic acid is due to release of inorganic iron. It is possible that other polyvalent cations could also be prooxidants in meat and play a role in warmed-over flavor (WOF). However, Sato and Hegarty (1971) demonstrated that cupric salts actually inhibited WOF, apparently by the reaction of free radicals with cupric ions. Thus, non-heme iron appears to be the major prooxidant in the development of WOF (Igene et al., 1979).

Antioxidant Role of Nitrite in Cured Meats

Nitrite has been shown to retard lipid oxidation or development of WOF in cooked meat and processed meat products. Sato and Hegarty (1971) were able to eliminate WOF in cooked ground beef by adding sodium nitrite at a concentration of 2,000 mg/kg of beef and inhibit WOF at a sodium nitrite concentration of 50 mg/kg, as indicated by 2-thiobarbituric acid (TBA) values, a measure of lipid oxidation. Fooladi et al. (1979) investigated the role and function of nitrite in preventing the development of WOF in cooked beef, pork, and chicken. Added sodium nitrite (156 mg/kg) effectively inhibited the development of WOF in the cooked meat, resulting in a twofold reduction in TBA values in beef and chicken and a fivefold reduction in pork. Sensory panel scores confirmed the protective effect of added nitrite in meat from all three species.

Using cooked hams, MacDonald et al. (1980b) studied the effects on lipid oxidation resulting from the addition of sodium nitrite at 50, 200, or 500 mg/kg, butylated hydroxytoluene (BHT), or citric acid. Their data indicate that there is a significant reduction in TBA values in pork cured with sodium nitrite. Treatment of meat samples with BHT and citric acid reduced TBA levels, but these compounds were not as effective as the lower concentration of sodium nitrite (50 mg/kg).

The role of nitrite in minimizing WOF in cooked cured meats is not yet thoroughly understood, although Pearson et al. (1977) suggested that nitrite may either stabilize the lipid components of the membranes or inhibit the natural prooxidants in the muscle. Zipser et al. (1964) reported that nitrite exerts its effect by chelating iron, thereby preventing it from catalyzing oxidation. This concept has been investigated by MacDonald et al. (1980a), who found that dialysis of an aqueous extract of pork removed a fraction largely responsible for catalysis of lipid oxidation. The dialysate contained iron, and

the investigators concluded that nitrite acts by binding iron and other trace metals. Similarly, Igene et al. (1979) showed that the addition of 2% EDTA chelated non-heme iron effectively, resulting in a significant reduction in lipid oxidation in cooked meat.

The antioxidant properties of other nitrite derivatives in meat have also been demonstrated. S-Nitrosocysteine, a compound generated during the curing of meat, has been shown to act as an antioxidant both in aqueous linoleate model systems and in ground cooked turkey meat (Kanner and Juven, 1980). Kanner et al. (1979) also reported antioxidant activity of nitric oxide myoglobin (NOMB) in linoleate and β -carotene-linoleate aqueous model systems. The specific antioxidant activity of NOMB was maintained, even in the presence of prooxidants such as heme proteins and lipoxygenase.

Health Implications of Lipid Oxidation

Malonaldehyde (OHCH_2CHO) is produced by the oxidation of polyunsaturated fatty acids. Interest in its possible effects on human health has been stimulated by reports that it is mutagenic (Mukai and Goldstein, 1976). Brooks and Klammerth (1968) reported that malonaldehyde may react with DNA, thereby providing a possible rationale for its mutagenic activity. However, Marnett and Tuttle (1980) suggested that the observed mutagenicity of malonaldehyde may be due in substantial part to impurities resulting from the method used to prepare it. According to Shamberger et al. (1974), malonaldehyde is carcinogenic in mouse skin, when it is dissolved in acetone and applied topically.

Malonaldehyde was also tested as a complete carcinogen in Swiss mice by Apaja (1980), who applied the compound dermally in methanol and administered it orally in drinking water. When the compound was given to the mice in drinking water, it induced toxic effects in the stomach, where there was destruction, inflammation, and fibrosis of the glandular mucosa. Results of the chronic studies showed no tumors that could be attributed to malonaldehyde. Apaja (1980) concluded that malonaldehyde is not a complete carcinogen for Swiss mice.

A recent survey of the malonaldehyde content of 96 samples of fresh and processed meat and fish indicated that 92% of the processed or cured meats and 38% of the fresh meat contained less than 1 mg/kg. Sixty percent of the fresh meat samples contained concentrations of malonaldehyde ranging from 1 to 6 mg/kg (Siu and Draper, 1978). Whether these reported concentrations of malonaldehyde in meats have significance for human health is unknown, but reports that this compound may be toxic emphasize the desirability of minimizing its formation during storage and marketing.

Discussions and Conclusions

Concern about the implications of lipid oxidation products in meats has focused on changes in consumer acceptability and loss of marketability rather than on the health hazard that they could present. Nevertheless, lipid oxidation should be considered in the overall safety assessment of meat, and its occurrence should be minimized in consumer products. Nitrite added to meat products inhibits oxidation and is particularly important in comminuted products into which air may be incorporated during manufacture. Some evidence indicates that the antioxidant effect of nitrite is greatest in pork products. The efficacy of alternative antioxidants will be discussed in the second report of this committee.

EFFECTS OF NITRITE ON SENSORY PROPERTIES OF MEAT PRODUCTS: FLAVOR, COLOR, AND TEXTURE

Chemical Aspects of the Formation of Cured Meat Flavor

The flavor of meat is a complex combination of characteristics such as taste, odor (aroma), texture, and temperature (Lawrie, 1974). Among these, aroma is of special significance, which is discussed below. Some studies have provided important insights into the development of flavor, but the precise origin of flavor remains uncertain. Studies conducted during the last 20 years, indicate that flavor results from a combination of volatile compounds produced during the heating of meat (MacLeod and Coppock, 1976). Heating also releases precursors to flavor from fat structures and allows intimate mixing of fat-soluble and water-soluble components (Herz and Chang, 1970).

Meat flavor is derived from both water-soluble (Hornstein and Crowe, 1960; Lawrie, 1974) and fat-soluble (Sink, 1973) nonvolatile precursors. The water-soluble precursors are low molecular weight compounds, including glycoproteins, reducing sugars, amino acids, and their degradation products (Batzer et al., 1960, 1962; Hornstein and Crowe, 1964; Wasserman and Gray, 1965). Precursors to meat flavor per se are similar in all species, whereas the difference in flavor among species is associated with the lipid fraction (Hornstein et al., 1960).

According to Chang and Peterson (1977), precursors to flavor, which may be unique to a given type of meat, are leached out of the aqueous phase during cooking and stored in the fat. In support of this suggestion is the fact that refined animal fat cooked by itself does not produce characteristic meat flavors or aromas. However, lipids themselves are not responsible for the formation of the sulfur- and nitrogen-containing heterocyclic compounds present in the volatile fraction of cooked meat.

Improved gas chromatographic and mass spectrometric techniques have facilitated the isolation and identification of many compounds from different meats. For example, more than 300 different compounds have been identified in the volatile fraction of cooked beef (MacLeod and Coppock, 1976). Aliphatic and aromatic hydrocarbons, saturated alcohols, carboxylic acids, esters, ethers, aldehydes, and ketones are probably not primary contributors to meat flavor, according to a report by Chang and Peterson (1977). However, these investigators suggested that lactones, acyclic sulfur-containing compounds (mercaptans and sulfides), nonaromatic nitrogen-, oxygen-, and sulfur-containing heterocyclic compounds (e.g., hydrofuranoids), and sulfur-, nitrogen-, and oxygen-containing aromatic heterocyclic compounds (pyrazines and thiophenes) are probably the main contributors to meat flavor.

Despite the fact that many compounds have been identified in meats, none have been shown to be responsible for the specific characteristic flavors of the various products (Chang and Peterson, 1977; Herz and Chang, 1970). Many of the volatile components thus far identified in cured meats have also been found in uncured meats. No specific component or components possess the characteristic "cured" flavor of cured meat.

There have been many sensory analyses of cured meat flavor (Gray et al., 1981), but relatively few reports on the chemical interactions of nitrite and meat constituents that influence flavor (Bailey and Swain, 1973; MacDougall et al., 1975). Although nitrite is closely associated with cured meat flavor, especially that of ham (discussed later in this chapter), the chemical changes responsible for the unique flavor are not entirely understood. As a result, many investigators have attempted to identify the volatile compounds produced during the curing of meat.

Ockerman et al. (1964) conducted the first major study in this area. They extracted volatile compounds from dry-cured hams by vacuum distillation, collected them in a series of cold traps, and analyzed them by gas chromatographic retention times and infrared spectroscopy. The investigators identified aldehydes, ketones, acids, bases, and sulfur compounds, all of which are known to contribute to uncured meat flavor (Landmann and Batzer, 1966). The contribution of carbonyl compounds to the flavor of uncured meats has also been reported by Hornstein and Crowe (1963), Jacobsen and Koehler (1963), and Sanderson et al. (1966).

Cross and Ziegler (1965) isolated volatile compounds from both cured and uncured ham. They reported that the volatile compounds (mainly aldehydes) were qualitatively similar, but that the concentrations of these compounds varied among the products. For example, concentrations of pentanal and hexanal were higher in the uncured product than in the cured product. These investigators also observed that the volatile compounds retained a characteristic cured ham aroma

after passage through a solution of 2,4-dinitrophenylhydrazine, regardless of whether they originated from cured or uncured ham. After similar treatment, volatile compounds extracted from cured and uncured chicken and beef also had an aroma similar to that of cured ham. These authors proposed that cured meat flavor was derived from nonglyceride precursors, rather than from carbonyl compounds. Chang and Peterson (1977) also believe that carbonyl compounds are not major contributors to the flavor.

Using a vacuum distillation system equipped with cold traps, Lillard and Ayres (1969) extracted carbonyl compounds (e.g., alkanals, alk-2-enals, alk-2,4-dienals, and ketones), alcohols, and esters from country cured hams. The odor of the distillate, which collected in the traps, was reminiscent of cured ham. However, many of these compounds have been identified in the volatile fractions from other cooked meats. In another comprehensive study of the volatile constituents from cured ham, Swain (1972) reported that the aroma from ether-extracted ham resembled that of boiled ham. He also found that gas chromatograms obtained by capillary columns indicated that volatile components from cured hams treated with and without nitrite were qualitatively similar but quantitatively different. Among the classes of compounds identified were acids, aldehydes, alcohols, furans, ketones, hydrocarbons, nitrogen and sulfur compounds, and aromatics. The formation of several of the higher molecular weight aldehydes ($>C_5$) appears to be retarded by nitrite.

Piotrowski et al. (1970) isolated and identified cured ham aromas by studying various extracts from hams. They also observed changes in the constituents of pork during curing, cooking, and smoking. A trained panel characterized aqueous extracts and diffusates of cured and uncured hams. The panelists also assessed the odor produced upon heating. Aqueous extraction of all types of hams isolated the precursors of basic meaty aroma, whereas components or precursors of cured and smoky aromas were extracted from hams with a mixture of chloroform-methanol (2:1, v/v). The volatile substances developed during the heating of ham diffusates and lipid extracts were analyzed by gas chromatography, which indicated that there were some variations among the patterns of volatile compounds from six types of hams. But no single component had a meaty or cured aroma. These investigators concluded that compounds of intermediate volatility may be important contributors to meat flavor as well as better indicators of the differences between cured and uncured hams.

Few studies have been conducted on volatile compounds extracted from bacon. Using gas chromatography, Mottram and Rhodes (1974) found no clear-cut difference in the patterns of peak retention times for extracts of volatile substances obtained from cured and uncured boiled Wiltshire bacon. Many observed peaks were complex and represented more than one substance.

In summary, most studies on the chemical nature of cured meat flavor have been conducted with gas chromatography using packed columns. Compounds have not generally been identified by mass spectrometry or other rigorous analytical methods.

There appears to be a general consensus that carbonyl compounds are involved in the difference detected between the aromas of cured and uncured meats, but that other, as yet unidentified, components may also contribute to cured flavor. There are few data pertaining to the chemical basis for any contribution nitrite might make to cured meat flavor. MacDougall *et al.* (1975) suggested that cured meat flavor is probably a composite sensation derived from the contributions of many different odiferous compounds. The development of more sophisticated gas chromatographic procedures for use in conjunction with identification methods, such as mass spectrometry, could provide the stimulus for further studies in this area.

Chemical Aspects of the Formation of Cured Meat Color

The bright appearance created by the oxymyoglobin in fresh red muscle and the reddish-pink hue of denatured nitrosylmyohemochrome in cured meat products are attributes recognized by the consumer. Although the color of a meat product does not necessarily predict good texture and flavor, the shopper tends to make such an association (Giddings, 1977a; Jeremiah *et al.*, 1972).

The color of both fresh and cured meat products is attributable primarily to the hemoprotein pigment, myoglobin (Clydesdale and Francis, 1971; Fox, 1966; Giddings, 1974, 1977a,b; Govindarajan, 1973). Figure 3-2 portrays the dynamic equilibrium between the various forms of myoglobin and demonstrates that the resulting hue of a meat product is dependent upon the oxidation state of the heme iron in the pigment and the type of functional group of the sixth ligand of the iron (Fox, 1966). The color of raw or fresh muscle tissue, such as beef or pork, is due to the dark red pigment, myoglobin (Mb); the cherry-red pigment, oxymyoglobin (O_2Mb); and the brown pigment, metmyoglobin (MMb) (Clydesdale and Francis, 1971; Reith and Szakaly, 1967a). Many factors influence the stability of these pigments (Clydesdale and Francis, 1971; Fox, 1966; Giddings, 1977a, b), and it is well known that they are not at all stable when the muscle tissue is heated (Reith and Szakaly, 1967a). To obtain a more stable red pigment in heated commercial meat products, nitrite is added before heating. Biochemical reactions in the meat reduce the nitrite to nitric oxide and the heme iron in myoglobin to the ferrous state. The interaction of these two species results in the formation of nitric oxide myoglobin (NOMb), a bright red pigment. When the meat product is then heated, the protein portion of NOMb is denatured and a rather stable pigment is formed, namely nitric oxide myohemochrome (DNOMb) (Clydesdale and Francis, 1971; Fox, 1966; MacDougall *et al.*,

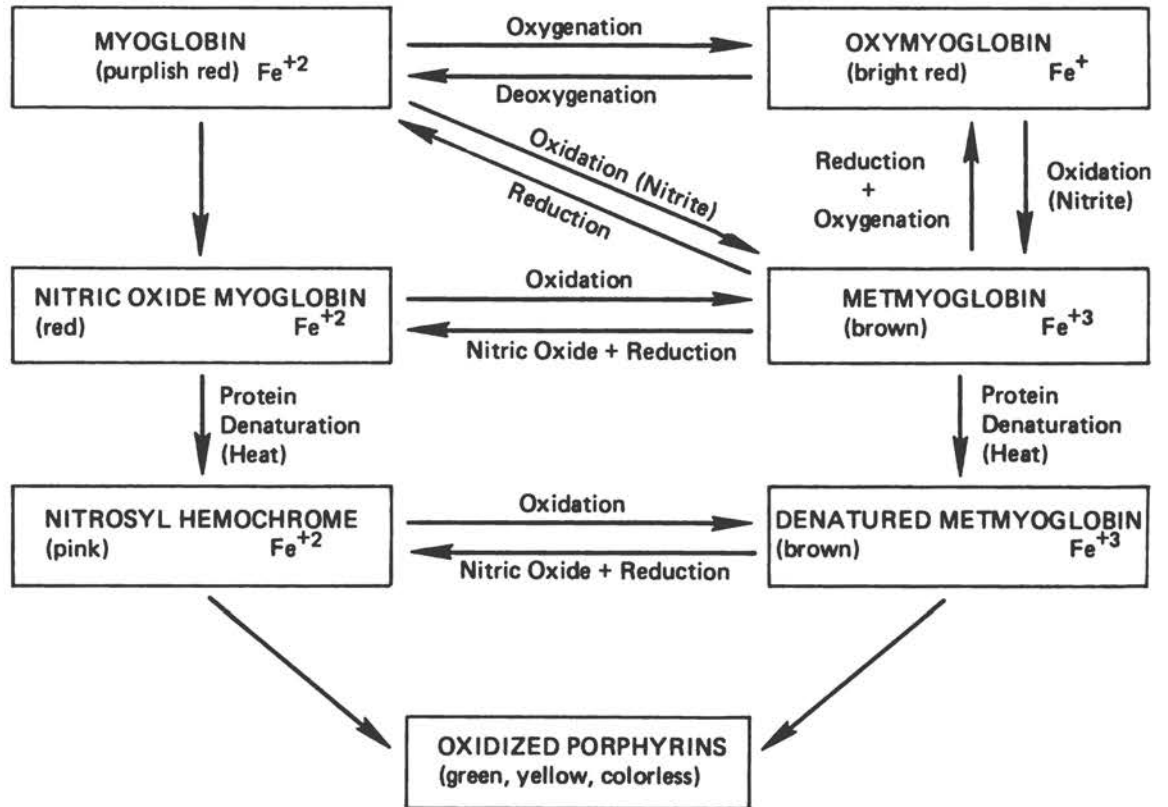
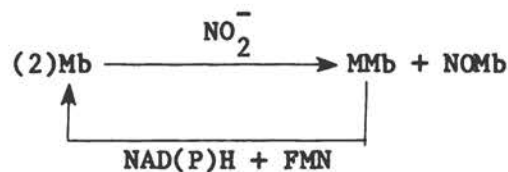


FIGURE 3-2. Some of the possible curing reactions that result from the addition of nitrite (Bard and Townsend, 1971).

1975; Reith and Szakaly, 1967a). Lee and Cassens (1976) reported that the amount of ^{15}N from labelled nitrite bound by heated samples of myoglobin was twice that bound by unheated samples. They postulated that the number of binding sites for nitric oxide doubled upon heating.

The precise sequence of events resulting in the formation of the cured meat pigment is not fully understood. It is known that nitrite and myoglobin react under anaerobic conditions to produce NOMb and MMb (Giddings, 1977a). This implies that Mb can reduce nitrite to nitric oxide directly. In the presence of excess nitrite and a reducing system, the MMb formed is readily converted to the reduced form to participate again in the formation of nitric oxide and NOMb.

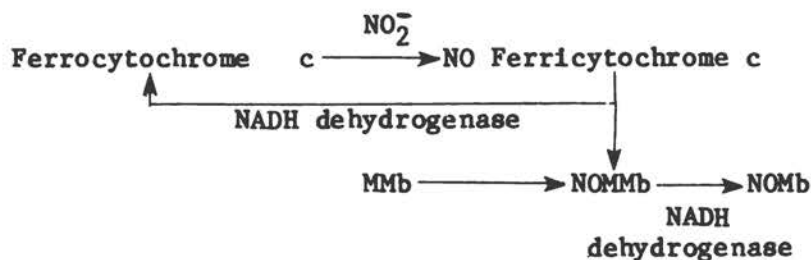
Several mechanisms have been hypothesized for the reduction of MMb in the presence of nitrite. The coenzyme systems, NADH (reduced nicotinamide adenine dinucleotide) or NADPH (reduced nicotinamide adenine dinucleotide phosphate) plus FMN (flavin mononucleotide), were studied by Koizumi and Brown (1971), who observed that these systems readily produced NOMb from MMb. This mechanism for the formation of NOMb can be shown as follows:



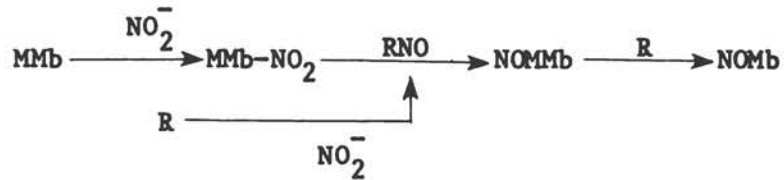
FAD (flavin adenine dinucleotide) and riboflavin can also promote the formation of NOMb by NADH, but nitrite was not reduced to nitric oxide in the absence of Mb.

Koizumi and Brown (1971) also studied a model enzyme-reducing system, diaphorase-methylene blue-NADH, which was effective in the formation of NOMb, but did not reduce nitrite in the absence of Mb. This proposed mechanism differs radically from the chemical scheme or enzymatic reactions proposed by others.

Walters and coworkers studied the participation of endogenous enzymes of the mitochondria in the reduction mechanism (Walters and Taylor, 1963; Walters *et al.*, 1967). They observed that the formation of NOMb can proceed via nitric oxide metmyoglobin (NOMMb) by the transfer of nitric oxide from nitric oxide ferricytochrome c to MMb. Nitric oxide ferricytochrome c is formed from the reaction of nitrite and ferrocytochrome c. The NOMMb formed is then reduced to NOMb by NADH dehydrogenase. The cytochrome system is recycled by an NADH dehydrogenase. This system can be summarized as follows:



Fox and Thomson (1963) showed that after MMB was formed by the action of nitrite, it formed the ionic complex metmyoglobin-nitrite (MMb-NO₂⁻), which then reacted with a semistable nitric oxide reductant intermediate to give NOMMb, which then reduced to NOMb:



In this scheme, R represents a naturally occurring or added reducing compound. The scheme does not rely on enzymes, but, rather involves coenzymes and/or other reducing compounds, such as cysteine, hydroquinone, or ascorbic acid (Fox and Ackerman, 1968). This mechanism explains how color formation can occur before and after the product is cooked, whereas heating would eliminate the possibility of enzyme-coupled reductions, which were suggested by Koizumi and Brown (1971) and by Walters and Taylor (1963). Because ascorbic acid has been reported to be a very effective reductant in promoting color formation, it is usually incorporated into cure formulations to accelerate the development and increase the stability of cured meat color (Fox, 1966; Fox et al., 1967; Watts and Lehman, 1965).

All of these hypothesized mechanisms must be considered in light of the numerous factors that influence the rate and extent of NOMb formation in model and meat systems. Such factors include the type and/or relative concentrations of exogenous reductants, pH, the presence of salt/metal ions, storage temperature, level of nitrite added, temperature reached during heating or cooking processes, exclusion of oxygen during formulation, and the original pigment concentration in the meat (Acton et al., 1979; Fox et al., 1967; Giddey, 1966; Reith and Szakaly, 1967a,b; Renner and Rougie, 1979; Siedler and Schweigert, 1959; Watts and Lehmann, 1965; Weiss et al., 1953).

Once formed, the complex of myoglobin and nitric oxide is very stable in the absence of oxygen. In the presence of oxygen, which rapidly oxidizes free nitric oxide to nitrite, the stability of the complex is limited by the rate of nitric oxide dissociation since oxygen does not react directly with the bound nitric oxide (Giddings, 1977a,b). This dissociation rate is generally low, occurring by autoxidation in air (Walsh and Rose, 1956), oxidation by nitrous acid (Walsh and Rose, 1956), lipid-peroxide-induced oxidation (Younathan and Watts, 1959), or by photocatalyzed oxidation (Bailey et al., 1964; Walsh and Rose, 1956). The underlying principle for all such mechanisms of nitric oxide-heme dissociation is believed to involve the withdrawal of electron density from iron to porphyrin, which weakens the Fe-NO bond. The nitric oxide group dissociates, leaving the iron susceptible to oxidation by the "electronegative groups"

present in the medium (Tarladgis, 1962a,b). Such color loss is believed to be delayed by providing stronger reducing conditions in the medium (Bailey et al., 1964; Lin et al., 1980; Reith and Szakaly, 1967a; Tarladgis, 1962a,b), by incorporating nitrite exceeding the Mb level (Reith and Szakaly, 1967a,b; Tarladgis, 1962a,b; Walsh and Rose, 1956), by preventing exposure to energy-generating electronic excitation, e.g., light (Tarladgis, 1962a,b; Walsh and Rose, 1956), by replacing NO-based curing salts with nitrogenous compounds possessing strong electron-donor power (Siedler and Schweigert, 1959; Tarladgis, 1962a,b), by eliminating oxygen during storage (Fox, 1966; Reith and Szakaly, 1967b), by using packaging films with low oxygen permeability ($7 \text{ cm}^3/\text{m}^2/24 \text{ hr bar}$) combined with a maximum initial vacuum level of 687 to 737 mm Hg (Kraft and Ayres, 1954; Lin and Sebranek, 1979; Lin et al., 1980), and/or by increasing the pH of the product (Bailey et al., 1964; Reith and Szakaly, 1967a; Walsh and Rose, 1956).

The level of nitrite necessary to produce an "acceptable" color in various cured meats is discussed below.

Chemical Aspects of Cured Meat Texture

The reaction of nitrite with the non-heme proteins of meat probably alters the texture of the meat. However, this association is difficult to prove since measurements of the reactions with the protein are much more sensitive than the relative crude measurements used for the meat's texture. Moreover, the salt in cured meat also plays a major role in determining the physical properties of the product. Randall and Voisey (1977) concluded that nitrite did not affect the texture of ham and frankfurters.

Sensory Evaluation of Cured Meat Flavor

Although we typically speak of "tasting" flavors in foods, smell is the sense most involved in the identification of flavors (Mozell et al., 1969). The only qualities universally accepted as being discernible by taste are sweetness, saltiness, sourness, and bitterness. On the other hand, through the sense of smell we perceive such a complex array of qualities that no universally accepted classification scheme has been developed (Cain, 1978). When food is placed in the mouth, volatile compounds ascend the rear of the oral cavity to the olfactory mucosa, thereby effectively stimulating the sense of smell.

In 1940, Brooks et al. suggested that sodium nitrite produces a characteristic "cured" flavor. Subsequent studies on the sensory characteristics of cured meats have attempted to determine if a cured meat flavor really exists and, if so, what produces it. Understanding the methods used in these studies and the difficulties in interpreting observations is essential to evaluating the sensory studies that have addressed "cured meat flavor." These issues are discussed below:

Methods. Sensations vary in quality and intensity. Methods have been designed to measure these sensory experiences.

Discriminative methods of sensory evaluation deal with the ability to detect differences. For example, the classic triangle test is a standard test in food technology in which a subject attempts to choose the odd sample from a group of three in which two samples are identical but the third differs. If a subject can choose the odd sample more often than would occur by chance, the odd sample is perceived to be different. However, this test does not indicate how the samples differ.

Descriptive methods of sensory evaluation permit an assessment of the way in which substances differ. The critical part of these methods is the choice of the attribute(s) to be described. For example, lemonade and milk have different tastes. There are many sensory qualities on which the two substances could be compared (e.g., sweetness and sourness). The selection of the relevant attributes is complex when foods are compared. The classic solution to this problem has been to train panels to recognize specific characteristics.

Scaling is also used in sensory evaluation. Once the attributes have been selected, one can simply ask for discriminative judgments (e.g., which sample is sweeter?), or the selected attribute can be quantified with a scaling procedure. The most common scaling procedures in use with foods are category scales. These scales are called "ordinal" scales because they can only order stimuli. For example, a person might be asked to assign the numbers 1 through 9 to a series of salt solutions on the basis that "1" means "weak," "5" means "medium," and "9" means "very strong." Ordinal scales express only order; they do not reflect the size of differences. For example, on a 9-point ordinal scale, a solution with a value of "4" is not necessarily twice as salty as one with a scale value of "2." The "4" indicates only that the solution is saltier than the one numbered "2."

When the size of the difference is important, then ratio scales are appropriate. One of the most commonly used is "magnitude estimation." With this method, subjects assign numbers that are proportional to the perceived intensities of the stimuli. In this scale, a value of "4" would reflect a stimulus with an intensity twice as great as one producing a value of "2."

Panel Selection. Panels used in the sensory evaluation of foods differ in their experience with foods and, possibly, with regard to sensory ability. Consumer panels are comprised of consumers with no special training beyond the specific instructions required by the task. Trained panel members do receive special training with food samples and may also be tested to ensure that their sensory abilities meet some standard. For example, this training might involve tasting

a variety of nitrite-cured meats in order to familiarize them with the attributes of "cured meat flavor." Such training often involves group discussions to develop descriptive terms that can be adopted by all participants. An individual who consistently disagrees with the majority, or who cannot perceive attributes generally agreed upon, is often dropped from such a panel. Expert panels tend to be composed of individuals with extensive experience in tasting the food products of interest (Amerine et al., 1965, pp. 275-320). Obviously, the type of panel used for sensory evaluation will influence the results.

Individual Differences and the Search for Cured Meat Flavor.

Identical food samples do not have the same flavors for all individuals (Amoore, 1977; Bartoshuk, 1979). This interindividual variation in sensory perception may contribute to the variability observed in different sensory studies of cured meat flavor. Chemosensory sensitivity must influence studies of the ability to discriminate nitrite-cured from nitrite-free samples. If insensitive individuals are routinely eliminated from trained or expert panels, then these panels could be expected to detect differences more reliably than would a random sample of the population. This is acceptable when such a panel is used for quality control, but the results cannot be used to predict consumer behavior.

Methods Used in Studies on the Effect of Nitrite. Discriminative methods have commonly been used to determine whether or not nitrite-treated samples can be distinguished from controls.

Cho and Batzler (1970) were the first to use these methods systematically to study the contribution of nitrite to the flavor of cured meat. They used two sensory tests: the "triangle test," which tests panelists' ability to discriminate between samples, and the "two-sample test," in which panelists are asked to select the sample with more "cured flavor." The meat products used in these tests were pork roasts, i.e., porcine longissimus dorsi muscles. The panelists compared nitrite-cured and nitrite-free samples with variations in the amount of sodium chloride, the presence or absence of sugar, and smoking.

The comparison of a roast cured in a nitrite-containing pickle with its pairmate "cured" in distilled water produced the most dramatic results. In three replications of the triangle test, 14 of 23, 11 of 19, and 11 of 18 panelists correctly discriminated between nitrite-containing and nitrite-free samples, i.e., nine, eight, and seven panelists made errors. In three replications of the two-sample test, 19 of 23, 16 of 19, and 14 of 18 panelists judged that the nitrite-cured sample had the most "cured flavor." For this test, four, three, and four panelists selected a sample of pork roast "cured" in distilled water as having the most "cured flavor."

Results obtained from both the triangle test and the two-sample test provide statistically significant evidence for discrimination. That is, more panelists correctly distinguished between nitrite-cured and nitrite-free samples (in the triangle tests) and identified the nitrite-cured sample (in the two-sample tests) than would have been expected if panelists were selecting randomly. But why did any of the panelists make errors? There are two possibilities. Some individuals may be insensitive to the "cured flavor" imparted by nitrite, whereas others recognize it easily. Alternatively, the magnitude of the "cured flavor" imparted by nitrite may have been very small. One could easily determine which of these alternatives applied simply by noting whether or not specific individuals were selecting nitrite-containing samples reliably.

A number of studies using ordinal scaling methods have been conducted since Cho and Batzler reported their studies in 1970. These studies are discussed below in the section in which the contribution of nitrite to different products is assessed. Since ordinal methods only rank the cured flavor of one sample as greater than that of another sample, they do not indicate the magnitude of the difference. Thus, a study may indicate, in a statistically reliable sense, that a nitrite-cured meat has more cured flavor than a nitrite-free control, but the difference could be very small.

To the best of the committee's knowledge, no ratio scaling methods have been used to evaluate the magnitude of the contribution of nitrite to cured meat flavor. Nor was it able to find any reports of studies in which ratio scaling methods were used to assess the degree to which the flavors of two samples varied.

The color of the product can influence evaluations of cured flavor (DuBose et al., 1981). Thus, the color should be concealed when such a sensory evaluation is being conducted.

Contributions to Cured Meat Flavor by Factors Other Than Nitrite. In some products, cured meat flavor can be produced by sodium chloride alone (Greene and Price, 1975; MacDougall et al., 1975; Wasserman et al., 1977; Williams and Green, 1979). Smoking also plays an important role in cured meat flavor in some products (Wasserman and Talley, 1972). The generation of smoke may result in the simultaneous generation of nitrogen oxides, which are available for the production of color and flavor in the final product in a manner similar to that of added nitrite. The extent of such reactions is not known. Thus, the contribution of smoking to the overall color and flavor of cured meat products should be investigated further.

Because of the many variables in the type of meat product and processing, and because addition of the other flavoring agents, e.g., spices, may vary among product types, the contribution of nitrite to cured meat flavor must be assessed product-by-product.

Principles of Chemoreception That are Relevant to the Perception of Cured Meat Flavor. As compounds in cured meats are identified, there is an understandable tendency to smell or taste them to determine if they are sources of cured meat flavor. The failure of this approach to identify a nitrite-specific flavor compound or compounds would not disprove that nitrite contributes to cured meat flavor.

"Cured meat flavor" is probably produced by a volatile compound or compounds and is discerned by the sense of smell, which appears to "synthesize" or "fuse" at least some aroma mixtures. For example, the aromas of two compounds may be perceived as one that is qualitatively distinct from either of the component aromas alone (Amerine *et al.*, 1965, pp. 147- 148). Thus, "cured meat flavor" could be an aroma produced by a specific mixture of volatile compounds emitted by nitrite-cured meat. A compound-by-compound search might miss such an aroma mixture.

Hedonic Evaluation of Cured Meats

Hedonic Scaling. Rather than ask subjects to judge the sensory characteristics of meat samples, one can ask them to rate the product's appeal. They can either be asked to select the sample they prefer, or a scaling method (like those described for sensory tests) can be used to quantify how much a given sample is preferred. Both sensory and hedonic scales can be either ordinal or ratio scales.

Although hedonic testing asks the questions most pertinent to consumer preference, this method cannot easily produce answers to sensory questions. When asked to select the most preferred samples, consumers base their responses on color, flavor, texture, and probably several other criteria.

Other Measures of Preference. The behavior of consumers can be studied directly to provide information about acceptance. For example, Williams and Greene (1979) studied the amount of uneaten nitrite-free and nitrite-containing bacon left on plates in order to determine acceptance. As in other hedonic testing, this method cannot easily produce information on the sensory characteristics of products.

Product-by-Product Evaluation of Nitrite and Cured Meat Flavor

Bacon. Bacon with an acceptable flavor can be prepared without nitrite. In studies with untrained panelists, Huhtanen *et al.* (1981) and Wasserman *et al.* (1977) found no preference differences between nitrite-free and nitrite-cured bacon. These results were supported by Williams and Greene (1979), who found no differences between the amounts of nitrite-free and nitrite-cured bacon left on plates by breakfasting students.

Paquette et al. (1980) varied the amount of sodium nitrite in bacon samples from 0 to 120 mg/kg. Samples containing nitrite had a significantly more desirable flavor than did nitrite-free samples; however, the desirabilities of the various samples with nitrite did not differ significantly, regardless of the concentration added. Although the nitrite-free bacon had a less desirable flavor than the nitrite-cured bacon, it was still acceptable.

Both the nitrite-free and the nitrite-cured bacon samples in the studies cited above contained sodium chloride. Kimoto et al. (1976a,b) reported that sodium chloride is more important than nitrite to the flavor of bacon produced in the United States. The importance of sodium chloride was also demonstrated by MacDougall et al. (1975) in studies of English (Wiltshire) bacon. They compared the bacon flavor of sodium-chloride-free and nitrite-free bacon as well as that of bacon cured with varying amounts of nitrite. The sodium-chloride-free samples had almost no bacon flavor, but the salted, nitrite-free bacon did.

Frankfurters. During the preparation of frankfurters, salt and nitrite are added to meat emulsions along with spices, sugars, and seasonings. Often, these products are also smoked.

Wasserman and Talley (1972) demonstrated that smoking is an important determinant of the flavor associated with frankfurters. Their panelists gave equivalent ratings of such flavor to nitrite-free and nitrite-cured samples when both were smoked. When unsmoked, the nitrite-cured frankfurters had more "frankfurter" flavor than the nitrite-free frankfurters.

Simon et al. (1973) found that all-beef frankfurters with no nitrite or with varying levels of nitrite were judged to have equivalent flavor quality, whereas the quality of half-pork, half-beef frankfurters varied greatly with nitrite level.

The contribution of sodium chloride to the flavor of frankfurters has not been evaluated. However, Greene and Price (1975) found that salt was the major contributor to cured meat flavor in samples of ground pork, whereas sodium nitrite alone produced very little cured meat flavor when used at a level of 200 mg/kg.

Ham. Brown et al. (1974), MacDonald et al. (1980c), and DuBose et al. (1981) confirmed the results of Greene and Price (1975) that sodium chloride can produce cured flavor. For example, MacDonald et al. (1980c) showed that "nitrite-free" ham samples containing salt possessed a slight cured ham flavor. This explains findings that acceptable country-style hams (i.e., dry-cured hams) can be produced without nitrite (Eakes and Blumer, 1975; Eakes et al. 1975; Kemp et al., 1974).

Nitrite does make a contribution to cured flavor in hams cured in pickling solution. MacDonald *et al.* (1980c) cured hams with sodium nitrite levels of 50, 200, and 500 mg/kg. The lowest nitrite level, 50 mg/kg, was sufficient to produce a significant increase in cured meat flavor when compared to samples containing only salt.

DuBose *et al.* (1981) evaluated the influence of color in hams on flavor ratings. When color was concealed, low-nitrite samples were given higher ratings. When less red color was perceived in some samples, a lower rating was given to cured flavor. This "color-cueing" effect on flavor occurs at a level of nitrite that is lower than that currently used to cure ham.

Sensory Evaluation of Nitrite Levels Needed for Cured Color Formation

Only a small fraction of the nitrite added to a meat product is utilized for color fixation. Theoretically, only 3 mg of sodium nitrite per kilogram of product should provide a 50% conversion of Mb to NOMb (MacDougall *et al.*, 1975). However, more is usually necessary to provide color stability because of the effects of the many above-mentioned factors, which influence the stability of the nitrosyl hemoprotein pigments, and also because of the reaction of nitrite with other meat components, such as sulfhydryl or amino groups (Cassens *et al.*, 1974, 1979; Woolford and Cassens, 1977).

Kerr *et al.* (1926) noted that incomplete color formation resulted from insufficient nitrite penetration into the meat and/or unusually low myoglobin concentrations. This is exemplified by the fact that the minimum level of nitrite necessary to produce the desired color varies with the type of meat product, method of preparation, and presence of reductants such as ascorbate (MacDougall *et al.*, 1975; Sofos *et al.*, 1979a).

Using hedonic scales, Kemp *et al.* (1974, 1975) Eakes and Blumer (1975), and Eakes *et al.* (1975) reported that the application of sodium nitrite (at >250 mg/kg), potassium nitrate (up to 3,300 mg/kg), or their combination, to dry-cured hams resulted in color that was ranked more desirable (darker red) than the brownishgray hue observed in the salt and sugar-treated (control) sample. In both dry-cured hams and pork loins cured with sodium nitrite and/or potassium nitrate at 70-160 mg/kg, color was ranked significantly more acceptable when compared on a hedonic scale to that of products "cured" with no nitrate or nitrite (Eakes and Blumer, 1975). DuBose *et al.* (1981) reported that pickle-cured, smoked hams (prepared with sodium nitrite at 25, 75, or 156 mg/kg and rated on a hedonic acceptability scale) were not significantly different from each other in color, but did rank significantly more acceptable on color than did nitrite-free hams. However, Brown

et al. (1974) reported a difference in the color intensity of pickle-cured hams prepared with sodium nitrite at 91 or 182 mg/kg; the color was darker for the product containing the higher concentration of nitrite. The color of smoked turkey drumsticks cured in a pickling solution containing nitrite was found to be more acceptable than their nitrite-free counterparts (Olson et al., 1979).

In comparison to pickle- or dry-cured products, comminuted meats require lower nitrite levels for color development because the chopping/emulsification process increases the available surface area and enhances the distribution of nitrite. Wasserman and Talley (1972) and Hustad et al. (1973) reported gray color in unsmoked frankfurters prepared without nitrite in the cure. Similar results were found in the sensory evaluation of salami sausage (Skjelkvale et al., 1974) and Thüringer sausage (Dethmers et al., 1975). Hustad et al. (1973) reported no significant difference in the color of frankfurters prepared with sodium nitrite concentrations of 50, 100, or 156 mg/kg. The lowest level of nitrite, plus smoking, imparted a characteristic color. Concentrations of sodium nitrite as low as 40 mg/kg resulted in acceptable color in chicken frankfurters (J. I. Gray, personal communication, 1981) and in turkey frankfurters (Sales et al., 1980), whereas 50 mg/kg was necessary for characteristic color to appear in a beef-pork bologna product (Lin and Sebranek, 1979) and in Thüringer sausage (Dethmers et al., 1975). In general, as the level of nitrite is increased, color acceptability of products increases (Sebranek et al., 1977). The colors, as indicated by Hunter color values, become more red and less yellow (Sales et al., 1980).

EFFECTS OF NITRATE IN CURED PRODUCTS

The committee found no evidence that nitrate had any direct effects in cured products, but believes that certain of its indirect effects, in addition to its capacity to yield nitrite by bacterial reduction, may have practical or sensory implications for such products as fermented sausages and dry-cured cuts.

Since nitrate is less reactive than nitrite (Chapter 4) and is not generally altered in foods, except by bacterial action, it can act as a reservoir from which nitrite can be produced over time. In certain cured products requiring long production times, e.g., fermented sausages and dry-cured cuts, it may be more practical to use nitrate than nitrite.

Certain species of bacteria, such as some micrococci (Buchanan and Gibbons, 1974), can reduce nitrate to nitrite. In the production of certain types of fermented sausage, especially European-style products (some of which are made in the United States), the availability of nitrate at the outset of the fermentation will promote the development

of a mixed microflora containing such organisms, which are capable of reducing nitrate to nitrite. The metabolic products (e.g., acids), and possibly other contributors to flavor produced by these organisms in the subsequent fermentation and processing steps, are most probably different from those of the microflora that develops or is added (as starter culture) to products containing only nitrite. Thus, nitrate probably contributes indirectly to the traditional and characteristic flavor of these products (B. Tompkin, Swift and Co., personal communication, 1981).

Catalase, an enzyme produced by micrococci in the mixed flora, also appears to reduce peroxide development, thereby enhancing color stability and reducing the development of rancidity (Andres, 1977).

For products other than those noted above, the committee concludes that nitrite added at the minimum level necessary to achieve the desired effect could be substituted for nitrate.

The committee's findings on the effects of nitrate in fermented sausages and dry-cured cuts are based on limited information. The way in which nitrate acts in these and other products needs further investigation to substantiate conclusions discussed above.

SUMMARY: FINDINGS AND CONCLUSIONS

In the United States, nitrite and nitrate are added to cured red meats, poultry, and fish. The effects they exert depend upon the product to which they are added.

The committee found no evidence that nitrate had any direct effects in these products. However, it believes that certain of its indirect effects, in addition to its capacity to yield nitrite by bacterial reduction, may have practical or sensory implications for certain cured products, such as fermented sausages and dry-cured cuts.

Nitrite is used predominantly in cured meats. The motivation for its use is multifaceted. The effects of nitrite at current levels of use are shown in Table 3-7 for the major classes of cured red meats and poultry. The rankings of the relative importance of the various factors inhibiting spoilage microorganisms or pathogens are judgments of the committee based on data pertaining to U. S. products and, in a few cases, on data from studies in other countries. Additional references to the information in this table can be found in the applicable sections of this chapter.

TABLE 3-7

The Effects of Nitrite in U.S. Cured Red Meat and Poultry Products

| Products ^a | Recommended Storage Temperature, °C ^b | Potential for Temperature Abuse or Contamination by Processor (P), Distributor (D), Consumer Before (CB) or After (CA) Opening | Spoilage Microorganisms | | Inhibited by: ^c |
|---|--|--|---|--|---|
| | | | Reduced or Controlled by NO ₂ ⁻ | Not, or Poorly, Controlled by NO ₂ ⁻ | |
| Raw, Cured Products^f | | | | | |
| High Water Activity: | | | | | |
| Bacon | < 4.4 | D - Low CB - Low CA - Very low | Aerobic mesophiles Corynebacteria ^g | Lactics ^h Micrococci | Low temperature NaCl Anaerobic packaging NO ₂ ⁻ pH ² |
| Other pickled-cured products (e.g., smoked ham) | < 4.4 | D - Low CB - Moderate CA - High | Clostridia and Bacilli | Psychrotrophs ^k Lactics ^h Micrococci | Heating ^f followed by low temperature NO ₂ ⁻ NaCl Anaerobic packaging pH |
| Low Water Activity: | | | | | |
| Dry-cured cuts (e.g., country ham) | 0 to ambient (i.e., ~15-35) | D - Moderate ¹ CB - Moderate ¹ CA - Moderate ¹ | Clostridia | Molds Yeasts | Low a _w NO ₂ ⁻ NaCl Anaerobic packaging |
| Dry, semidry, and fermented sausage (e.g., Lebanon bologna, salami) | 0 to ambient (i.e., ~15-35) | P - Moderate CB - Low CA - Low | | Molds Yeasts | Acid or low a _w Heating NO ₂ ⁻ Anaerobic packaging |
| Cooked, Cured Products | | | | | |
| Packaged After Heating:^m | | | | | |
| Sausages (e.g., beef or chicken frankfurters) and some cold cuts | < 4.4 | P or D - Low CB - Low CA - Very Low | Psychrotrophs ^k | Psychrotrophs ^k Lactics ^h Yeast | Heating followed by low temperature Anaerobic packaging |
| Canned: | | | | | |
| Perishable (e.g., canned ham) | < 4.4 | D - Moderate CB - High CA - High | Clostridia and other putrefactive anaerobes | Some clostridia | Pasteurization with NO ₂ ⁻ followed by low temperature Sealed container NaCl |
| Shelf stable (e.g., luncheon meat) | Ambient (i.e., ~15-35) | P or D - Very Low CB - Extremely Low CA - Low | Clostridia, thermophiles | | Thermal process Sealed container NO ₂ ⁻ NaCl |
| Commercially sterile (e.g., deviled ham) | Ambient (i.e., ~15-35) | P or D - Extremely Low CB - Negligible CA - Very Low | Spore-formers (with faulty processing) | | Thermal process Sealed container (NO ₂ ⁻ /NaCl if processing faulty) |

^aFurther examples of products of these categories can be found in the text (p. 3-6).

^bMost U.S. producers recommended < 4.4°C (40°F), but < 7.2°C (45°F) is still within some local laws.

^cAscorbate may potentiate the antimicrobial effects of nitrite, but is not effective by itself.

^dColor fixation by nitrite is selective for the muscle tissue of meat products.

^eReferences to nitrite's contribution to flavor in various product classes are as follows: bacon: Huhtanen et al., 1981; Kimoto et al., 1976a,b; MacDougall et al., 1975; Paquette et al., 1980; Wasserman et al., 1977; Williams and Greene, 1979; ham and ham-based products, including those canned: Brown et al., 1974; DuBois et al., 1981; MacDonald et al., 1980c; dry-cured cuts: Eakes and Blumer, 1975; Eakes et al., 1975; Kemp et al., 1974; fermented sausages: Dethmers et al., 1975; frankfurters: Simon et al., 1973; Wasserman and Talley, 1972.

^fSome raw cured products may be mildly heated during smoking (International Commission on Microbial Specifications for Foods, 1980, pp. 136-159, 333-409; Nitrite Safety Council, 1980).

TABLE 3-7 (Continued)

| Pathogens | | | Effects of Nitrite on: | | |
|--|---|---|------------------------|---|---|
| Reduced or Controlled by NO ₂ ⁻ | Not or Poorly, Controlled by NO ₂ ⁻ | Inhibited by ^c | Color ^d | Flavor ^e | Lipid Oxidation |
| <u>C. botulinum</u> <u>Staphylococci</u> ¹ | Staphylococci ¹ | Low temperature NO ₂ ⁻ Fermentable carbohydrate (if added) NaCl Frying ^j | Color fixation | Inconsequential contribution (salt major contributor) | Inhibits |
| <u>C. botulinum</u> <u>Staphylococci</u> ¹ <u>B. cereus</u> | Staphylococci ¹ Salmonellae | Low temperature NO ₂ ⁻ NaCl Anaerobic packaging ¹ | Color fixation | Important contri- bution (salt also major contributor) | Inhibits |
| <u>C. botulinum</u> | | Low a _w NO ₂ ⁻ NaCl Anaerobic packaging ¹ | Color fixation | Inconsequential con- tribution (salt and lipid oxidation major contributors) | May limit after some oxidation has occurred |
| <u>C. botulinum</u> <u>Staphylococci</u> ¹ | Staphylococci ¹ Salmonellae | Acid or low a _w | Color fixation | Important contribution (lactic acid produc- tion and salt also major contributors) | Inhibits |
| <u>C. botulinum</u> | | Heating Low temperature NO ₂ ⁻ Fermentable carbohydrate (if added) Packaging NaCl | Color fixation | Important contribution, if product not smoked; NO ₂ ⁻ inconsequential, if smoked (spices also contribute) | Inhibits |
| <u>C. botulinum</u> | | NO ₂ ⁻ Low temperature Heating Sealed container NaCl | Color fixation | Important contribution ⁿ | Inhibits |
| <u>C. botulinum</u> | | Thermal process with NO ₂ ⁻ Sealed container | Color fixation | Important contribution ⁿ | Inhibits |
| <u>C. botulinum</u> | | Thermal process Sealed container (NO ₂ ⁻ /NaCl if processing faulty) | Color fixation | Important contribution ⁿ | Inhibits |

⁸See text (p. 3-34). Dempster (1980) studied corynebacteria sensitive to heating at 63°C for 30 minutes.

^bLactics include lactic-acid-producing bacteria, "mainly atypical streptococci" whose speciation remains to be satisfactorily defined (Reuter, 1975).

ⁱSee text (p. 3-33); Golis and Cassolari, 1979; Labota, 1976.

^jFrying or other cooking by consumer or processor (as with prefried bacon).

^kPsychrotrophs could include gram-negative bacteria such as pseudomonads and coliforms as well as yeasts (see Terrell, 1974).

^lThis ranking is for packaged slices; for whole hams the potential is very low.

^mThus, subject to possible contamination during packaging.

ⁿMany of these products are ham-based; thus, studies on ham flavor are pertinent (see footnote e).

Conclusions concerning the minimum levels of nitrite required to achieve the specific effects described below will be discussed in the second report of the committee. In that report, the committee will explore alternative approaches to achieving the effects currently resulting from the use of nitrate and nitrite.

Effects on Pathogenic and Spoilage Microorganisms

The specific contribution of nitrite to the inhibition of potential pathogens and spoilage microorganisms varies with the product in which it is used and with variations in their production, handling, and abuse.

Nitrite, in association with other components in the curing salt mix, exerts a concentration-dependent antimicrobial effect in cured products including, but not limited to, inhibition of the outgrowth of spores of putrefactive and pathogenic clostridia, including Clostridium botulinum. Nitrite thus provides protection against the risk to health posed by botulism. Under conditions of excessive contamination or prolonged temperature abuse, nitrite does not indefinitely prevent such outgrowth, and spoilage and/or toxin production may ultimately ensue.

Residual nitrite appears to be an important determinant of the degree of protection provided by nitrite. Thus, any product or process changes that result in a lower level of residual nitrite, e.g., adding less nitrite or increasing its rate of depletion, will increase the likelihood of the product becoming toxic if contaminated and abused. However, other factors that influence the risk of botulism, e.g., contamination or the timing and duration of temperature abuse, are not predictable. Thus, it is not possible to derive a quantitative relationship between the protection provided by nitrite and the risk of botulism or to determine the degree of protection that is necessary to ensure the safety of a particular product. The committee believes that it is not practicable to produce raw meat, meat products, or fish products with a guarantee that they do not contain microbial contamination. Under these circumstances, the prudent approach to protecting the public health is to base precautions on the assumption that product contamination by pathogens and opportunities for their growth (temperature abuse) are both likely to occur.

Depending on a number of factors, including the concentration of nitrite, environmental conditions, and the type of food product, nitrite may also contribute to the control of pathogens other than C. botulinum, for example, Staphylococcus aureus, Bacillus cereus, and C. perfringens. Nitrite retards microbial spoilage of cured meats by inhibiting the growth of a variety of organisms, especially anaerobic and aerobic spore-forming bacteria, such as clostridia and bacilli.

The mechanism by which nitrite inhibits spore outgrowth and the growth of vegetative cells of certain microorganisms is not fully understood, but in some bacteria it appears to involve reaction with iron-containing enzymes. A more thorough knowledge of the mechanism would facilitate a search for alternative antimicrobial agents.

Special considerations are relevant to the use of nitrate and nitrite in fish products. Most important among these are the higher frequency of contamination of fish with C. botulinum and the fact that the most common contaminating strains are able to grow at lower temperatures.

Various cured products differ in the opportunities they present for microbial multiplication during production, in the extent to which control is exerted over pathogens by factors other than nitrite (e.g., pH, brine concentration, and thermal processing), and in the care taken during consumer handling. Based on these considerations, product types can be ranked according to the degree to which the addition of nitrite is desired to ensure their freedom from microbial hazard to health. The committee will present such an evaluation in its second report, which will discuss alternative approaches to the current use of nitrite.

Effects of Nitrite on Lipid Oxidation

Another preservative property of nitrite in cured meat products is its ability to minimize lipid oxidation, which yields products that cause rancidity and may be toxic. This effect of nitrite is particularly important to preserve flavor in comminuted products, but may not be necessary for this purpose in dry-cured cuts.

Effects of Nitrite on Sensory Characteristics

Nitrite produces a distinctive color in the muscle tissue of cured meats as a result of its reaction with myoglobin.

The contribution of nitrite to flavor has not yet been fully determined for all cured meat products. Because of differences among products, this contribution should be examined on a product-by-product basis. Nitrite appears to make a significant contribution to the flavor of pickle-cured hams and ham-based products.

Sodium chloride is largely responsible for the "cured" flavor in some products, especially bacon. Other product ingredients (e.g., spices) or processes (e.g., smoking) may also be important contributors to flavor. It is possible that flavor characteristics may not be attributable to a specific chemical component or components of cured meat since the olfactory system can "synthesize" or "fuse" individual aromas into one that is different from that of the individual components.

RECOMMENDATIONS

Chemistry

For reasons described in this chapter and also in Chapter 5, the committee recommends that:

Methods should be developed for the rapid and accurate determination of residual nitrite in meats at levels less than 10 mg/kg.

Research should be conducted to determine the potential of cured meats to nitrosate amino substrates in vivo. Furthermore, investigations should be conducted to determine the potential of the nitrogen-containing compounds arising from added nitrite (e.g., "residual" nitrite, nitrosothiols, nitric oxide myoglobin, nitrosated amides, peptide linkages, and nitrosated lipids) to act as nitrosating agents.

Antimicrobial Action

The mechanism(s) of action by which nitrite delays clostridial spore outgrowth and inhibits the growth of susceptible vegetative cells should be investigated further. The reasons that some bacterial groups are not susceptible to nitrite should also be determined. This work should be accorded high priority in view of its importance in developing alternatives to nitrite.

The contribution of nitrite to the control of pathogens other than C. botulinum in cured meats should also be determined. Moreover, further definition of the role of nitrite in controlling spoilage organisms in cured meats is desirable, especially for genera other than clostridia.

High priority should also be accorded to investigations of the interaction of factors controlling pathogens and spoilage organisms in different commercial products in order to develop methods for predicting the degree of control gained or lost through alteration of any of those factors. However, predictions from these models should be verified by testing under commercial conditions, before changes in production practices are introduced.

As a contribution to assessing the microbial hazard to health from cured products, surveys using serial sampling techniques should be conducted to determine the frequency with which C. botulinum spores contaminate different classes of raw meats and cured products under diverse production conditions and in different geographic locations.

Antioxidant Effects

The mechanism by which nitrite inhibits lipid oxidation in cured meat products should be investigated.

Sensory Studies

Psychophysical methods, with the capacity to determine the magnitude of any flavor differences, should be used to investigate the contribution of nitrite to the flavor of cured meats to determine if a dose-response relationship exists and what form it takes. Such studies should initially be conducted on ham because evidence indicates that nitrite contributes to the flavor of this product. The contributions of sugar, salt, and other potential contributors to flavor should also be investigated.

To derive the maximum potential from sensory studies, research should be conducted to determine the distribution of the ability to discriminate "cured meat flavor" in the general population.

Studies should be conducted to determine the extent to which nitrogen oxides from "smoke" contribute to color and flavor of meats that do not contain nitrite and to elucidate how smoking affects the overall sensory characteristics and other properties of meat.

Use of Nitrate

For products other than fermented sausages and dry-cured cuts, the committee recommends that nitrite added at the minimum level necessary to achieve the desired effect should be substituted for nitrate.

It also recommends that further investigation be conducted to determine the need for, and mode of action of, nitrate in fermented sausages and dry-cured cuts.

REFERENCES

- Acton, J. C., R. L. Dick, and A. K. Torrence. 1979. Turkey ham properties on processing and cured color formation. *Poult. Sci.* 58:843-847.
- American Meat Institute. 1980. *Meatfacts*, 80th edition. American Meat Institute, Washington, D.C. 27 pp.
- Amerine, M. A., R. M. Pangborn, and E. B. Roessler. 1965. *Principles of Sensory Evaluation of Food*. Academic Press, New York.
- Amoore, J. E. 1977. Specific anosmia and the concept of primary odors. *Chem. Senses Flavor* 2:267-281.
- Andres, C. 1977. Starter culture for sausage has two microorganisms for better performance. *J. Food Protect.* 38:132.
- Apaja, M. 1980. Evaluation of toxicity and carcinogenicity of malonaldehyde. *Acta Univ. Oul.* D55. 1980. *Anat. Path. Microbiol.* 8:1-61.
- Ashworth, J., L. L. Hargreaves, and B. Jarvis. 1973. The production of an antimicrobial effect in pork heated with sodium nitrite under simulated commercial pasteurization conditions. *J. Food Technol.* 8:477-484.
- Bailey, M. E., and J. W. Swain. 1973. Influence of nitrite on meat flavor. Pp. 29-45 in *Proceedings of the Meat Industry Research Conference, March 22-23, 1973, Center for Continuing Education, University of Chicago, American Meat Institute Foundation, Chicago, Illinois.*
- Bailey, M. E., R. W. Frame, and H. D. Naumann. 1964. Cured meat pigments: Studies of the photooxidation of nitrosomyoglobin. *J. Agric. Food Chem.* 12:89-93.
- Baird-Parker, A. C., and B. Freame. 1967. Combined effect of water activity, pH and temperature on the growth of Clostridium botulinum from spore and vegetative cell inocula. *J. Appl. Bacteriol.* 30:420-429.
- Banwart, G. J. 1979. *Basic Food Microbiology*. AVI Publishing Company, Inc., Westport, Connecticut. 781 pp.
- Bard, J., and W. E. Townsend. 1971. Meat curing. Pp. 452-483 in *The Science of Meat and Meat Products*. J. F. Price and B. S. Schweigert, eds. Freeman, San Francisco, California.

- Bartoshuk, L. M. 1979. Bitter taste of saccharin related to the genetic ability to taste the bitter substance 6-n-propylthiouracil. *Science* 205:934-935.
- Batzer, O. F., A. T. Santoro, M. C. Tan, W. A. Landmann, and B. S. Schweigert. 1960. Meat flavor chemistry. Precursors of beef flavor. *J. Agric. Food Chem.* 8:498-501.
- Batzer, O. F., A. T. Santoro, and W. A. Landmann. 1962. Identification of some beef flavor precursors. *J. Agric. Food Chem.* 10:94-96.
- Benedict, R. C. 1980. Biochemical basis for nitrite-inhibition of Clostridium botulinum in cured meat. *J. Food Protect.* 43:877-891.
- Binkerd, E. F., and O. E. Kolari. 1975. The history and use of nitrate and nitrite in the curing of meat. *Food Cosmet. Toxicol.* 13:655-661.
- Brathen, G., and A. Svensen. 1973. Determination of nitrite and nitrate in cheese. *Meieriposten* 62:620-632.
- Brooks, B. R., and O. L. Klammerth. 1968. Interaction of DNA with bifunctional aldehydes. *European J. Biochem.* 5:178-182.
- Brooks, J., R. B. Haines, T. Moran, and J. Pace. 1940. The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Department of Scientific and Industrial Research, Food Investigation Board Special Report No. 49. His Majesty's Stationery Office, London, United Kingdom.
- Brown, C. L., H. B. Hedrick, and M. E. Bailey. 1974. Characteristics of cured ham as influenced by levels of sodium nitrite and sodium ascorbate. *J. Food Sci.* 39:977-979.
- Bryan, F. L. 1979. Infections and intoxications caused by other bacteria. Pp. 211-279 in H. Riemann and F. Bryan, eds. *Foodborne Infections and Intoxications*, 2nd ed. Academic Press, New York. 748 pp.
- Bryan, F. L. 1980. Foodborne diseases in the United States associated with meat and poultry. *J. Food Protect.* 43:140-150.
- Buchanan, R. E., and N. E. Gibbons, eds. 1974. *Bergey's Manual of Determinative Bacteriology*, Eighth Edition. Williams and Wilkins Company, Baltimore, Maryland. 1,268 pp.
- Buchanan, R. L., and M. Solberg. 1972. Interaction of sodium nitrate (sic) [nitrite], oxygen, and pH on growth of Staphylococcus aureus. *J. Food Sci.* 37:81-85.

- Cain, W. S. 1978. History of research on smell. Pp. 197-229 in E. C. Carterite and M. P. Friedman, eds. Handbook of Perception, Volume VIA. Academic Press, New York.
- Cantafora, A., D. A. Villalohos, and R. Monacelli. 1974. Colorimetric method for a global evaluation of N-nitrosamines in dairy products. Rivista Della Societa Italiana di Scienza Dell' Alimentazione 3:213-217. (Chem. Abs. 82:56134g, 1975).
- Cassens, R. G., G. Woolford, S. H. Lee, and R. Goutefonga. 1977. Fate of nitrite in meat. Pp. 95-100 in B. J. Tinbergen and B. Krol, eds. Proceedings of the 2nd International Symposium on Nitrite in Meat Products. PUDOC, Wageningen, the Netherlands. 320 pp.
- Cassens, R. G., M. L. Greaser, T. Ito, and M. Lee. 1979a. Reactions of nitrite in meat. Food Technol. 33:46-56.
- Cassens, R. G., T. Ito, and M. Lee. 1979b. A Research Note. Morphology of bacon and its possible role in formation of nitrosamines. J. Food Sci. 44:306-307.
- Cassens, R. G., J. G. Sebranek, G. Kubberod, and G. Woolford. 1974. Where does the nitrite go? Food Prod. Develop. 8:50-56.
- Castellani, A. G., and C. F. Niven. 1955. Factors affecting the bacteriostatic action of sodium nitrite. Appl. Microbiol. 3:154-159.
- Center for Disease Control. 1979. Botulism in the United States, 1899-1977. Handbook for Epidemiologists, Clinicians, and Laboratory Workers. Center for Disease Control, U.S. Public Health Service, Atlanta, Georgia. 41 pp.
- Centers for Disease Control. 1981. Foodborne Disease Outbreaks-- Annual Summary, 1979. HHS Publication No. (CDC) 81:8185. Centers for Disease Control, U.S. Public Health Service, Atlanta, Georgia. 40 pp.
- Cerutti, G., R. Zappavigna, and P. L. Santini. 1975. [In Italian.] N-Alchil-nitrosammine in formaggi nazionali e di importazione. Latte 3:224-227.
- Cervený, J. G. 1980. Effects of changes in the production and marketing of cured meats on the risk of botulism. Food Technol. 34:240-243.
- Chang, S. S., and R. J. Peterson. 1977. Symposium: The basis of quality in muscle foods: Recent developments in the flavor of meat. J. Food Sci. 42:298-305.

- Cho, I. C., and L. J. Bratzler. 1970. Effect of sodium nitrite on flavor of cured pork. *J. Food Sci.* 35:668-670.
- Christiansen, L. N. 1980. Factors influencing botulinal inhibition by nitrite. *Food Technol.* 34:237-239.
- Christiansen, L. N., and E. M. Foster. 1965. Effect of vacuum packaging on growth of Clostridium botulinum and Staphylococcus aureus in cured meats. *J. Appl. Microbiol.* 13:1023-1025.
- Christiansen, L. N., R. W. Johnston, D. A. Kautter, J. W. Howard, and W. J. Aunan. 1973. Effect of nitrite and nitrate on toxin production by Clostridium botulinum and on nitrosamine formation in perishable canned comminuted cured meat. *Appl. Microbiol.* 25:357-362. (Erratum 26:653).
- Christiansen, L. N., R. B. Tompkin, A. B. Shaparis, T. V. Keuper, R. W. Johnston, D. A. Kautter, and O. J. Kolarik. 1974. Effect of sodium nitrite on toxin production by Clostridium botulinum in bacon. *Appl. Microbiol.* 27:733-737.
- Christiansen, L. N., R. B. Tompkin, A. B. Shaparis, R. W. Johnston, and D. A. Kautter. 1975. Effect of sodium nitrite and nitrate on Clostridium botulinum growth and toxin production in a summer style sausage. *J. Food Sci.* 40:488-490.
- Christiansen, L. N., J. C. Manuel, and L. S. Divilbiss. 1977. Effect of sodium nitrite in cured poultry products. In Special Poultry Research Committee, Response to FDA Notice Published in September 2, 1977 Federal Register (42 Fed. Reg. 44376; Docket No. 77N-0222).
- Christiansen, L. N., R. B. Tompkin, and A. B. Shaparis. 1978. Fate of C. botulinum in perishable canned meat at abuse temperature. *J. Food Protect.* 41:354-355.
- Clydesdale, F. M., and F. J. Francis. 1971. Color measurement of foods. XXVII. Chemistry of meat color. *Food Prod. Develop.* 5:81-82, 87-90.
- Cross, C. K., and P. Ziegler. 1965. A comparison of the volatile fractions from cured and uncured meat. *J. Food Sci.* 30:610-614.
- Crowther, J. S., R. Holbrook, A. C. Baird-Parker, and B. L. Austin. 1977. Role of nitrite and ascorbate in the microbiological safety of vacuum-packed sliced bacon. Pp. 13-20 in B. J. Tinbergen and B. Krol, eds. *Proceedings of the 2nd International Symposium on Nitrite in Meat Products*, Zeist. PUDOC, Wageningen, the Netherlands. 320 pp.

- Dempster, J. F. 1980. The microbiology of "back bacon" manufactured in "nitrate-free" brines after storage at 5°C. Pp. 272-275 in Proceedings of the 26th European Meeting of Meat Research Workers, Vol. 2. American Meat Sciences Association, Chicago, Illinois.
- Dethmers, A. E., H. Rock, T. Fazio, and R. W. Johnston. 1975. Effect of added sodium nitrite and sodium nitrate on sensory quality and nitrosamine formation in thuringer sausage. *J. Food Sci.* 40:491-495.
- DuBose, C. N., A. V. Cardello, and O. Maller. 1981. Factors affecting the acceptability of low-nitrite smoked, cured ham. *J. Food Sci.* 46:461-463.
- Duncan, C. L. 1970. Arrest of growth from spores in semi-preserved foods. *J. Appl. Bacteriol.* 33:60-73.
- Duncan, C. L., and E. M. Foster. 1968. Effect of sodium nitrite, sodium chloride, and sodium nitrate on germination and outgrowth of anaerobic spores. *Appl. Microbiol.* 16:406-411.
- Eakes, B. D., and T. N. Blumer. 1975. Effect of various levels of potassium nitrate and sodium nitrite on color and flavor of cured loins and country-style hams. *J. Food Sci.* 40:977-980.
- Eakes, B. D., T. N. Blumer, and R. J. Monroe. 1975. Effect of nitrate and nitrite on color and flavor of country-style hams. *J. Food Sci.* 40:973-976.
- Eklund, M. W., and F. T. Poysky. 1970. Distribution of Clostridium botulinum on the Pacific Coast of the United States. Pp. 304-308 in M. Herzberg, ed. Proceedings of the First U.S.-Japan Conference on Toxic Microorganism, Honolulu, Hawaii, October 7-10, 1968. U.S. Department of the Interior, Washington, D.C.
- Eklund, M. W., D. I. Wieler, and F. T. Poysky. 1967. Outgrowth and toxin production of nonproteolytic type B C. botulinum at 3.3 to 5.6°C. *J. Bacteriol.* 93:1461-1462.
- Emi-Miwa, M., A. Okitani, and M. Fujimaki. 1976. Comparison of the fate of nitrite added to whole meat, meat fractions and model systems. *Agric. Biol. Chem.* 40:1387-1392.
- Fooladi, M. H., A. M. Peterson, T. H. Coleman, and R. A. Merkel. 1979. The role of nitrite in preventing development of warmed-over flavour. *Food Chem.* 4:283-292.
- Fox, J. B., Jr. 1966. The chemistry of meat pigments. *J. Agric. Food Chem.* 14:207-210.
- Fox, J. B., and S. A. Ackerman. 1968. Formation of nitrosylmyoglobin. Mechanism of reaction with various reductants. *J. Food Sci.* 33:364.

- Fox, J. B., Jr., and R. A. Nicholas. 1974. Nitrite in meat: Effect of various compounds on loss of nitrite. *J. Agric. Food Chem.* 22:302-306.
- Fox, J. B., Jr., and J. S. Thompson. 1963. Formation of bovine nitrosylmyoglobin. I. pH 4.5-6.5. *Biochemistry* 2:465-470.
- Fox, J. B., W. E. Townsend, S. A. Ackerman, and C. E. Swift. 1967. Cured color development during frankfurter processing. *Food Technol.* 21:386-392.
- Frouin, A. 1977. Nitrates and nitrites: Reinterpretation of analytical data by means of bound nitrous oxide. In B. J. Tinbergen and B. Krol, eds. *Proceedings of the 2nd International Symposium on Nitrite in Meat Products*, Zeist. PUDOC, Wageningen, the Netherlands. 320 pp.
- Fujimaki, M., M. Emi, and A. Okitani. 1975. Fate of nitrate in meat-curing model systems composed of myoglobin, nitrite and ascorbate. *Agric. Biol. Chem.* 39:371-377.
- Galesloot, T. E. 1961. Concerning the action of nitrate in preventing butyric acid fermentation in cheese. *Neth. Milk Dairy J.* 15:395-410.
- Galesloot, T. E. 1964. Some factors affecting the efficiency of nitrate in controlling the butyric acid fermentation in Edam and Gouda cheese. *Neth. Milk Dairy J.* 18:127-138.
- Galesloot, T. E., J. Stadhouders, and R. H. C. Elgersma. 1975. On the occurrence of nitrosamines and the use of nitrate in the production of Gouda and Edam cheese. Report NOV-470, 2nd Version, Netherlands Institute for Dairy Research.
- Genigeorgis, C., and H. Riemann. 1979. Food processing and hygiene. Pp. 613-713 in H. Riemann and F. L. Bryan, eds. *Foodborne Infections and Intoxications*, 2nd Ed. Academic Press, New York. 748 pp.
- Giddey, C. 1966. The change in meat pigments in sausage making processes. *J. Sci. Food Agric.* 17:14-17.
- Giddings, G. C. 1974. Reduction of ferrimyoglobin in meat. *Crit. Rev. Food Technol.* 5:143-173.
- Giddings, G. C. 1977a. The basis of color in muscle foods. *Crit. Rev. Food Sci. Nutrition* 8:81-114.
- Giddings, G. G. 1977b. Symposium: The basis of quality in muscle foods. The basis of color in muscle foods. *J. Food Sci.* 42:288-294.

- Giolitti, G., C. A. Cantoni, M. A. Bianchi, and P. Renon. 1971. Microbiology and chemical changes in raw hams of Italian type. *J. Appl. Bacteriol.* 34:51-61.
- Gola, S., and A. Casolari. 1979. Antimicrobial activity of nitrite-dependent compounds. Pp. 267-279 in B. Jarvis, ed. *Food Microbiology and Technology: Proceedings of the International Meeting on Food Microbiology and Technology, Medicina viva Servizio Congress, Srl., Parma, Italy.*
- Goodhead, K., T. A. Gough, K. S. Webb, J. Stadhouders, and H. C. Elgersma. 1976. The use of nitrate in the manufacture of Gouda cheese. Lack of evidence of nitrosamine formation. *Neth. Milk Dairy J.* 30:207.
- Gough, B. J., and J. A. Alford. 1965. Effect of curing agents on the growth and survival of food-poisoning strains of Clostridium perfringens. *J. Food Sci.* 30:1025-1028.
- Gould, G. W. 1964. Effect of food preservatives on the growth of bacteria from spores. Pp. 17-24 in N. Molin and A. Erichsen, eds. *Microbial Inhibitors in Food.* Almquist and Wiksell, Stockholm, Sweden.
- Goutefongea, R., R. G. Cassens, and G. Woolford. 1977. Distribution of sodium nitrite in adipose tissue during curing. *J. Food Sci.* 42:1637-1641.
- Govindarajan, S. 1973. Fresh meat color. *Crit. Rev. Food Technol.* 4:117-140.
- Gray, J. I., D. M. Irvine, and Y. Kakuda. 1979. Nitrates and N-nitrosamines in cheese. *J. Food Protect.* 42:263-272.
- Gray, J. I., B. MacDonald, A. M. Pearson, and I. D. Morton. 1981. Role of nitrite in cured meat flavor: A review. *J. Food Protect.* 44:302-312, 319.
- Greenberg, R. A. 1972. Nitrite in the control of Clostridium botulinum. Pp. 25-34 in *Proceedings of the Meat Industry Research Conference, Chicago, Illinois.* American Meat Institute, Washington, D.C.
- Greene, B. E., and L. G. Price. 1975. Oxidation-induced color and flavor changes in meat. *Agric. Food Chem.* 23:164-167.
- Greenwood, D. A. 1940. Some further studies on the destruction of sodium nitrite by heating. Pp. 41-46 in *Proceedings of the Chemistry and Operating Section, American Meat Institute 35th Annual Convention.* American Meat Institute, Washington, D.C.

- Gross, C. E., C. Vinton, and C. R. Stumbo. 1946a. Bacteriological studies relating to thermal processing of canned meats. V. Characteristics of putrefactive anaerobe used in thermal resistance studies. *Food Res.* 11:405-410.
- Gross, C. E., C. Vinton, and C. R. Stumbo. 1946b. Bacteriological studies relating to thermal processing of canned meats. VI. Thermal death-time curve for spores of test putrefactive anaerobe in meat. *Food Res.* 11:411-418.
- Haurowitz, F., P. Schwerin, and M. M. Yensen. 1941. Destruction of hemin and hemoglobin by the action of unsaturated fatty acids and oxygen. *J. Biol. Chem.* 140:353-359.
- Hauschild, A. H. W. 1980. Microbial problems in food safety with particular reference to Clostridium botulinum. Pp. 68-107 in H. D. Graham, ed. *The Safety of Foods*, 2nd Ed. AVI Publishing, Westport, Connecticut. 774 pp.
- Hauschild, A. H. W., and R. Hilsheimer. 1980. Incidence of Clostridium botulinum in commercial bacon. *J. Food Protect.* 43:564-565.
- Hayes, S., J. M. Craig, and K. S. Pilcher. 1970. The detection of Clostridium botulinum type E in smoked fish products in the Pacific Northwest. *Can. J. Microbiol.* 16:207-209.
- Health and Welfare Canada. 1975. Food and Drug Regulations, Amendment. Privy Council Order No. 1975-774. *Canada Gazette Part II*, 109:757-762 (April 23, 1975).
- Henry, M., L. Joubert, and P. Goret. 1954. Mécanisme biochimique de l'action du nitrite dans la conservation des vivandes. Conditions physicochimiques favorables à son action bactériostatique. *Ct. Rd. Soc. Biol.* 148:819-821.
- Herz, K. D., and S. S. Chang. 1970. Meat flavor. *Adv. Food Res.* 18:1-83.
- Holley, R. A. 1978. Botulism Hazard in Cured Meat Treated with Reduced Concentrations of Nitrite: A Report Prepared for the Industry/Government Committee on Nitrites and Nitrosamines. Food Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada. 20 pp.
- Holley, R. A. 1981. Review of the potential hazard from botulinum in cured meats. *Can. Inst. Food Sci. Technol. J.* 16:183-195.
- Hornstein, I. 1967. Flavor in red meats. Pp. 228-250 in H. W. Schultz, E. A. Day, and L. M. Libbey, eds. *Symposium on Foods: The Chemistry and Physiology of Flavors*. AVI Publishing Company, Westport, Connecticut.

- Hornstein, I., and P. F. Crowe. 1960. Meat flavor chemistry. Flavor studies on beef and pork. *J. Agric. Food Chem.* 8:494-498.
- Hornstein, I., and P. F. Crowe. 1963. Meat flavor: Lamb. *J. Agric. Food Chem.* 11:147-149.
- Hornstein, I., and P. F. Crowe. 1964. Meat flavor: A review. *J. Gas Chromatog.* 2:128-131.
- Hornstein, I., P. F. Crowe, and J. M. Heimberger. 1960. Constituents of meat flavor: Beef. *J. Agric. Food Chem.* 8:65-67.
- Huhtanen, C. N., F. B. Talley, J. Feinberg, and J. G. Phillips. 1981. Sensory and antibotulinal evaluation of sorbic acid-containing bacon. *J. Food Sci.* 46:1796-1800.
- Hustad, G. O., J. G. Cervený, H. Trenk, R. J. Deibel, D. A. Kautter, T. Fazio, R. W. Johnston, and O. E. Kolari. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in weiners. *Appl. Microbiol.* 26:22-26.
- Igene, J. O., J. A. King, A. M. Pearson, and J. I. Gray. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. *J. Agric. Food Chem.* 27:838-842.
- Ingram, M. 1974. The microbiological effects of nitrite. Pp. 63-75 in B. Krol and B. J. Tinbergen, eds. *Proceedings of the International Symposium on Nitrite in Meat Products*, Zeist. PUDOC, Wageningen, the Netherlands.
- Ingram, M., and T. A. Roberts. 1971. Application of the D-concept to heat treatments involving curing salts. *J. Food Technol.* 6:21-28.
- International Commission on Microbial Specifications for Foods. 1980. *Microbial Ecology of Foods*, Volumes 1 and 2. Academic Press, New York. Volume 1, pp. 1-322; Volume 2, pp. 323-998.
- Jacobsen, M., and H. H. Koehler. 1963. Components of the flavor of lamb. *J. Agric. Food Chem.* 11:336-339.
- Jarvis, B., A. C. Rhodes, S. E. King, and M. Patel. 1976. Sensitization of heat-damaged spores of Clostridium botulinum, type B to sodium chloride and sodium nitrite. *J. Food Technol.* 11:41-50.
- Jeremiah, L. E., Z. L. Carpenter, and G. C. Smith. 1972. Beef color as related to consumer acceptance and palatability. *J. Food Sci.* 37:476-479.

- Johnston, M. A., and R. Loynes. 1971. Inhibition of Clostridium botulinum by sodium nitrite as affected by bacteriological media and meat suspensions. *Can. Inst. Food Technol. J.* 4:179-184.
- Johnston, R. W., and R. P. Elliott. 1976. Meat and Poultry Products. Pp. 540-548 in M. L. Speck, ed. *Compendium of Methods for the Microbiological Examination of Foods*. American Public Health Association, Washington, D.C.
- Kanner, J., and B. J. Juven. 1980. S-Nitrosocysteine as an anti-oxidant, color-developing, and anticlostridial agent in comminuted turkey meat. *J. Food Sci.* 45:1105-1112.
- Kanner, J., I. Ben-Gera, and S. Berman. 1979. Nitric oxide myoglobin as inhibitor of lipid oxidation. Abstr. Paper 22, 39th Annual Meeting of the Institute of Food Technologists (IFT), St. Louis, Missouri, June 1979. Institute of Food Technologists, Chicago, Illinois.
- Kemp, J. D., J. D. Fox, and W. G. Moody. 1974. Cured ham properties as affected by nitrate and nitrite and fresh pork quality. *J. Food Sci.* 39:972-976.
- Kemp, J. D., B. E. Langlois, J. D. Fox, and W. Y. Varney. 1975. Effects of curing ingredients and holding times and temperatures on organoleptic and microbiological properties of dry-cured sliced ham. *J. Food Sci.* 40:634-636.
- Kendrick, J., and B. M. Watts. 1969. Acceleration and inhibition of lipid oxidation of heme compounds. *Lipids* 4:454-458.
- Kerr, R. H., C. T. N. Marsh, W. F. Schroeder, and E. A. Boyer. 1926. The use of sodium nitrite in the curing of meat. *J. Agric. Res.* 33:541-551.
- Kimoto, W. I., A. E. Wasserman, and F. B. Talley. 1976a. Effects of sodium nitrite and sodium chloride on the flavor of processed pork bellies. *Lebensm. Wiss. Technol.* 9:99-101.
- Kimoto, W. I., A. E. Wasserman, and F. B. Talley. 1976b. Sensory evaluation of flavor development in lean and adipose tissues of bacon. *Lebensm. Wiss. Technol.* 9:274-276.
- Koizumi, C., and W. D. Brown. 1971. Formation of nitric oxide myoglobin by nicotinamide adenine dinucleotides and flavines. *J. Food Sci.* 36:1105-1109.
- Komarik, S. L., D. K. Tressler, and L. Long. 1974. *Food Products Formulary Vol. 1: Meats, Poultry, Fish, and Shellfish*. AVI Publishing Company, Westport, Connecticut. 348 pp.

- Kraft, A. A., and J. C. Ayres. 1954. Color changes in packaged bologna. *Food Technol.* 8:22-26.
- Kramlich, W. E., A. M. Pearson, and F. W. Tauber. 1973. *Processed Meats*. AVI Publishing Company, Westport, Connecticut.
- Labots, H. 1976. Effect of nitrite on the development of Staphylococcus aureus in fermented sausages. Pp. 21-27 in B. J. Tinbergen and B. Krol, eds. *Proceedings of the 2nd International Symposium on Nitrite in Meat Products*, Zeist. PUDOC, Wageningen, the Netherlands.
- Landmann, W. A., and O. F. Batzer. 1966. Influence of processing procedures on the chemistry of meat flavor. *J. Agric. Food Chem.* 14:210-214.
- Lawrie, R. A. 1974. *Meat Science*. 2nd edition, Pergamon Press, Toronto, Canada. 419 pp.
- Lechowich, R. V., W. L. Brown, R. H. Deibel, and I. I. Somers. 1978. The role of nitrite in the production of canned cured meat products. *Food Technol.* 32:45-58.
- Lee, S. H., and R. G. Cassens. 1976. Nitrite binding sites in myoglobin. *J. Food Sci.* 41:969-970.
- Lillard, D. A., and J. C. Ayres. 1969. Flavor compounds in country cured hams. *Food Technol.* 23:251-254.
- Lin, H.-S., and J. G. Sebranek. 1979. Effect of nitrite concentration and packaging conditions on color stability and rancidity development on sliced bologna. *J. Food Sci.* 44:1451.
- Lin, H.-S., J. G. Sebranek, D. E. Gallaway, and K. D. Lind. 1980. Effect of sodium erythorbate and packaging conditions on color stability and rancidity development in sliced bologna. *J. Food Sci.* 45:115.
- Love, J. D., and A. M. Pearson. 1974. Metmyoglobin and non-heme iron as prooxidants in egg yolk phospholipid dispersions and cooked meat. *J. Agric. Food Chem.* 22:1032-1034.
- Lundberg, W. O. 1962. Mechanisms. Pp. 31-50 in H. W. Schultz, E. A. Day, and R. O. Sinnhuber, eds. *Symposium on Foods: Lipids and Their Oxidation*. AVI Publishing Company, Westport, Connecticut.
- MacDonald, B., J. I. Gray, and L. N. Gibbins. 1980a. Role of nitrite in cured meat flavor: Antioxidant role of nitrite. *J. Food Sci.* 45:893-897.

- MacDonald, B., J. I. Gray, Y. Kakuda, and M. L. Lee. 1980b. Role of nitrite in cured meat flavor: Chemical Analysis. *J. Food Sci.* 45:889-892.
- MacDonald, B., J. I. Gray, D. W. Stanley, and W. R. Usborne. 1980c. Role of nitrite in cured meat flavor: Sensory analysis. *J. Food Sci.* 45:885-904.
- MacDougall, D. B., D. S. Mottram, and D. N. Rhodes. 1975. Contribution of nitrite and nitrate to the colour and flavour of cured meats. *J. Sci. Food Agric.* 26:1743-1754.
- MacLeod, G., and B. M. Coppock. 1976. Volatile flavor compounds of beef boiled conventionally and by microwave radiation. *J. Agric. Food Chem.* 24:835-843.
- McKay, A. J. 1976. The determination of nitrate and nitrite in cheese. *Aust. J. Dairy Technol.* 29:34-36.
- Mottram, D. S., and D. N. Rhodes. 1974. Nitrite and the flavour of cured meat. I. Pp. 161-171 in B. Krol and B. J. Tinbergen, eds. *Proceedings of the International Symposium on Nitrite in Meat Products.* PUDOC, Wageningen, the Netherlands.
- Mozzell, M. M. 1969. Nasal chemoreception in flavor identification. *Arch. Otolaryng.* 90:131-137.
- Mukai, F. H., and B. D. Goldstein. 1976. Mutagenicity of malonaldehyde, a decomposition product of peroxidized polyunsaturated fatty acids. *Science* 191:868-869.
- Mundt, J. O., and H. M. Kitchen. 1951. Taint in southern country-style hams. *Food Res.* 16:233-238.
- National Oceanic and Atmospheric Administration. 1980. *Processed Fishery Products, Annual Summary, 1979.* Current Fisheries Statistics No. 8003. Department of Commerce, Washington, D.C.
- Nitrite Safety Council. 1980. A survey of nitrosamines in sausages and dry-cured meat products. *Food Technol.* 34:45-51, 53, 103.
- Nordin, H. R. 1969. The depletion of added sodium nitrite in ham. *Can. Inst. Food Sci. Technol. J.* 2:79-85.
- Nordin, H. R., T. Burke, G. Webb, L. J. Rubin, and D. van Binnendyk. 1975. Effect of pH, salt, and nitrite in heat processed meat on destruction and out-growth of P.A. 3679. *Can. Inst. Food Sci. Technol. J.* 8:58-66.

- Ockerman, H. W., T. N. Blumer, and H. B. Craig. 1964. Volatile chemical compounds in dry-cured hams. *J. Food Sci.* 29:123-129.
- Ohye, D. F., and W. J. Scott. 1953. The temperature relations of Clostridium botulinum types A and B. *Austral. J. Biol. Sci.* 6:178-189.
- Olson, V. M., N. A. King, J. A. Langbein, and W. J. Stadelman. 1979. Acceptability of smoked turkey drumsticks with and without nitrite addition. *Poult. Sci.* 58:587-590.
- Page, G. V., and M. Solberg. 1979. Redox potential-dependent nitrite metabolism by Salmonella typhimurium. *Appl. Environ. Microbiol.* 37:1152-1156.
- Paquette, M. W., M. C. Robach, J. N. Sofos, and F. F. Busta. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on color and sensory qualities of commercially prepared bacon. *J. Food Sci.* 45:1293-1296.
- Pearson, A. M., J. D. Love, and F. B. Shorland. 1977. Warmed-over flavor in meat, poultry, and fish. *Adv. Food Res.* 23:1-74.
- Perigo, J. A., and T. A. Roberts. 1968. Inhibition of clostridia by nitrite. *J. Food Technol.* 3:91-94.
- Perigo, J. A., E. Whiting, and T. E. Bashford. 1967. Observations on the inhibition of vegetative cells of Clostridium sporogenes by nitrite which has been autoclaved in a laboratory medium discussed in the context of sub-lethally processed cured meats. *J. Food Technol.* 2:377-397.
- Piotrowski, E. G., L. L. Zaika, and A. E. Wasserman. 1970. Studies on aroma of cured ham. *J. Food Sci.* 35:321-325.
- Pivnick, H. 1970. The inhibition of heat-damaged spores of C. botulinum by sodium chloride and sodium nitrite. Symposium on the Microbiology of Semi-Preserved Foods, October 5-7, Prague. 17 pp. + tables and figures.
- Pivnick, H., and P-C. Chang. 1973. Perigo effect in pork. Pp. 111-116 in B. Krol and B. J. Tinbergen, eds. Proceedings of the International Symposium on Nitrite in Meat Products, Zeist. PUDOC, Wageningen, the Netherlands.
- Pivnick, H., and A. Petrasovits. 1973. A rationale for the safety of canned shelf-stable cured meats: Protection = destruction + inhibition. Pp. 1086-1095 in Proceedings of the 19th Annual Meeting of European Meat Research Workers, Paris, France.

- Pivnick, H., H. W. Barnett, H. R. Nordin, and L. J. Rubin. 1969. Factors affecting the safety of canned, cured, shelf-stable luncheon meat inoculated with Clostridium botulinum. Can. Inst. Food Sci. Technol. J. 2:141-148.
- Pivnick, H., M. A. Johnston, C. Thacker, and R. Loynes. 1970. Effect of nitrite on destruction and germination of Clostridium botulinum and putrefactive anaerobes 3679 and 3679h in meat and in buffer. Can. Inst. Food Sci. Technol. J. 3:103-109.
- Price, J. F., and B. S. Schweigert. 1971. The Science of Meat and Meat Products. 2nd edition, W. H. Freeman and Company, San Francisco, California.
- Rammell, C. G., and M. M. Joerin. 1972. Determination of nitrite in cheese and other dairy products. J. Dairy Res. 39:89-94.
- Randall, C. J., and P. W. Voisey. 1977. A method for measuring the texture of meat and the effect of nitrite and salt addition on the texture of cured meats. J. Texture Studies 8:49-60.
- Reith, J. F., and M. Szakaly. 1967a. Formation and stability of nitric oxide myoglobin. I. Studies with model systems. J. Food Sci. 32:188-193.
- Reith, J. F., and M. Szakaly. 1967b. Formation and stability of nitric oxide myoglobin. II. Studies on meat. J. Food Sci. 32:194-196.
- Renerre, M., and P. Rougie. 1979. (In French; English summary) [Effect of heating on nitrite binding to myoglobin.] Ann. Technol. Agric. 28:423-431.
- Reuter, G. 1975. Classification problems, ecology and some biochemical activities of lactobacilli of meat products. Pp. 221-229 in J. G. Carr, C. V. Cutting, and G. C. Whiting, eds. Lactic Acid Bacteria in Beverages and Food. Academic Press, New York.
- Riemann, H. 1963. Safe heat processing of canned cured meats with regard to bacterial spores. Food Technol. 17:39-49.
- Roberts, T. A., and C. E. Garcia. 1973. A note on the resistance of Bacillus spp., faecal streptococci and Salmonella typhimurium to an inhibitor of Clostridium spp. formed by heating sodium nitrite. J. Food Technol. 8:463-466.
- Roberts, T. A., and G. Hobbs. 1968. Low temperature growth characteristics of clostridia. J. Appl. Bacteriol. 31:75-88.

- Roberts, T. A., and M. Ingram. 1966. The effect of sodium chloride, potassium nitrate and sodium nitrite on the recovery of heated bacterial spores. *J. Food Technol.* 1:147-163.
- Roberts, T. A., and M. Ingram. 1973. Inhibition of growth of C. botulinum at different pH values by sodium chloride and sodium nitrite. *J. Food Technol.* 8:467-475.
- Roberts, T. A., and M. Ingram. 1977. Nitrite and nitrate in the control of Clostridium botulinum in cured meats. Pp. 29-38 in B. J. Tinbergen and B. Krol, eds. *Proceedings of the 2nd International Symposium on Nitrite in Meat Products*, Zeist. PUDOC, Wageningen, Netherlands. 320 pp.
- Roberts, T. A., and J. L. Smart. 1976a. The occurrence of clostridia, particularly Clostridium botulinum in bacon and pork. Pp. 911-915 in J. Wolf, A. N. Barker, D. J. Ellar, G. J. Doing, and G. W. Gould, eds. *Spore Research*. Academic Press, London, United Kingdom.
- Roberts, T. A., and J. L. Smart. 1976b. The occurrence and growth of Clostridium spp. in vacuum-packed bacon with particular reference to C. perfringens (welchii) and C. botulinum. *J. Food Technol.* 11:229-244.
- Roberts, T. A., R. J. Gilbert, and M. Ingram. 1966. The effect of sodium chloride on heat resistance and recovery of heated spores of Clostridium sporogenes (P.A. 3679/S₂). *J. Appl. Bacteriol.* 29:549-555.
- Roberts, T. A., B. Jarvis, and A. C. Rhodes. 1976. Inhibition of Clostridium botulinum by curing salts in pasteurized pork slurry. *J. Food Technol.* 11:25-40.
- Roberts, T. A., A. M. Gibson, and A. Robinson. 1981a. Factors controlling the growth of Clostridium botulinum types A and B in pasteurized, cured meats. I. Growth in pork slurries prepared from "low" pH meat (pH range 5.5-6.3). *J. Food Technol.* 16:239-266.
- Roberts, T. A., A. M. Gibson, and A. Robinson. 1981b. Factors controlling the growth of Clostridium botulinum types A and B in pasteurized, cured meats. II. Growth in pork slurries prepared from "high" pH meat (pH range 6.3-6.8). *J. Food Technol.* 16:267-281.
- Roberts, T. A., A. M. Gibson, and A. Robinson. 1981c. Prediction of toxin production by Clostridium botulinum in pasteurized pork slurry. *J. Food Technol.* 16:337-355.

- Rowe, J. J., J. M. Yarbrough, J. B. Rake, and R. G. Eagon. 1979. Nitrite inhibition of aerobic bacteria. *Current Microbiol.* 2:51-54.
- Sakaguchi, G. 1979. Botulism. Pp. 389-442 in H. Riemann and F. L. Bryan, eds. *Foodborne Infections and Intoxications*, 2nd Ed. Academic Press, New York. 748 pp.
- Sales, C. A., J. A. Bowers, and D. Kropf. 1980. Consumer acceptability of turkey frankfurters with 0, 40, and 100 ppm nitrite. *J. Food Sci.* 45:1060-1061.
- Sanderson, A., A. M. Pearson, and B. S. Schweigert. 1966. Effect of cooking procedure on flavor components of beef. Carbonyl compounds. *J. Agric. Food Chem.* 14:245-247.
- Sato, K., and G. R. Hegarty. 1971. Warmed-over flavor in cooked meats. *J. Food Sci.* 36:1098-1102.
- Schack, W. R., R. A. Greenberg, G. A. Blodgett, and J. H. Silliker. 1959. Thermal processing characteristics of canned non-comminuted meats. *Food Res.* 24:112-118.
- Sebranek, J. G., R. G. Cassens, W. G. Hoekstra, W. G. Winder, E. Y. Podebradsky, and E. W. Kielsmeier. 1973. N-Tracer studies of nitrite added to a comminuted meat product. *J. Food Sci.* 38:1220-1226.
- Sebranek, J. G., B. G. Schroder, R. E. Rust, and D. G. Topel. 1977. A Research Note: Influence of sodium erythorbate on color development, flavor and overall acceptability of frankfurters cured with reduced levels of sodium nitrite. *J. Food Sci.* 42:1120-1121.
- Shamberger, R. J., T. L. Andreone, and C. E. Willis. 1974. Antioxidants and cancer. IV. Initiating activity of malonaldehyde as a carcinogen. *J. Natl. Cancer Inst.* 53:1771-1773.
- Siedler, A. J., and B. S. Schweigert. 1959. Effects of heat, nitrite level, iron salts and reducing agents on formation of denatured nitrosomyoglobin. *J. Agric. Food Chem.* 7:271-274.
- Silliker, J. H. 1959. The effect of curing salts on bacterial spores. Pp. 51-60 in *Proceedings of the 11th Research Conference*. American Meat Institute Foundation, Chicago, Illinois.

- Simon, S., D. E. Ellis, B. D. MacDonald, D. G. Miller, R. C. Waldman, and D. O. Westerberg. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. *J. Food Sci.* 38:919-923.
- Sink, J. D. 1973. Lipid-soluble components of meat flavors/odors and their biochemical origin. *J. Am. Oil Chem. Soc.* 50:470-473.
- Siu, G. M., and H. H. Draper. 1978. A survey of the malonaldehyde content of retail meats and fish. *J. Food Sci.* 43:1147-1149.
- Skjelkvale, R., T. B. Tjaberg, and M. Valland. 1974. Comparison of salami sausage produced with and without addition of sodium nitrite and sodium nitrate. *J. Food Sci.* 39:520-524.
- Skovgaard, N. 1980. Nitrate, nitrite, and nitrosamines in foods--improved production hygiene as an alternative to nitrite. *Nord. Veterinaermed.* 32:387-399.
- Smith, L. DS. 1977. *Botulism: The Organism, Its Toxins, The Disease.* Charles C Thomas, Springfield, Illinois. 236 pp.
- Sofos, J. N., and F. F. Busta. 1980. Alternatives to the use of nitrite as an antibotulinal agent. *Food Technol.* 34:244-251.
- Sofos, J. N., F. F. Busta, and C. E. Allen. 1979a. Botulism control by nitrite and sorbate in cured meats: A review. *J. Food Protect.* 42:739-770.
- Sofos, J. N., F. F. Busta, K. Bhothipaksa, and C. E. Allen. 1979b. Sodium nitrite and sorbic acid effects on Clostridium botulinum toxin formation in chicken frankfurter-type emulsions. *J. Food Sci.* 44:668-672, 675.
- Sofos, J. N., F. F. Busta, K. Bhothipaksa, C. E. Allen, M. C. Robach, and M. W. Paquette. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on Clostridium botulinum toxin production in commercially prepared bacon. *J. Food Sci.* 45:1285-1292.
- Stumbo, C. R., C. E. Gross, and C. Vinton. 1945a. Bacteriological studies relating to thermal processing of canned meats. *Food Res.* 10:260-282.

- Stumbo, C. R., C. E. Gross, and C. Vinton. 1945b. Bacteriological studies relating to thermal processing of canned meats. II. Influence of meat curing agents upon thermal resistance of spores of a putrefactive anaerobic bacterium in meat. *Food Res.* 10:283-292.
- Stumbo, C. R., C. E. Gross, and C. Vinton. 1945c. Bacteriological studies relating to thermal processing of canned meats. III. Influence of meat curing agents upon growth of a putrefactive anaerobic bacterium in heat-processed meat. *Food Res.* 10:293-302.
- Swain, J. W. 1972. Ph.D. Dissertation, University of Missouri, Columbia.
- Tappel, A. L. 1962. Hematin compounds and lipoxidase as biocatalysts. Pp. 122-138 in H. W. Schultz, E. A. Day, and R. O. Sinnhuber, eds. *Symposium on Foods: Lipids and Their Oxidation*. AVI Publishing Company, Westport, Connecticut.
- Tarladgis, B. G. 1961. An hypothesis for the mechanism of the heme catalyzed lipid oxidation in animal tissues. *J. Am. Oil Chem. Soc.* 38:479-483.
- Tarladgis, B. G. 1962a. Interpretation of the spectra of meat pigments. I. Cooked meats. *J. Sci. Food Agric.* 13:481-484.
- Tarladgis, B. G. 1962b. Interpretation of the spectra of meat pigments. II. Cured meats. The mechanism of colour fading. *J. Sci. Food Agric.* 13:485-491.
- Tarr, H. L. A. 1941a. The action of nitrites on bacteria. *J. Fish. Res. Board Can.* 5:265-275.
- Tarr, H. L. A. 1941b. Bacteriostatic action of nitrites. *Nature (London)* 147:417-418.
- Tarr, H. L. A. 1942. The action of nitrites on bacteria: Further experiments. *J. Fish. Res. Board Can.* 6:74-89.
- Tarr, H. L. A. 1944. Action of nitrates and nitrites on bacteria. *J. Fish. Res. Board Can.* 6:233-242.
- Terrell, R. N. 1974. Theory and practice of preblending for use in comminuted meat food mixes. Pp. 23-33 in *Proceedings of Meat Industry Research Conference*. American Meat Institute Foundation, Washington, D.C.

- Tims, M. J., and B. M. Watts. 1958. Protection of cooked meats with phosphates. *Food Technol.* 12:240-243.
- Tompkin, R. B. 1978. The role and mechanism of the inhibition of *C. botulinum* by nitrite--is a replacement available? Pp. 135-147 in Proceedings of the 31st Annual Reciprocal Conference of the American Meat Science Association, Chicago, Illinois.
- Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis. 1977. Variation in inhibition of *C. botulinum* by nitrate in perishable canned comminuted cured meats. *J. Food Sci.* 42:1046-1048.
- Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis. 1978a. Enhancing nitrite inhibition of *Clostridium botulinum* with isoascorbate in perishable canned cured meat. *Appl. Environ. Microbiol.* 35:59-61.
- Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis. 1978b. Causes of variation in botulinal inhibition in perishable canned cured meat. *Appl. Environ. Microbiol.* 35:886-889.
- Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis. 1978c. The effect of iron on botulinal inhibition in perishable canned cured meat. *J. Food Technol.* 13:521-527.
- Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis. 1979a. Iron and the antibotulinal efficacy of nitrite. *Appl. Environ. Microbiol.* 37:351-353.
- Tompkin, R. B., L. N. Christiansen, and A. B. Shaparis. 1979b. Isoascorbate level and botulinal inhibition in perishable canned cured meat. *J. Food Sci.* 44:1147-1149.
- U.S. Department of Agriculture. 1979. Food safety and quality service four plant study to investigate the use of 40 ppm sodium nitrite and 0.26 percent potassium sorbate in bacon. Food Safety Quality Service, U.S. Department of Agriculture, Washington, D.C. 54 pp.
- U.S. Department of Agriculture. 1980. Statistical Summary: Federal Meat and Poultry Inspection for Fiscal Year 1979. Food Safety and Quality Service, U.S. Department of Agriculture, Washington, D.C.
- Vinton, C., S. Martin, Jr., and C. E. Gross. 1947a. Bacteriological studies relating to thermal processing of canned meats. VII. Effect of substrate upon thermal resistance of spores. *Food Res.* 12:173-183.

- Vinton, C., S. Martin, Jr., and C. E. Gross. 1947b. Bacteriological studies relating to thermal processing of canned meats. VIII. Thermal resistance of spores normally present in meat. *Food Res.* 12:184-187.
- Walsh, K. A., and D. Rose. 1956. Meat pigments: Factors affecting the oxidation of nitric oxide myoglobin. *J. Agric. Food Chem.* 4:352-355.
- Walters, C. L., and A. M. Taylor. 1963. Biochemical properties of pork muscle in relation to curing. *Food Technol.* 17(3):354-359.
- Walters, C. L., R. J. Casselden, and A. McM. Taylor. 1967. Nitrite metabolism by skeletal muscle mitochondria in relation to haem pigments. *Biochim Biophys. Acta* 143:310.
- Walters, C. L., R. J. Hart, and S. Perse. 1979. The possible role of lipid pseudonitrosites in nitrosamine formation in fried bacon. *Z. Lebensm. Unters. Forsch.* 168:177-180.
- Wasserman, A. E., and N. Gray. 1965. Meat flavor. I. Fractionation of water soluble precursors. *J. Food Sci.* 30:801-807.
- Wasserman, A. E., and F. Talley. 1972. The effect of sodium nitrite on the flavor of frankfurters. *J. Food Sci.* 37:536-538.
- Wasserman, A. E., W. Kimoto, and J. G. Phillips. 1977. Consumer acceptance of nitrite-free bacon. *J. Food Protect.* 40:683-685.
- Watts, B. M., and B. T. Lehmann. 1965. The effect of ascorbic acid on the oxidation of hemoglobin and the formation of nitrosylhemoglobin. *Food Res.* 17:100.
- Weiss, T. J., R. Green, and B. M. Watts. 1953. Effect of metal ions on the formation of nitric oxide hemoglobin. *Food Res.* 18:11-16.
- Williams, J. C., and B. E. Greene. 1979. Plate waste of bacon cured with and without nitrite. *J. Food Sci.* 44:1260, 1262.
- Wilson, B. R., A. M. Pearson, and F. B. Shorland. 1976. Effect of total lipids and phospholipids on warmed-over flavor in red and white muscle from several species as measured by thiobarbituric acid. *J. Agric. Food Chem.* 24:7-10.
- Woods, L. F. J., and J. M. Wood. In press. The effect of nitrite inhibition on the metabolism of Clostridium botulinum. *J. Appl. Bacteriol.*

- Woods, L. F. J., J. M. Wood, and P. A. Gibbs. 1981. The involvement of nitric oxide in the inhibition of the phosphoroclastic system in Clostridium sporogenes by sodium nitrite. *J. Gen. Microbiol.* 125:399-406.
- Woolford, G., and R. G. Cassens. 1977. The fate of sodium nitrite in bacon. *J. Food Sci.* 42:586-589, 596.
- Woolford, G., R. G. Cassens, M. L. Greaser, and J. G. Sebranek. 1976. The fate of nitrite: Reaction with protein. *J. Food Sci.* 41:585-588.
- Yarbrough, J. M., J. B. Rake, and R. G. Eagon. 1980. Bacterial inhibitory effects of nitrite: Inhibition of active transport, but not of group translocation, and of intracellular enzymes. *Appl. Environ. Microbiol.* 39:831-834.
- Younathan, M. T., and B. M. Watts. 1959. Relationship of meat pigments to lipid oxidation. *Food Res.* 24:728-734.
- Zipser, M. W., T. W. Kwon, and B. M. Watts. 1964. Oxidative changes in cured and uncured frozen cured pork. *J. Agric. Food Chem.* 12:105-109.

CHAPTER 4

THE CHEMISTRY OF NITRATE, NITRITE, AND NITROSATION

| | <u>Page</u> |
|--|-------------|
| NITRATE AND NITRITE..... | 4-3 |
| Chemical Transformation..... | 4-3 |
| Biological Transformation..... | 4-6 |
| NITROSATION..... | 4-8 |
| Nitrosation by Aqueous Nitrous Acid..... | 4-9 |
| Nitrosation by Nitrate and Nitrite Salts..... | 4-18 |
| Nitrosation by Nitrogen Oxides..... | 4-19 |
| Transnitrosation: Nitrosation by Organic Nitroso and Nitro Compounds..... | 4-23 |
| Nitrosation of Primary, Tertiary, and Quarternary Amino Compounds..... | 4-28 |
| Factors Influencing Nitrosation in Foods..... | 4-31 |
| SUMMARY..... | 4-32 |
| Nitrate and Nitrite..... | 4-32 |
| Nitrosation..... | 4-32 |
| CONCLUSIONS AND RECOMMENDATIONS..... | 4-34 |
| REFERENCES..... | 4-35 |

THE CHEMISTRY OF NITRATE, NITRITE, AND NITROSATION

Elemental nitrogen and oxygen combine in various proportions to form entities such as nitrate (NO_3^-), nitrite (NO_2^-), hyponitrite ($\text{N}_2\text{O}_2^{2-}$), and several nitrogen oxides, including nitrous oxide (N_2O), nitric oxide (NO), dinitrogen trioxide or nitrous anhydride (N_2O_3), nitrogen dioxide (NO_2), dinitrogen tetroxide (N_2O_4), and dinitrogen pentoxide (N_2O_5). Both nitric oxide and nitrogen dioxide are relatively stable free radicals and are therefore sometimes denoted NO^\bullet and NO_2^\bullet , respectively.

This chapter is limited to the chemistry of those compounds which can directly or indirectly participate in nitrosation reactions, especially in the formation of N-nitroso compounds containing the group $> \text{N}-\text{N}=\text{O}$. These compounds are referred to in the text as nitroso derivatives of the parent amine compound without the prefix, e.g., nitrosodimethylamine, rather than N-nitrosodimethylamine.

NITRATE AND NITRITE

Nitrate salts of several metallic elements (MNO_3) are distributed widely in nature, whereas nitrite salts (MNO_2) are less common because of their higher chemical reactivity. The underlying reason for this difference is that nitric acid (HNO_3 ; $\text{pK}_a \sim 1.3$) is a stronger acid than nitrous acid (HNO_2 ; $\text{pK}_a 3.4$) and, therefore, forms less readily from its salts. Both the occurrence of nitrite and nitrate salts and exposure of humans to these compounds have been thoroughly reviewed (Archer, in press; Green et al., in press; National Academy of Sciences, 1978; White, 1975, 1976). Estimates of current exposure are presented in Chapter 5.

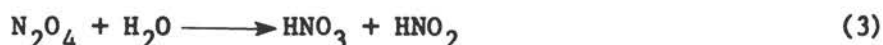
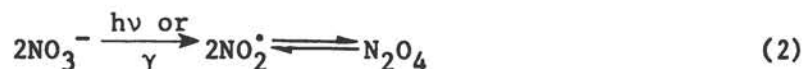
Chemical Transformation

Nitrate salts are often hydrated, and nearly all are soluble and highly dissociated in aqueous media (Mellor, 1928). Both the salts and the nitrate ion are relatively chemically inert. The reactions that are most important in this discussion, i.e., the reduction of nitrate ion to either nitrogen dioxide or nitrite ion (NO_2^-), do not occur readily under neutral or mildly acidic conditions. Generally, such reactions require temperatures in excess of 500°C or treatment with a reducing agent such as iodide ion (I^-) or metals in the presence of strong acid (Mellor, 1928). The only transformations of potential biochemical interest appear to be the reduction of alkali metal nitrates to the corresponding nitrites by photolysis (Bayliss

and Bucat, 1975; Shuali *et al.*, 1969) and radiolysis (Kiwi and Daniels, 1978; Muhammad and Maddock, 1978) of aqueous solutions at ambient temperatures (equation 1). Both transformations are considered to



proceed via nitrogen dioxide, which hydrolyzes to a mixture of nitrous and nitric acids following dimerization to dinitrogen tetroxide (equations 2 and 3):



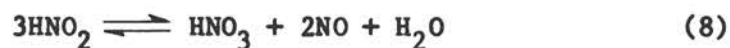
The reductive radiolysis proceeds at dose levels commonly used in the sterilization of foods by radiation, and the reductive photolysis can be induced under atmospheric conditions (Petroni *et al.*, 1967). Significantly, dinitrogen tetroxide is a powerful nitrosating agent toward amino compounds in aqueous and other solvents (discussed below in the section on nitrosation by nitrogen oxides).

Nitrite salts are much more reactive than the corresponding nitrate salts because the nitrite ion is a stronger nucleophile than the nitrate ion (Edwards, 1954) and because nitrous acid is weaker than nitric acid. Thus, nitrous acid is generated under mildly acidic conditions ($\text{pH} < 5$) and much of the chemistry of nitrite salts relates to the presence of nitrous acid.

In aqueous solution, nitrous acid exists in equilibrium with nitrous anhydride (dinitrogen trioxide, N_2O_3), as shown in equation 4, where $K_E = 0.2$ at 20°C (Turney, 1960):



Dinitrogen trioxide is unstable and decomposes, especially when heated, to a mixture of nitric oxide and nitrogen dioxide (equation 5). Dimerization of nitrogen dioxide to dinitrogen tetroxide and subsequent hydrolysis (equations 6 and 7) produces a mixture of nitrous and nitric acids. Thus, after standing, aqueous acidic solutions ($\text{pH} < 5$) of nitrite salts tend to decompose to the nitrate salt, thereby releasing nitric oxide, as summarized by equation 8 (Mellor, 1928):

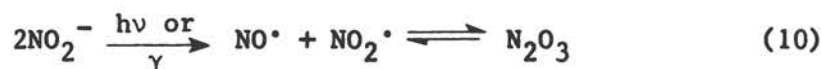


Nitrous acid (and, inter alia, nitrite salts) can be either oxidized to nitrate ion ($\text{HNO}_2 + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + 3\text{H}^+ + 2\text{e}^-$; $E^\circ = -0.94 \text{ V}$) or reduced to nitric oxide ($\text{HNO}_2 + \text{H}^+ + \text{e}^- \rightarrow \text{NO} + \text{H}_2\text{O}$; $E^\circ = -1.0 \text{ V}$). Oxidation of the acid requires relatively strong reagents such as manganese dioxide (MnO_2) or chlorine (Cl_2), but weaker oxidants are effective in alkaline media ($\text{NO}_2^- + 2\text{OH}^- \rightarrow \text{NO}_3^- + \text{H}_2\text{O} + 2\text{e}^-$; $E^\circ = -0.01 \text{ V}$) and the spontaneous decomposition of nitrous acid (equation 8) also converts it to nitric acid. Nitrous acid is readily reduced to nitric oxide by reaction with a wide range of inorganic materials such as copper (Cu^+), iron (Fe^{2+}), iodide (I^-), and bisulfite (HSO_3^-) ions, and organic compounds such as ascorbic acid, polyphenols, and thiols. Thus, nitrous acid is a useful oxidant (Turney and Wright, 1959). The behavior of nitrous acid in the presence of reducing agents is not predictable from the reduction to nitric oxide alone. Other products may result, depending on the reductant selected, the acidity, and the temperature. Thus, reaction of nitrite ion with hydrogen sulfide (H_2S) yields nitric oxide and sulfur in acidic solution, ammonia (NH_3) and sulfur in bicarbonate buffer, and ammonia, sulfur, and thiosulfate ($\text{S}_2\text{O}_3^{2-}$) in unbuffered nitrite salt solutions (Moeller, 1952). Furthermore, nitrous acid is reduced to nitrogen in the presence of compounds containing primary amino groups, e.g., ammonia, primary amines (RNH_2) and amides (RCO.NH_2), hydrazine (H_2NNH_2), urea ($\text{H}_2\text{NCO.NH}_2$), sulfamic acid ($\text{NH}_2\text{SO}_3\text{H}$), or hydroxylamine (NH_2OH) (equation 9):



Aqueous solutions of alkali metal nitrites are also reduced by radiolysis (Dainton and Logan, 1965; Grätzel et al., 1970) and photolysis (Treinin and Hayon, 1970) in a manner analogous to the corresponding nitrates. The reactions differ, however, insofar as a mixture of

nitric oxide and nitrogen dioxide is obtained from nitrites (equations 10 and 11), but only nitrogen dioxide is obtained from nitrates.



Thus, there is no net loss of nitrite in a closed system, but generation of dinitrogen trioxide as an intermediate results in nitrosation reactions under nonacidic conditions. Many of the redox reactions of nitrous acid and nitrite salts are relevant to their biochemical behavior. They explain why nitrate salts exist at much higher concentrations than nitrite salts in the environment and the mechanisms by which many compounds inhibit the formation of N-nitroso compounds.

Both nitrous acid and, to a lesser extent, nitrite salts can nitrosate a variety of inorganic and organic materials. These reactions, which proceed via several reactive nitrosating agents, are discussed in detail below. They are relevant to biochemical processes, especially the formation of N-nitroso compounds. Furthermore, several of the redox processes in which nitrous acid is reduced either to nitric oxide or to nitrogen involve an initial nitrosation of the reductant.

Biological Transformation

Biological or enzymatic transformations of both nitrate and nitrite salts are well-known components of the nitrogen cycle. They are redox processes associated with both the conversion of atmospheric nitrogen to the nitrate-plant nutrient and its conversion to ammonia during assimilation by plants and microorganisms.

Much of our knowledge concerning the microbial oxidation of nitrogen comes from studies of the autotrophic microorganisms Nitrosomonas and Nitrobacter (Schmidt, 1978). Nitrosomonas catalyzes the oxidation of ammonia and hydroxylamine (NH_2OH) to nitrite, whereas Nitrobacter catalyzes the oxidation of nitrite to nitrate, which is relevant to the present discussion. This oxidation is known to involve a flavomolybdoprotein and cytochrome, which probably act sequentially. The activity is linked to the respiratory chain and is associated with the cell membrane (Nicholas, 1978). Some of the physical properties of cytochrome isolated from Nitrobacter resemble mammalian cytochrome (Butt and Lees, 1958). Similar oxidations have been demonstrated in heterotrophic bacteria (Focht and Verstraete, 1977). Nitrite is oxidized to nitrate in red blood cells, where the

conversion of 1 mole of oxyhemoglobin to methemoglobin is coupled with the oxidation of 1 mole of nitrite to nitrate (Kosaka et al., 1979; Parks et al., 1981).

In plants and microorganisms, nitrate is reduced to ammonia by two, well-defined enzymatic processes. In the first, nitrate is reduced to nitrite in a reaction that is catalyzed by the flavomolybdo-protein, nitrate reductase (EC 1.6.6.3). In the second, the reduction of nitrite to ammonia is catalyzed by the iron-containing protein, nitrite reductase (EC 1.6.6.4).

Nitrate reductases have been isolated from bacteria, fungi, algae, and plants. Their molybdenum (Mo) content is approximately one atom per mole, and they have a molecular weight of 1.6 to 6×10^5 daltons and a K_m for nitrate from 0.015 to 1 mM (Hewitt, 1975). In bacteria, two different nitrate reductases have been identified (Hewitt, 1974), and their properties are discussed in a review written by Green et al. (in press). Nitrate reductase activity has also been demonstrated in various rat tissues (Cohen and Weinhouse, 1971). Its properties are similar to those of xanthine oxidase, which can also reduce nitrate to nitrite (Rajagopalan et al., 1962). Nitrite reductase has also been isolated from bacteria, algae, fungi, and plants. This enzyme contains iron, has a molecular weight of 0.6 to 1.2×10^5 daltons (but 3.5 to 6.7×10^5 daltons for *E. coli* and yeast), and a K_m for nitrite of 0.005 to 0.07 mM (Hewitt, 1975). In plants and algae, enzymes are usually specific for donors of single electrons (ferrodoxin, methyl viologen) and do not utilize reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Hucklesby and Hewitt, 1970). In yeast and bacteria, however, the enzyme is specific for NADPH and is flavin dependent (Prabhakararao and Nicholas, 1970).

E. coli also has two nitrite reductases, one of which is specific for reduced nicotinamide adenine dinucleotide (NADH) and is unable to reduce sulfite (SO_3^{2-}), whereas the other is specific for NADPH and can reduce sulfite (Abou-Jaoudé et al., 1979). An alternative mechanism has been proposed for nitrite reduction by bovine heart cytochrome c, whereby cyclic turnover by the cytochrome yields nitric oxide from nitrite ion and an electron donor (Ori and Shimada, 1978). As nitric oxide is produced during the anaerobic incubation of fresh pig muscle with nitrite ion at pH 6 (Walters and Taylor, 1964), this reaction may be general for muscle tissue.

There is good evidence that nitrate and nitrite salts are both formed and destroyed endogenously by microflora or by metabolic action. For example, nitrate ion is reduced to nitrite ion by microflora normally present in the mouth (Goaz and Biswell, 1961; Ishidate et al., 1974; Keith et al., 1930; Spiegelhalder et al., 1976; Tannenbaum et al., 1979; Ville and Mestrezat, 1907), by microorganisms that colonize the abnormally hypochlorhydric or achlorhydric stomach (Jones et al., 1978; Ruddell et al., 1976,

1978; Sander and Seif, 1969; Schlag et al., 1980), and by flora that inhabit the infected urinary tract or bladder (Cruickshank and Moyes, 1914; Guignard and Torrado, 1978; Jørgart, 1978; Kumin and DeGroot, 1977; Scheifele and Smith, 1978; Sinaniotis et al., 1978).

Nitrate salts are also reduced by mammalian enzymes in vitro (Cohen and Weinhouse, 1971; Rajagopalan et al., 1962). As noted above, nitrate ion forms from nitrite ion via oxyhemoglobin (Kosaka et al., 1979) and, possibly, from more reduced nitrogen compounds by mammalian systems (Green et al., in press). These reactions, together with the metabolism, distribution, and excretion of nitrate and nitrite salts in animals and humans, have been discussed by Green et al. (in press) and Archer (in press).

NITROSATION

Nitrosation usually results from reaction of compounds that can generate a nitrosonium ion (NO^+) and, less often, nitric oxide (NO). Free nitrosonium ion can be obtained only in strong acids or in solid salts such as nitrosonium tetrafluoroborate (NO^+BF_4^-), but it is readily available from labile nitrosating agents (represented as YNO, where Y is a nucleophilic catalyst) generated in nitrite solutions and by gaseous nitrogen oxides. These nitrosating agents react with almost any nucleophile (i.e., electron-rich entity) such as amines, alcohols, and anionic species.

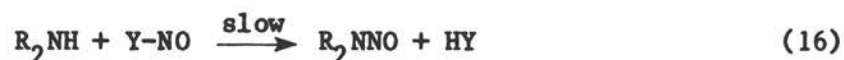
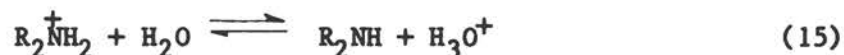
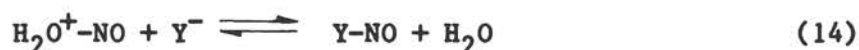
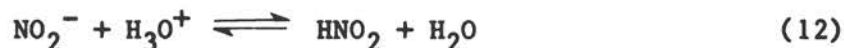
Reactions with amines and amides are of primary interest because they may produce carcinogenic compounds including nitrosamines [$\text{R}(\text{R}')\text{NNO}$] and nitrosamides [$\text{R}(\text{R}')\text{CONNO}$]. However, nitrosation of compounds in food and in vivo has to be considered in connection with catalytic and inhibitory effects. The formation of nitrosamines from nitric oxide is less common, but it does occur with amine anions (R_2N^-) and when oxidants generate either amino radicals ($\text{R}_2\text{N}^\bullet$) or radical cations ($\text{R}_2\text{N}^{\bullet+}$).

Carcinogenic N-nitroso products derive from many different amino compounds including most secondary (R_2NH) and tertiary (R_3N) amines, secondary and tertiary amides ($\text{RCO.NHR}'$, $\text{RCO.NR}'\text{R}''$), N-substituted ureas ($\text{R}'\text{HNCO.NH}_2$), guanidines [$\text{R}'\text{HNC}(=\text{NH})\text{NH}_2$], and urethanes ($\text{RR}'\text{N.COOR}$). The most common N-nitroso compounds are derived from either secondary amines ($\text{RR}'\text{NH}$) or their N-acylated analogues [$\text{R}(\text{R}')\text{CO.NH}$].

The chemistry of N-nitrosation in vitro is relatively well understood (Challis, 1981; Ridd, 1961). The principles can be applied to reactions in food and in vivo to explain both catalysis and inhibition. Nitrosation is best discussed in relation to the type or source of nitrosating agent.

Nitrosation by Aqueous Nitrous Acid

Aqueous acidic solutions of nitrite salts (or nitrous acid) at $\text{pH} < 5$ are the best known nitrosating media, and their reactions have been widely investigated (Challis, 1981; Ridd, 1961). Neither nitrous acid nor nitrite ion reacts directly with the amino substrate. The effective nitrosating agent (Y-NO) forms from the nucleophilic catalyst (Y^- , e.g., NO_2^- , Cl^- , SCN^-) and protonated nitrous acid in a rapid, preequilibrium step (equations 12, 13, and 14).



Only the unprotonated amino substrate, which is in equilibrium with its conjugate acid (equation 15), reacts with Y-NO (equation 16). Thus, reaction rates are dependent on pH, the basicity of the amino substrate, and the presence of catalytic anions (Y^-) in addition to the concentrations of amino substrate and nitrite ion.

In the absence of other nucleophiles, nitrite ion acts as the catalyst, Y^- , in which case the reactive species is dinitrogen trioxide (nitrous anhydride) formed in equilibrium with nitrous acid (equation 17).



For basic secondary amines ($\text{pK}_a > 5$), the rates of nitrosamine formation calculated from the gross amounts of amine and nitrite salt added (equation 18) have a characteristic dependence on pH, reaching a maximum at approximately pH 3.4 and, for amino acids, at approximately pH 2.5 (Mirvish, 1975). This reflects the

$$\text{Rate} = k_1 [\text{amine}] [\text{nitrite}]^2 \quad (18)^1$$

¹Brackets refer to concentration.

counteracting effects of acidity on the concentrations of dinitrogen trioxide and unprotonated amine. The level of observed rates over the entire pH range, however, is dependent on the amine basicity (pK_a), which determines the proportion of unprotonated amine available for reaction. Data in Table 4-1 show clearly that N-nitrosamines form most rapidly (i.e., largest k_1 values) from the least basic amino substrates. However, rate coefficients k_2 in Table 4-1, calculated from the actual concentrations of nitrous acid and unprotonated amine in solution, have similar values irrespective of the amine pK_a .

$$\text{Rate} = k_2 [R(R')NH][HNO_2]^2 \quad (19)^1$$

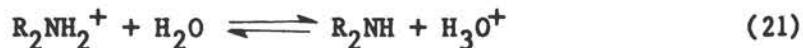
Thus, the reactivity of these compounds is probably governed by factors such as diffusion through the solvent and is not due solely to the ease with which the unprotonated amine attacks dinitrogen trioxide. This suggestion has important ramifications in explaining the effect of catalysts.

Reaction via dinitrogen trioxide seems to apply to most secondary alkyl and heterocyclic amines and amino acids at pH 2 to 5 in the absence of catalysts such as thiocyanate (SCN^-) or iodide (I^-) ions. It is important that neither the type nor concentration of buffer seems to have an appreciable influence, suggesting that similar rates may apply to reactions in gastric contents.

Weakly basic amino compounds (e.g., amides, ureas, and some aromatic amines) are too unreactive to combine readily with dinitrogen trioxide. At approximately pH 2 or lower, however, they undergo nitrosation by another pathway, which is attributed to a direct reaction of the neutral substrate with either hydrated or unhydrated nitrosonium ion, i.e., H_2ONO^+ or NO^+ (Challis, 1981; Mirvish, 1975; Ridd, 1961) (equation 20).

$$\text{Rate} = k_3 [R(R')NH][HNO_2][H_3O^+] \quad (20)$$

Equations 21 and 22 show acid-catalyzed nitrosation of a weakly basic amino compound by acidified nitrous acid. Usually, these reactions



¹Brackets refer to concentration.

TABLE 4-1

Rate Constants k_1 and k_2 (Equations 18 and 19) for the Nitrosation of Amines at the Optimum pH and 25°C

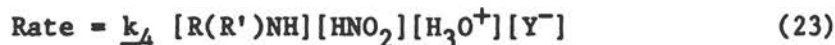
| <u>Amine</u> | <u>pK_a</u> | <u>Optimum pH</u> | <u>k_1 (M⁻²sec⁻¹)</u> | <u>$k_2 \times 10^{-5}$ (M⁻²sec⁻¹)</u> | <u>Reference</u> |
|------------------------------|-----------------------|-----------------------|--|---|---|
| Piperidine | 11.2 | 3.0 | 0.00045 | 1.4 | Mirvish, 1972 |
| Dimethylamine | 10.72 | 3.4 | 0.0017 | 1.5 | Mirvish, 1970 |
| Pyrrolidine | 11.27 | 3.0 | 0.0053 | 21.0 | Mirvish, 1975 |
| N-Methylethanol- amine | 9.5 | 3.2 | 0.010 | 0.62 | Mirvish, 1975 |
| N-Methylbenzyl- amine | 9.54 | 3.0 | 0.013 | 0.92 | Mirvish, 1975 |
| Proline | - | 2.5 | 0.037 | 1.4 | Mirvish <u>et al.</u> , 1973 |
| Sarcosine | - | 2.5 | 0.23 | 2.6 | Mirvish <u>et al.</u> , 1973 |
| Prolylglycine | 8.97 | 3.0 | 0.25 | 5.0 | Mirvish <u>et al.</u> , 1973 |
| Hydroxyproline | - | 2.5 | 0.31 | 2.1 | Mirvish <u>et al.</u> , 1973 |
| Prolylleucylglycin- amide | 8.97 | 3.4 | 0.38 | 6.2 | Mirvish <u>et al.</u> , 1973 |
| Morpholine | 8.7 | 3.4 | 0.42 | 2.3 | Fan and Tannenbaum, 1973; Mirvish, 1972 |
| Mononitrosopiper- azine | 6.8 | 3.0 | 6.7 | 0.38 | Mirvish, 1972 |
| Aminopyrine | 5.04 | 2.0 | 80 | 1.0 | Mirvish <u>et al.</u> , 1974 |
| Piperazine | 5.57 | 3.0 | 83 | 0.62 | Mirvish, 1972 |
| N-Methylaniline | 4.85 | - | 250 ^a | 18.0 ^a | Kalatzis and Ridd, 1966 |

^aCalculated from rates at pH 1 and 0°C.

are quite slow at $\text{pH} > 3$, but they become progressively faster with increasing acidity. Furthermore, the reactivity of these substances is strongly dependent on their structure. This is shown in Table 4-2 for reaction at $\text{pH} 2$ and 25°C , where the fastest reactions (largest k_3) tend to apply to the most basic compounds (Mirvish, 1975).

To sum up this section, the rate of nitrosation of most secondary amines is proportional to the square of nitrite concentration (equation 19) and shows a maximum value at $\text{pH} 3$ to 3.4 (equation 19). The rate of nitrosation of N-alkylureas, N-alkylcarbamates, simple amides, and some aromatic amines is proportional to the nitrite and hydrogen ion concentrations. Thus, it does not show a pH maximum, but increases steadily as the pH drops (equation 20). Despite these differences, it can be stated that, in general, weakly basic secondary amines, N-alkylureas, and N-alkylcarbamates are most readily nitrosated, and that strongly basic secondary amines, simple N-alkylamides, and guanidines are nitrosated more slowly. Primary, tertiary, and quaternary amines usually yield nitrosamines still more slowly, with the exception of the tertiary amine compound aminopyrine, which is extremely rapidly nitrosated (Table 4-1). However, the relative ease of nitrosation will vary according to the conditions, especially the pH and the nitrite concentration, both of which affect reactions differently, as shown in equations 19 and 20 (pH below 3, and low nitrite concentrations favor the reaction shown in equation 20).

Catalysis. In the presence of anionic (Y^-) or nucleophilic entities (HY), nitrous acid forms additional Y-NO reagents (equations 14 and 16) and nitrosamine formation follows equation 23:



The formation of N-nitroso compounds is accelerated principally by raising the concentration of nitrosating agents (Fan and Tannenbaum, 1973; Williams, 1977). Some of these reagents may also be more reactive than dinitrogen trioxide. Examples of the Y-NO species include nitrosyl thiocyanate (SCN-NO), which is formed from the thiocyanate ion, and nitrosyl iodide (I-NO), which is formed from the iodide ion. The rate of nitrosation according to equation (23) is proportional to the concentration of nitrite.

Strong accelerations by thiocyanate and iodide ions have attracted attention because of their possible relevance in vivo (Boyland and Walker, 1974; Boyland et al., 1971; Fan and Tannenbaum, 1973). In smokers, salivary thiocyanate ion levels are known to be elevated. Iodide ion is present in gastric secretions. The degree of catalysis is dependent on the structure and basicity of the amino substrates.

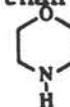
TABLE 4-2

Rate Constant k_3 (Equation 20) for the Nitrosation of Amides at 25°C and pH 2

| Amide | k_3 ($M^{-2}sec^{-1}$) | Nitrosamides: | | Reference |
|--|-------------------------------|--|--|---------------|
| | | UV Absorption in Water [λ in nm (ϵ)] | | |
| N-Methylbenzamide | 0.0014 | 406 (75) | | Mirvish, 1975 |
| N-Methylacetamide | 0.0025 | 402 (66) | | Mirvish, 1975 |
| Dihydrothymine | 0.0035 | 406 (95) | | Mirvish, 1975 |
| Methylguanidine | 0.004 | - | | Mirvish, 1971 |
| 1-Methyl-3-nitroguanidine | 0.008 | 420 (109) | | Mirvish, 1975 |
| Dihydrouracil | 0.010 | 406 (94) | | Mirvish, 1975 |
| Hydantoin | 0.042 | 417 (66) | | Mirvish, 1972 |
| Ethyl N-ethylcarbamate | 0.10 | 420 (64) | | Mirvish, 1971 |
| Hydantoic acid | 0.18 | 390 (85) | | Mirvish, 1975 |
| Ethyl N-methylcarbamate | 0.37 | 410 (71) | | Mirvish, 1971 |
| 2-Ureidoethanol | 0.38 | 398 (89) | | Mirvish, 1975 |
| DL-Citrulline | 0.72 | 396 (83) | | Mirvish, 1971 |
| Phenylurea | 2.2 | 400 (74) | | Mirvish, 1975 |
| Ethylurea | 3.0 | 400 (78) | | Mirvish, 1971 |
| Trimethylurea | 7.0 | 400 (36) | | Mirvish, 1975 |
| Ethylcyanamide | 8.0 | 388 (126) | | Mirvish, 1975 |
| Methylurea | 10.5 | 400 (79) | | Mirvish, 1971 |
| Ethoxyphenylurea | 13 | 420 (49) | | Mirvish, 1975 |
| 2(1H)-Tetrahydropyrimidinone (propylene urea) | 18 | 411 (96) | | Mirvish, 1975 |
| 1,3-Dimethylurea | 200 | 400 (72) | | Mirvish, 1975 |
| 2-Imidazolidinone (ethylene urea) | 400 | 400 (76) | | Mirvish, 1975 |

The accelerations for weakly basic secondary amines, e.g., N-methylaniline ($C_6H_5NHCH_3$), are greater (Fan and Tannenbaum, 1973) than

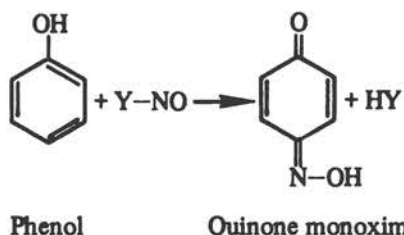
those for more strongly basic secondary amines, e.g., morpholine



(Boyland and Walker, 1974; Boyland et al., 1971). No anion catalysis is observed for amides (Berry and Challis, 1974), thioamides ($RCSNHR^-$) (Al-Mallah et al., 1974), ureas, and urethanes (Hallett et al., 1980). Berry and Challis (1974) proposed a mechanism to explain this.

A number of electron-rich compounds other than amines react with acidified nitrite to yield Y-NO compounds, but examples leading to catalysis of nitrosamine formation by the Y-NO are rare. Usually the nitroso product Y-NO is either too stable to react further or so unstable that spontaneous decomposition ensues before further reaction

with an amino substrate can occur. In both cases the outcome is inhibitory rather than catalytic. As a general rule, reactions involving carbon atoms give stable products, whereas reactions at hetero atoms produce unstable intermediates with catalytic potential. An interesting example of both is found with phenol (C_6H_5OH), which is known to be present in gastric secretions. Phenol reacts readily with aqueous nitrous acid at $pH < 4$ (equation 24) to give the stable quinone monoxime (Challis and Lawson, 1971):



(24)

Subsequently, it has been shown that quinone monoxime catalyzes

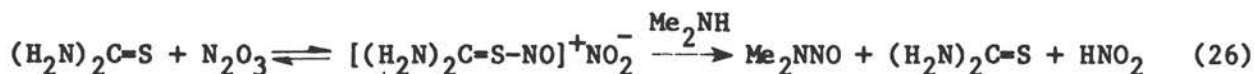
the nitrosation of pyrrolidine () (Davies and McWeeny,

1977) and diethylamine (Walker *et al.*, 1979), probably by rapid formation of an O-nitroso derivative, which then reacts with the secondary amine:



(25)

Under these circumstances, nitrosamine formation is proportional to $[\text{nitrite}]$ rather than to $[\text{nitrite}]^2$. The pathway will be important at high $[\text{nitrite}]/[\text{phenol}]$ ratios only. Other nucleophiles whose interactions with nitrous acid appear to catalyze nitrosamine formation are sulfur compounds and alkenes. Thus, nitrosation of dimethylamine $[(CH_3)_2NH]$ at $pH 4$ is strongly catalyzed by thiourea $[(NH_2)_2C=S]$ (Masui *et al.*, 1979):



Under certain conditions, thiols (e.g., cysteine) mildly catalyze the nitrosation of pyrrolidine, presumably via thionitrite esters (RSNO) (Davies *et al.*, 1978a,b). Furthermore, pseudonitrosites obtained by reaction of nitrogen oxides with alkenes (including the unsaturated lipid palmitodiolein) nitrosate morpholine in a lipid solvent (Walters *et al.*, 1979). However, sorbic acid ($\text{CH}_3\text{CH}=\text{CHCH}=\text{CHCOOH}$) produces structurally similar intermediates to pseudonitrosites when reacted with nitrous acid at pH 3.5, but is not reported to catalyze nitrosamine formation (Osawa *et al.*, 1979).

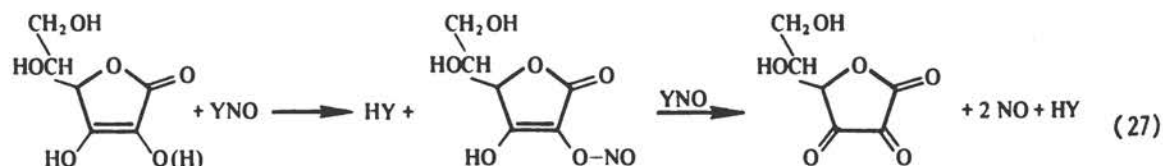
Substances capable of forming micelles also catalyze the formation of nitrosamines from nitrous acid. For example, at pH 3.5 there is an 800-fold increase in rate of reaction with di-n-hexylamine in the presence of decyltrimethylammonium bromide, but much smaller effects apply to reaction of secondary amines with shorter alkyl substituents (Okum and Archer, 1977). Catalysis by both microorganisms (Yang *et al.*, 1977) and bile acid conjugates (Kim *et al.*, 1980) has also been explained by hydrophobic interactions between the amine and either the microorganisms or the bile acid conjugates.

Inhibition. The simplest mode of inhibition is to convert amino substrates to their unreactive conjugate acids by raising the solvent acidity or to convert nitrosating agents to inactive nitrite ion by reducing the acidity above pH 6. Both methods meet with limited success because the two effects counteract each other. Hence, the maximum rate for aliphatic and alicyclic amines is attained at approximately pH 3.4, and some substrates, e.g., amides and ureas, undergo significant protonation only in relatively concentrated acids. Thus, effective inhibition requires materials (scavengers) that readily convert nitrosating agents to innocuous products. Generally, this implies compounds that either reduce nitrous acid to nitrogen or nitric oxide, or that bind the nitrosonium ion (NO^+) irreversibly.

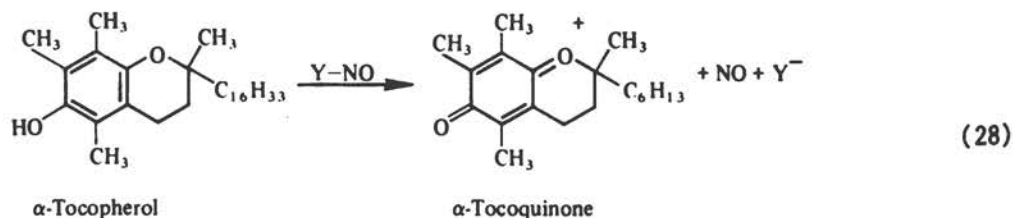
Nitrous acid is reduced to nitrogen in the presence of ammonia, primary amines, hydrazine, urea, sulfamic acid and its salts, hydroxylamine, and azides (XN_3). Ammonia is a poor inhibitor, however, because it is extensively protonated at low pH. A similar reservation applies to primary amines (except for aromatic amines), and alkylation of the primary amines concurrent with deamination produces small amounts of secondary amines and, ultimately, nitrosamines (Tannenbaum *et al.*, 1978). The remaining compounds are more useful, but urea and sulfamic acid appear to be effective only below pH 2 (Mirvish, 1975). Some studies suggest that sulfamic acid may enhance nitrosamine formation above pH 4 (Ziebarth and Teichmann, 1980). Hydroxylamine (Hughes and Stedman, 1963; Hussain *et al.*, 1968; Morgan *et al.*, 1968), hydrazoic acid (HN_3)

or azide ion (Stedman, 1959a,b), and hydrazine (H_2NNH_2) (Perrott et al., 1976) all react rapidly with nitrous acid over a wide range of pH.

A number of compounds reduce nitrous acid to nitric oxide under mildly acidic conditions. These include thiols (RSH), sulfur dioxide (SO_2), bisulfite (HSO_2^-), ascorbic acid (vitamin C), tocopherols (e.g., vitamin E), 1,2- and 1,4-dihydroxyphenols [$C_6H_4(OH)_2$], gallic acid [$C_6H_2(OH)_3COOH$], and other well-established synthetic and natural "antioxidants." Their ability to inhibit the formation of N-nitroso compounds both *in vitro* and *in vivo* has been examined assiduously, as summarized in a recent review by Douglass et al. (1978). Mirvish et al. (in press) have championed the application of ascorbic acid as an inhibitor. It is effective over a wide range of pH because both the free acid and the ascorbate ion rapidly reduce Y-NO to nitric oxide (Dahn and Loewe, 1960):



For lipophilic matrices, however, the lipid-soluble α -tocopherol may be superior (Fiddler et al., 1978; Mergens et al., 1978). This is discussed further in Chapter 6. α -Tocopherol acts like 1,2-dihydroxyphenols (see equations 28 and 29) in being oxidized by dinitrogen trioxide to a quinonoid product (Mirvish, 1981; Newmark and Mergens, in press).

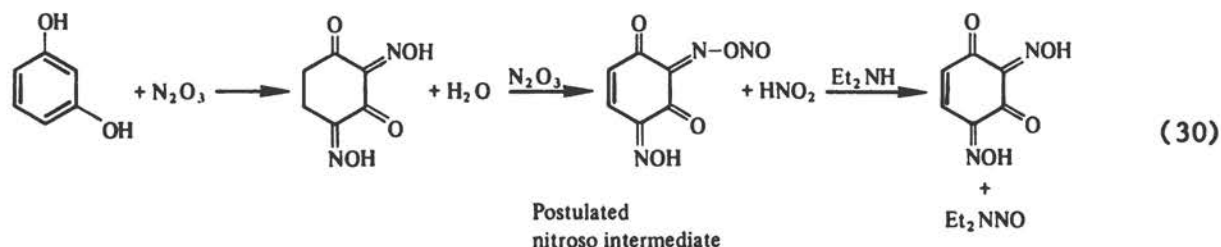


Other recent work has clarified confusing results concerning the effect of polyhydroxylated phenols on nitrosamine formation. In particular, Pignatelli et al. (1980) have shown that 1,2- and 1,4-dihydroxyphenols (including naturally occurring flavonols) inhibit nitrosamine formation at pH 4 and that earlier reports of catalysis

by 4-methylcatechol [$\text{CH}_3\text{C}_6\text{H}_3(\text{OH})_2$] (Challis and Bartlett, 1975) and gallic acid (Walker *et al.*, 1975) are incorrect because of the formation of artifacts. The inhibition results from reduction of the nitrosating agent, e.g., dinitrogen trioxide, to nitric oxide (NO):




1,3-Dihydroxyphenols (e.g., resorcinol) are powerful catalysts under similar conditions (Pignatelli *et al.*, 1980). This is attributed to rapid formation of a nitroso intermediate (equation 30), which interacts with more dinitrogen trioxide to generate a powerful nitrosating agent analogous to that proposed for catalysis by quinone monoxime (compare equation 25 with equation 30).



The reduction of nitrous acid to nitric oxide leads to inhibition because nitric oxide is an ineffectual nitrosating agent in the absence of catalysts. To be effective, however, it is necessary to add excess reducing agent because the ready oxidation of nitric oxide back to nitrogen dioxide and subsequent formation of dinitrogen trioxide ($\text{NO} + \text{NO}_2 \rightleftharpoons \text{N}_2\text{O}_3$) may quickly restore nitrosating capability. This effect has been noted for the formation of nitrosomorpholine in the presence of ascorbic acid (Archer *et al.*, 1975).

Production of nitric oxide can enhance nitrosation in a two-phase system, as has occurred inadvertently during the preparation of nitrosation mixtures (reviewed by Mirvish, 1981). Nitric oxide is extracted into the lipid phase and oxidized by oxygen to nitrogen dioxide. Lipid-soluble compounds, e.g., amines in an alkaline medium, can then be nitrosated in the lipid phase by the dinitrogen trioxide, which results from the reaction of nitric oxide and nitrogen dioxide. This system of nitrosation may be important in some industrial processes.

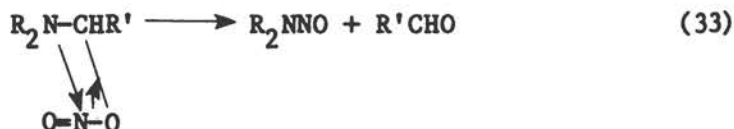
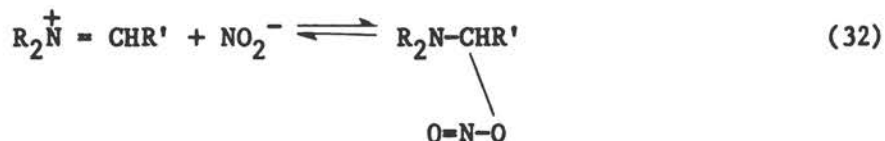
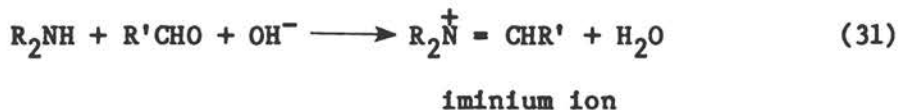
Inhibition by irreversible binding of the nitrosonium ion, e.g., formation of C-nitroso compounds, seems to be more difficult than the

reductive methods discussed above. Pyrrole (), however,

inhibits the formation of nitrosomorpholine (Groenen, 1976), and other reactive heteroaromatic compounds may act similarly.

Nitrosation by Nitrate and Nitrite Salts

Nitrosation by nitrite ion proceeds in the presence of certain carbonyl compounds, chlorinated solvents, metal salts, or radiation. Formaldehyde (HCHO), pyridoxal (C₈H₉NO₃), and benzaldehydes (RC₆H₄CHO), but not acetone (CH₃COCH₃) or acetaldehyde (CH₃CHO), produce nitrosamines from secondary amines in neutral and alkaline solutions of nitrite ion (Archer *et al.*, 1976; Keefer and Roller, 1973). The reaction rates vary with steric accessibility to the nitrogen atom, but all are very much slower than those for nitrous acid in acidic solutions. The first mechanism proposed involved nucleophilic attack by nitrite ion on an iminium ion intermediate (R₂N⁺=CHR') followed by collapse of the adduct to nitrosamine. Equations 31, 32, and 33 show the formation of N-nitrosamines from nitrite ion, carbonyl compounds, and secondary amines.



Alternative mechanisms have been discussed elsewhere (Keefer, 1979). This type of reaction may also explain the unexpected formation of nitrosamines from secondary amines and solid sodium nitrite (NaNO₂)

in halogenated solvents such as dichloromethane (CH_2Cl_2) (Roller et al., 1980) and small amounts of nitrosodiethanolamine $[(\text{HOCH}_2\text{CH}_2)_2\text{NNO}]$ in cosmetics containing the bactericide 2-bromo-2-nitropropane-1,3-diol ($\text{HOCH}_2\text{CBr}(\text{NO}_2)\text{CH}_2\text{OH}$; bronopol), which decomposes to release nitrite ion and formaldehyde (Schmeltz and Wenger, 1979).

Sodium nitrite has also been shown to produce nitrosamines from secondary amines at pH 11 in the presence of ferrocyanide ion $[\text{Fe}^{\text{II}}(\text{CN})_6]^{4-}$ (Maltz et al., 1971) and in 2,2'-bipyridine in the presence of cupric nitrate (Croisy et al., 1980). In both cases, interaction of nitrite ion with the metal salt is believed to generate a powerful nitrosating agent such as $[\text{Fe}^{\text{III}}(\text{CN})_5\text{NO}]^{2-}$ and $[\text{Cu}^{\text{II}}(\text{bipyr})(\text{ONO}_2)]$, respectively. Many transition metals other than iron are known to form diverse nitrosyl complexes, but their ability to nitrosate amino compounds has not been extensively investigated.

Nitrosation by nitrate salts or nitric acid requires reductive conditions and, in principle, the formation of either nitrite ion, nitrogen dioxide, or nitric oxide intermediates. This is achieved in the presence of certain microorganisms. For example, the formation of nitrosamines from aqueous solutions of nitrate salts and secondary amines may be mediated by bacteria (Hashimoto et al., 1975; Hill and Hawksworth, 1972). Reduction of nitrate ion is difficult to achieve chemically. Recent work, however, shows that secondary amides are rapidly converted to their N-nitroso derivatives by nitric acid in acetic acid containing copper powder (McQuinn et al., 1979). Reduction of nitric to nitrous acid is believed to occur, effecting nitrosation heterolytically.

Other recent studies reveal that nitrosamines are readily formed when neutral aqueous solutions of sodium nitrate and secondary amines are exposed to either γ -irradiation (Challis et al., 1980) or to ultraviolet photolysis (Challis and Li, in press). The highest yields apply to experiments with excess sodium nitrate. These reactions are believed to result from reduction of nitrate ion to nitrogen dioxide, which then dimerizes to form the dinitrogen tetroxide reagent. This conclusion is supported by the concurrent formation of nitramines (Challis and Li, in press; Challis et al., 1980).

Nitrosation by Nitrogen Oxides

Nitrogen oxides are generated by the chemical and microbial reduction of nitrite and nitrate salts and are common environmental pollutants produced by combustion. Four of these compounds have been implicated in the formation of N-nitroso compounds: nitrogen dioxide, dinitrogen tetroxide, dinitrogen trioxide, and nitric oxide. The first three react unaided, but nitric oxide requires either oxidation to nitrogen dioxide or the presence of certain metal salts, iodine, or hydrogen iodide. Acidic conditions are not required. The

formation of N-nitroso compounds from nitrogen oxides is usually faster and more extensive than from aqueous nitrous acid.

Gaseous dinitrogen trioxide and dinitrogen tetroxide exist in equilibrium with their nitric oxide and nitrogen dioxide constituents (Hisatsune, 1961):



Dissociation is less in condensed (liquid) than in gaseous phases (Grätzel *et al.*, 1969, 1970; Redmond and Wayland, 1968; Shaw and Vosper, 1971). Dinitrogen trioxide reacts with amino compounds to give the N-nitroso product (Challis and Kyrtopoulos, 1979; Lovejoy and Vosper, 1968):



whereas dinitrogen tetroxide gives a mixture of N-nitroso and N-nitro compounds (Challis and Kyrtopoulos, 1978; White and Feldman, 1957):



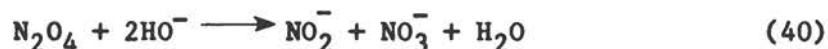
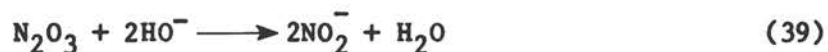
The extent of these reactions has not been widely recognized. They proceed in the gas phase, in organic (lipophilic) media, and in neutral and alkaline aqueous solutions.

Gas phase studies about smoking (Neurath *et al.*, 1976; Spincer and Westcott, 1976) or atmospheric pollution (Bretschneider and Matz, 1973; Gehlert and Rolle, 1977; Hanst *et al.*, 1977) show that both equimolar amounts of nitric oxide and nitrogen dioxide (i.e., dinitrogen trioxide) or nitrogen dioxide alone convert secondary amines to nitrosamines. Also, Pitts *et al.* (1978) and Atkinson *et al.* (1978) have detected both nitrosodiethylamine [(C₂H₅)₂NNO] and nitrodiethylamine [(C₂H₅)₂NNO₂] when either di- or triethylamine [(C₂H₅)₂NH; (C₂H₅)₃N] reacts with low concentrations of nitric oxide and nitrogen dioxide under simulated atmospheric conditions. The possibility of such reactions *in vivo* is raised by the observation of nitrosomorpholine in the carcasses of mice (Iqbal *et al.*, 1980) and the detection of lung adenomas (Van Stee *et al.*, 1980)

following inhalation of gaseous nitrogen dioxide. However, the nitrosomorpholine may form artifactually from a nitrosating agent produced in vivo from the nitrogen dioxide (Mirvish et al., in press).

In organic solvents, secondary amines react with gaseous dinitrogen trioxide to give high yields of N-nitrosamines (Challis and Kyrtopoulos, 1979; Lovejoy and Vosper, 1968), whereas gaseous dinitrogen tetroxide gives a mixture of N-nitroso and N-nitro compounds (Challis and Kyrtopoulos, 1978; White and Feldman, 1957). Furthermore, both dinitrogen trioxide and dinitrogen tetroxide have been recommended for the synthesis of N-nitrosamides in organic solvents (White, 1955). Both amines and amides are likely to react similarly in lipophilic media.

Nitrosation of amines by gaseous dinitrogen trioxide and dinitrogen tetroxide in aqueous solution is a recent discovery. Both would be expected to undergo rapid hydrolysis at pH > 5 to innocuous nitrite ion:



Hydrolysis does occur, but less rapidly than the nitrosation of many amines (Challis and Kyrtopoulos, 1978, 1979). Data in Table 4-3 show that substantial yields of nitrosamines are obtained within 4 minutes from secondary amines in neutral and alkaline solutions. With dinitrogen tetroxide, small amounts of nitramine form concurrently. Only the unprotonated amines react, similar nitrosamine ($\text{RR}'\text{NNO}_2$) yields are obtained for all but very basic amines ($\text{pK}_a < 1$), and, importantly, no reactions are observed with amides (Challis and Kyrtopoulos, 1978, 1979).

These results have been interpreted as evidence for tautomeric forms of dinitrogen trioxide ($\text{ONONO} \rightleftharpoons \text{ONNO}_2$) and dinitrogen tetroxide ($\text{ONONO}_2 \rightleftharpoons \text{O}_2\text{NNO}_2$), which appear to react with basic amines (Challis and Kyrtopoulos, 1978). Thus, nitrosation by gaseous dinitrogen trioxide and dinitrogen tetroxide follows the general mechanisms of equations 12 through 16, where NOY refers to ON-ONO or ON-ONO₂, respectively.

Subsequent work has shown that the reactions in aqueous media are inhibited by acid, sodium azide, ascorbic acid, some phenols, simple alcohols, and primary amines (Li, 1981). They are catalyzed,

TABLE 4-3

Nitrosation of Amino Compounds by Gaseous Dinitrogen Tetroxide and Dinitrogen Trioxide in Aqueous 0.1 M Sodium Hydroxide at 25°C^a

| Amine | pK _a | Percent Nitrosation ^b | |
|-------------------------|-----------------|----------------------------------|---------------------|
| | | Dinitrogen tetroxide | Dinitrogen trioxide |
| Piperidine | 11.12 | 39(0) ^c | 65(0) ^c |
| Morpholine | 8.33 | 19 | 52 |
| N-Methylpiperazine | 9.8, 5.11 | 33(44) ^c | 39(45) ^c |
| Aniline | 4.65 | 27 | 45 |
| N-Methyl-4-nitroaniline | 1.19 | 16 | 27 |
| 4-Nitroaniline | 0.99 | 24(38) ^c | 29(31) ^c |
| Diphenylamine | 0.78 | 6 | |
| 3,5-Dinitroaniline | 0.35 | 14 | |
| 2-Nitroaniline | -0.3 | 11 | |
| 2-Chloro-4-nitroaniline | -1.0 | 13 | 13 |
| 2,4-Dinitroaniline | -4.53 | 0 | |
| N-Butylacetamide | -0.29 | 0 | |

^aFrom Challis and Kyrtopoulos, 1979.

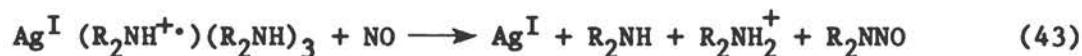
^bBased on [Amine].

^cFigures in parentheses refer to reaction in phosphate buffer at pH 6.85.

however, by nucleophilic anions such as thiocyanate (Li, 1981) and by 1,2-alkanolamines and 1,2-dihydroxy compounds, including carbohydrates (Challis and Shuker, 1979, 1980; Challis *et al.*, 1980). Tertiary amines such as triethylamine react to give lower, but nonetheless significant, yields of nitrosamines (Li, 1981).

In the absence of catalysts, nitric oxide reacts slowly with secondary amines and amides under anaerobic conditions (Challis and Kyrtopoulos, 1978). These reactions can be accelerated by injecting air, which converts nitric oxide to nitrogen dioxide. Furthermore, under anaerobic conditions, the formation of nitrosamines from secondary amines and nitric oxide in aqueous ethanol is promoted by zinc iodide (ZnI₂), zinc bromide (ZnBr₂), cuprous chloride (CuCl), cupric chloride (CuCl₂), ferrous nitrate [Fe(NO₃)₂], silver nitrate (AgNO₃), silver perchlorate (AgClO₄), and other metal salts (Challis *et al.*, 1978). Many salts appear to generate amino radicals (R₂N[•]) or radical cations (R₂NH^{•+}), which combine directly with nitric

oxide as shown for monovalent silver salts (Ag^{I}) (Challis and Outram, 1978):



Apart from oxygen or air, two of the best promoters for nitrosamine formation by nitric oxide are iodine (Challis and Outram, 1979) and hydrogen iodide (HI) (Outram, 1979). These, and zinc iodide, accelerate the reaction by forming nitrosyl iodide (NOI) (Outram, 1979):



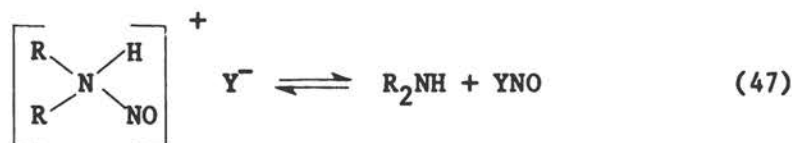
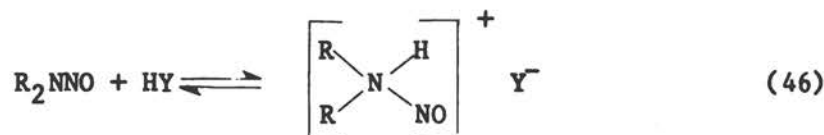
Transnitrosation: Nitrosation by Organic Nitroso and Nitro Compounds

In addition to nitrosation by nitrous acid and nitrogen oxides, certain nitroso compounds can act as nitrosating agents through the transfer of their nitroso group to an appropriate substrate (amines, amides, urea, amino acids). Depending on the substance that is nitrosated, these newly formed N-nitroso compounds may be carcinogenic. Nitrosation by nitrite esters (RONO), thionitrite esters [nitrosothiols (RSNO)], and certain other organic nitroso and nitro compounds has been known for many years, and some have been used in the synthesis of organic compounds. These compounds are formed by nitrosation pathways similar to those described for amino substrates.

Buglass et al. (1975) and, more recently, Singer et al. (1980) reported that transnitrosation is also effected by alicyclic nitrosamines, including certain derivatives of natural products such as nitrosoproline and nitrosopiperic acid, and by piperazines, morpholines, and alkylpiperidines (Singer et al., 1980). These authors have reported that all of the nitrosopiperazines tested can be effective transnitrosating agents; the nitrosomorpholines are also active, but to a lesser degree. Nitrosopiperidine is stable and will not act as a nitroso donor, but various derivatives of nitrosopiperidine are transnitrosating agents. Nitrosoamino acids are also active but vary in their reactivity, depending on the basicity of the amino nitrogen (Singer et al., 1980).

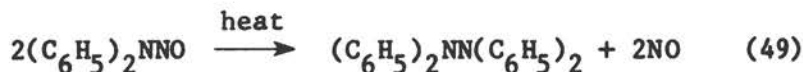
These transnitrosation reactions probably occur because N-nitroso compounds liberate nitrous acid upon treatment with acid. This reacts with the amino compounds to form N-nitroso compounds. Other organic N-nitroso and N-nitro compounds may liberate nitric oxide or nitrogen dioxide on heating, and may therefore serve as nitrosating agents.

Transnitrosation by nitrosamines is facilitated when the N-N(O) bond is weakened by electron-withdrawing substituents (Challis and Osborne, 1973; Singer *et al.*, 1980). Transnitrosation occurs in dilute acid (pH < 3) and is catalyzed by nucleophilic anions such as thiocyanate and iodide (Singer *et al.*, 1980; Thompson and Williams, 1977). This implies that release of Y-NO is involved:



Thus, the scope and limitations of these reactions will be similar to nitrosation by nitrous acid. Since the acidic reaction conditions are not too dissimilar to those in the stomach, compounds such as nitrosodiphenylamine [$(C_6H_5)_2NNO$] and nitrosamino acids [$RCH(N.NO)COOH$], which are weak carcinogens or noncarcinogens, may produce strong carcinogens by reacting with amino compounds *in vivo* (Cardy *et al.*, 1979).

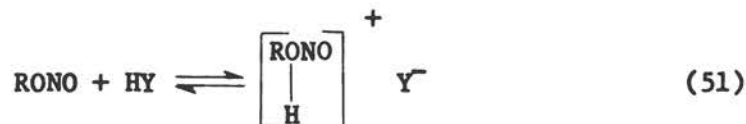
Transnitrosation can also be effected by heating aromatic N-nitrosamines to 50-80°C (Buglass *et al.*, 1975; Rappe and Rydström, 1980). The ensuing nitric oxide requires oxidation to nitrogen dioxide (which will be in equilibrium with dinitrogen tetroxide) or catalysis by metal salts, etc., to react with another amine (Outram, 1979).



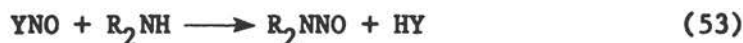
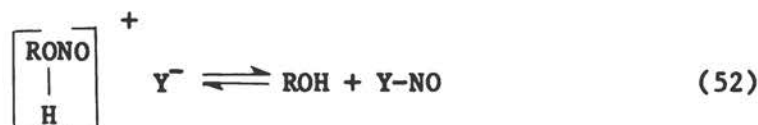
metal salt
catalysts

Simple nitrosamides (Berry and Challis, 1974; Challis and Jones, 1975), nitrososulfonamides [$C_6H_5SO_2N(NO)R$] (Williams, 1976), nitrosoureas [$H_2NCON(NO)R$] (Hallett *et al.*, 1980; Singer, 1980), and nitrosourethanes [$RO_2CN(NO)R'$] (Hallett *et al.*, 1980) also release nitrous acid in dilute acid ($pH < 4$) and, in principle, are capable of transnitrosation to another amino substrate.

Nitrite esters derived from simple monohydric alcohols (e.g., C_2H_5ONO , $C_5H_{11}ONO$) behave as transnitrosating agents like nitrosamines. Thus, decomposition ensues in the presence of dilute acid ($pH < 4$) and nucleophilic anions to give $Y-NO$:

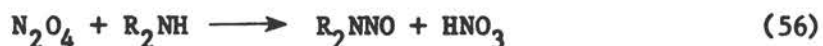
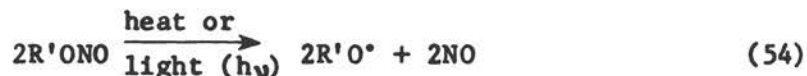


O-conjugate acid
intermediate



This can effect the nitrosation of amino compounds in the same way as for nitrous acid solutions (Aldred and Williams, 1981; Allen, 1954). Surprisingly, the O-conjugate acid intermediate does not seem to react directly with amino substrates (Aldred and Williams, 1981).

Decomposition of simple nitrite esters also proceeds thermally (Coombes, 1979) and photolytically (Forrest *et al.*, 1978) to generate alkoxy radicals (RO^\bullet) and nitric oxide (NO). These reactions rarely occur under anaerobic conditions, and it is likely that ensuing nitrosation of amino compounds proceeds via dinitrogen tetroxide following oxidation of nitric oxide to nitrogen dioxide (equations 54 through 56). Since nitrite esters are very soluble in organic solvents, these reactions may be important in lipophilic media.



Nitrite esters bearing electron-withdrawing β -substituents (X) are much more reactive, and they effect nitrosamine formation at ambient temperatures, in the absence of acid catalysts, by direct nucleophilic attack of the amine on the neutral nitrite ester (Challis *et al.*, 1980; Challis and Shuker, 1979, 1980):



2-Ethoxyethyl nitrite ($C_2H_5OCH_2CH_2ONO$), for example, reacts with both piperidine [$(CH_2)_5NH$] and morpholine [$O(CH_2CH_2)_2NH$] in 0.1 M sodium hydroxide and with N-methylpiperazinium ion [$HN(CH_2CH_2)_2NHCH_3^+$] at pH 6.85 and 25°C to give significant yields of the corresponding N-nitrosamines in approximately 30 minutes (Challis and Shuker, 1979). Related reactions apply to nitrite esters derived from several vicinal diols [$RCH(OH)CHR(OH)$] (Challis *et al.*, 1980), β -alkanolamines [$RCH(OH)CHRNHRR'$] (Challis and Shuker, 1980), and carbohydrates (Challis *et al.*, 1980). Table sugar (sucrose), milk sugar (lactose), and glucose, for example, form powerful nitrosating agents (presumably from the corresponding nitrite esters) on treatment with gaseous nitrogen dioxide, which rapidly converts secondary amines to their N-nitroso derivatives in aqueous alkali (Challis and Shuker, 1979; Challis *et al.*, 1980). It is not known whether these reactions proceed in food or under mildly acidic conditions.

Certain nitrosophenols (e.g., nitrosocresols) and nitrosothiols (e.g., S-nitrosocysteine) can participate in transnitrosation reactions and, thus, can enhance nitrosamine formation. The mechanism whereby these reactions catalyze nitrosation by phenols and thiols is discussed earlier in the chapter (see "Catalysis").

Phenols, which are present in cured meats at levels up to 280 mg/kg, are produced during the smoking process (Lustre and Issenberg, 1970). Knowles et al. (1975) demonstrated that the interaction of liquid smoke emulsions and nitrite, in the presence of casein, produces nitroso- and nitrocresols. Although many nitrosophenols are unstable under aerobic conditions and are oxidized to the corresponding C-nitrophenol (nitrophenols do not nitrosate secondary amines), *p*-nitroso-*o*-cresol has been shown to catalyze the nitrosation of pyrrolidine by nitrite at pH 5 (Davies and McWheeny, 1977; Davies et al., 1978a).

Cysteine, a thiol, is an important amino acid residue in meat protein, and its concentration in meat has been reported to be 21-25 mM (Hamm and Hofman, 1966). Protein-bound nitrite is in the form of nitrosothiol groups (Olsman, 1977), and these cysteine derivatives are capable of nitrosating amines (Dennis et al., 1979); however, when bound to a peptide chain, their reactivity is greatly reduced (Massey et al., 1980).

Thionitrite esters (RSNO) should be more reactive than regular nitrite esters because RS^- is a more stable group than RO^- . This conclusion is partially substantiated by reports that alkyl- and arylthionitrites convert piperidine to its N-nitroso derivative in organic solvents (Oae et al., 1978) and that S-nitrosocysteine produces nitrosamines in acidic, neutral, and alkaline aqueous solutions (Davies et al., 1978a; Massey et al., 1980). Thus, there is clear evidence that activation by acids, which would generate free nitrous acid, is not necessary. Catalysis by air and by light (Oae et al., 1978) suggests that release of nitric oxide followed by oxidation to nitrogen dioxide (which will be in equilibrium with dinitrogen tetroxide) occurs in some cases.

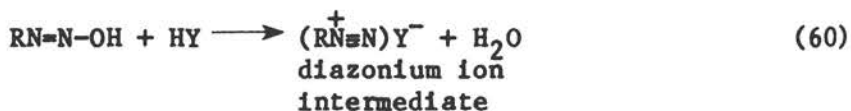
Early work reviewed by Fridman et al. (1971) showed that some aliphatic nitro compounds act as nitrosating as well as nitrating ($-NO_2$ donating) agents. More recently, the formation of nitroso-morpholine from tetranitromethane $[C(NO_2)_4]$, 2,2-dinitropropanol $[CH_3C(NO_2)_2CH_2OH]$, and 2-bromo-2-nitropropane-1,3-diol (bronopol) $[HOCH_2CBr(NO_2)CH_2OH]$ by heating with morpholine at 70°C has been described by Fan et al. (1978). The mechanism of these reactions is not clear, but one explanation is that release of nitrogen dioxide leads to formation of dinitrogen tetroxide, which then leads to nitrosation. It follows that any nitro compound that releases nitrogen dioxide may form nitrosamines from secondary and tertiary amines. Few other examples are known, but nitrodimethylamine transforms to nitrosodimethylamine upon heating or photolysis (Flournoy, 1962; Suryanarayanan and Bulusu, 1972). Furthermore, antianginal drugs containing a nitrate ester structure have been shown recently to produce nitrosamines in dilute acid, but these reactions are thought to proceed via the release of nitrous acid (Raisfeld et al., 1979).

In Vivo Significance of Transnitrosating Agents. Of special relevance in the committee's assessment of the in vivo nitrosation potential of transnitrosating agents are the conditions required to effect the reaction. As discussed above, aromatic nitrosamines require a pH of 1-3 and the presence of a nucleophilic catalyst (e.g., thiocyanate) for the reaction to occur (Singer et al., 1980; Thompson and Williams, 1977). Thus, such reactions could occur in the human stomach where the pH is sufficiently low and where nucleophilic catalysts are probably present. In contrast, nitrosothiols are present in meat products and are active at near neutral or alkaline pH's (Davies et al., 1978a; Dennis, personal communication), and could be involved in transnitrosation reactions in the duodenum and small intestine. That such nitrosation reactions may occur in vivo is suggested by a study of Love et al. (1977) in which rats fed high doses of mononitrosopiperazine developed tumors that were similar to those that developed in rats fed lower doses of dinitrosopiperazine. However, despite the plausibility of these reactions taking place in vivo, knowledge about the occurrence of such reactions endogenously is extremely limited.

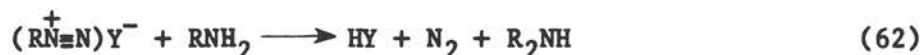
Nitrosation of Primary, Tertiary, and Quarternary Amino Compounds

At first sight, the formation of nitrosamines from these substrates seems unlikely, but there is good evidence to indicate otherwise. However, these reactions are usually less facile and/or less extensive than those with secondary amino compounds.

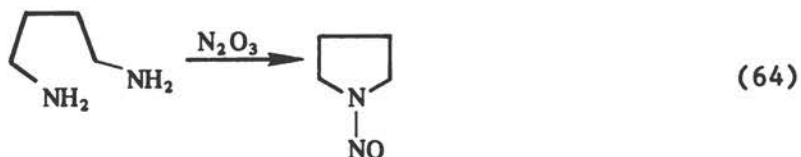
Nitrosation of primary aliphatic amines leads to deamination via an unstable diazonium ion intermediate, which reacts with nucleophiles to give substitution, elimination, and rearrangement products (Challis, 1981; Ridd, 1961):



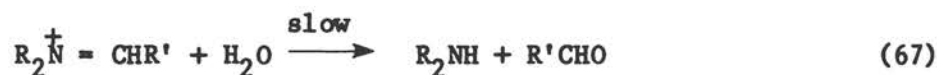
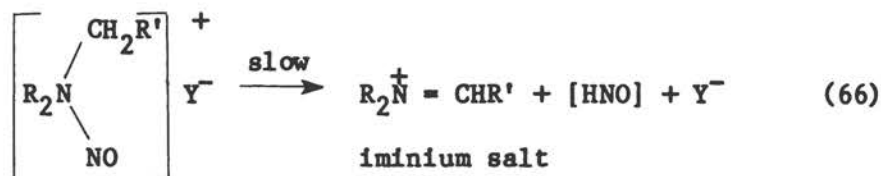
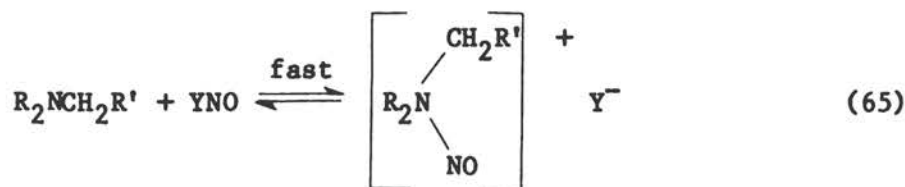
One of these decomposition pathways can result in alkylation of the primary amine to give a secondary amine and, subsequently, nitrosamine (Tannenbaum *et al.* 1978):



The kinetic characteristics of the initial deamination (including catalysis and inhibition) should be similar to nitrosamine formation, but yields of nitrosamines obtained from primary amines are very low because the diazonium ion intermediate reacts by several competitive pathways other than that shown in equations 62 and 63. Higher yields might be anticipated for reaction in organic (lipophilic) solvents, but this awaits confirmation. These reactions could be of some importance to the formation of heterocyclic nitrosamines from primary diamine precursors. Putrescine, for example, gives approximately 1.6% nitrosopyrrolidine on heating with nitrous acid (Warthesen *et al.*, 1975):



Early work on the interaction of tertiary amines with acidified nitrite has been reviewed by Hein (1963) and by Smith and Loeppky (1967). Subsequently, these reactions attracted further attention (Gowenlock *et al.*, 1979; Lijinsky and Singer, 1975) because of their role in the formation of nitrosamines, which is particularly facile for certain drugs such as aminopyrine (Lijinsky *et al.*, 1972; Mirvish *et al.*, 1974). The sequence of reactions presented in equations 65 through 68, in which an iminium salt undergoes hydrolysis to a secondary amine or reacts directly with nitrite ion to give a nitrosamine, was suggested by Smith and Loeppky (1967):



This scheme is supported by the recent identification of secondary amines (Singer, 1980) as coproducts with aldehydes, nitrous oxide (because $2HNO \rightarrow N_2O + H_2O$), and nitrosamines (Hecht *et al.*, 1978; Lijinsky and Singer, 1975). An alternative pathway involving electron transfer rather than nitrosation to form the iminium salt has been proposed by Michejda *et al.* (1976). This is a more likely mechanism with aromatic amines.

Temperatures between 50°C and 100°C are required for the nitrosation of tertiary alkylamines. Mirvish (1975) has estimated that these compounds are approximately 10,000 times less reactive than comparable secondary amines. This implies that either formation or hydrolysis of the iminium salt is rate-limiting (equations 65-68). Many investigators (e.g., Gowenlock *et al.*, 1979) have reported that the maximum rates of reaction with nitrous acid occur at pH 3-3.4 (similar to those of secondary amines), but there is considerable disagreement as to the dependence of kinetics on the concentration of nitrous acid (Gowenlock *et al.*, 1979; Ohshima and Kawabata, 1978; Singer, 1980).

Small amounts of nitrosodimethylamine have also been produced by the reactions of acidified nitrite with both quarternary methylammonium salts ($R_3NH_3^+Y^-$) and trimethylamine-N-oxide [$(CH_3)_3N^+ \rightarrow O^-$] (Eisenbrand *et al.*, 1975; Lijinsky and Singer, 1975; Ohshima and Kawabata, 1978). As for tertiary amines, these reactions require such conditions as high reagent concentrations and high temperatures. Surprisingly, trimethylamine-N-oxide seems to be more reactive than trimethylamine itself (Ohshima and Kawabata, 1978), whereas the quarternary salts are less reactive (Fiddler *et al.*, 1972). Very little is known about the mechanism of these reactions.

Tertiary amides and related compounds have only been superficially examined, but there is good evidence that nitrosamines (and in some cases nitrosamides) form when they react with acidified nitrite at elevated temperatures (Eisenbrand *et al.*, 1975; Elespuru and Lijinsky, 1973; Lijinsky *et al.*, 1972). Trialkylureas usually give the corresponding nitrosurea, whereas dialkyl- and trialkylthioureas, 1,1-dialkylureas, 1,1-dialkyl-3-phenylureas, and tetraalkylureas produce nitrosamines.

Factors Influencing Nitrosation in Foods

The extent to which nitrosation may occur in a food prior to ingestion is influenced by many factors. Nitrate or nitrite may be added deliberately for their preservative properties, or may be present naturally in water and some foods (see Chapter 5). Nitrate may be reduced to nitrite. Moreover, foods are exposed to nitrogen oxides that are either airborne contaminants or produced during certain processes such as the smoking, drying, or roasting of certain foods and baking in gas ovens. The extent of nitrosation in food is affected not only by the amount and type of nitrosatable compounds that are present, but also by the content of nitrosation catalysts and inhibitors and their solubility characteristics.

Smoking of Foods. Although wood-smoking is one of the oldest methods of food preservation, relatively little is known about the chemical interactions involved in this process. Most of the information pertains to the composition of the smoke and the components that affect the texture and flavor of the smoked products (Clifford *et al.*, 1980; Gilbert and Knowles, 1975). According to Foster and Simpson (1961), wood smoke consists of two phases--a disperse, liquid phase containing smoke particles and a dispersing gaseous phase (vapor). Direct deposition of smoke particles on the food is believed to be negligible compared to the absorption of vapors by surface and interstitial water.

Smoking involves combustion, so it is highly probable that nitrogen oxides are absorbed by the food; however, the amounts absorbed have not been adequately measured. The nitrogen oxides may be expected to act as nitrosating agents (Challis and Kyrtopoulos, 1978, 1979; Challis *et al.*, 1978) and, in principle, to produce N-nitroso compounds. It has been established that smoking produces up to a 55% reduction in the basic amino acid content, especially lysine (Clifford *et al.*, 1980; Hoffman *et al.*, 1977) and a smaller reduction of thio amino acids (Mauron, 1970). These losses could possibly be due to deamination by nitrogen oxides. In any event, the nitrogen oxides will undergo hydrolysis by surface and interstitial water, and nitrite and nitrate ions will be deposited in the food.

The vapors of wood smoke are a complex mixture whose principal organic constituents are phenolic compounds, carboxylic acids, carbonyl compounds, esters (including lactones), aromatic ethers, furans, and aromatic hydrocarbons (Gilbert and Knowles, 1975). The phenolic compounds, aromatic ethers, and furans react rapidly with nitrosating agents (Challis and Higgins, 1972; Challis and Lawson, 1971), and should therefore inhibit the formation of N-nitroso compounds (Challis, 1973). Inhibition by some of the phenolic constituents has been reported by Pignatelli *et al.* (1980), but the effect might be partly counteracted by the ability of nitrosophenols to catalyze the formation of nitrosamines (Davies and McWeeny, 1977; Walker *et al.*, 1979). Certain carbonyl compounds may also catalyze the formation of nitrosamines from nitrite salts (Keefer and Roller, 1973).

SUMMARY

Nitrate and nitrite undergo a number of chemical and biological transformations. Of most importance to human health is their participation in both in vitro and in vivo nitrosation reactions, i.e., reactions that lead to the formation of N-nitroso compounds. There are factors that catalyze or inhibit these reactions in foods prior to consumption and in vivo.

Nitrate and Nitrite

Nitrate salts are stable, and they are not easily reduced chemically to nitrite salts. Nitrite salts, which are less stable, are readily oxidized to nitrate salts or reduced to nitric oxide, nitrous oxide, or nitrogen. Both nitrate and nitrite salts are transformed to reactive nitrogen oxides by gamma radiation and by photolysis.

Enzymatic reduction of nitrate salts and both enzymatic oxidation and enzymatic reduction of nitrite salts are well-known components of the nitrogen cycle. These transformations are readily accomplished in certain bacterial, fungal, and plant systems. They permit the interconversion of nitrate and nitrite salts and the generation of reactive nitrogen oxides from those salts.

Nitrosation

Amino substrates are nitrosated by electrophilic nitrosating agents derived from nitrous acid, dinitrogen trioxide, dinitrogen tetroxide, and, occasionally, nitric oxide.

Nitrosation in aqueous nitrous acid at $\text{pH} < 5$ proceeds by reaction of the unprotonated amino substrate with an electrophilic nitrosating agent such as dinitrogen trioxide or the hydrated nitrosonium ion. Basic secondary amines react most rapidly at pH 3.4, but these reactions are generally slower than those for amides, ureas, and carbamates (e.g., urethane).

Nitrosation of secondary amines by aqueous nitrous acid is accelerated by nucleophilic anions such as thiocyanate and iodide and by some phenolic materials, thiols, and alkenes. Comparable reactions of amides, ureas, guanidines, and carbamates are not usually accelerated by these catalysts.

Nitrosation of secondary amines, amides, ureas, and guanidines by aqueous nitrous acid can be inhibited by compounds that reduce nitrous acid to nitrogen or nitric oxide. These inhibitors include ascorbic acid (vitamin C), α -tocopherol (vitamin E), and several naturally occurring polyphenolic antioxidants.

The nitroso group of certain nitrosamines, chiefly aromatic and alicyclic nitrosamines, can be transferred to amino substrates to form N-nitroso compounds. The catalysis of nitrosation by certain phenols and thiols, which proceeds via the formation of intermediate unstable nitroso compounds, may be termed transnitrosation. Transnitrosation has been demonstrated to occur in vitro, but little is known about its occurrence and, thus, its significance in nitrosation reactions in vivo.

Nitrosation of secondary amines by nitrite ion is exceptional, but it may occur slowly in aqueous solution in the presence of certain carbonyl compounds and in lipophilic solvents in the presence of metal salts. Nitrosamines form rapidly when aqueous solutions of secondary amines and either nitrite or nitrate salts are subjected to gamma radiation and to photolysis. After extended irradiation and photolysis, the nitrosamines are decomposed.

Nitrosamines form rapidly from reaction of secondary and tertiary amines with dinitrogen trioxide and dinitrogen tetroxide in organic and aqueous (neutral or alkaline) solutions. Nitric oxide forms nitrosamines only in the presence of oxygen, metal salts, or halide ions. These reactions are usually much faster than those occurring with aqueous nitrous acid, and they may explain the formation of nitrosamines by nitrogen oxide pollutants. Nitrosamine formation by dinitrogen trioxide and dinitrogen tetroxide is inhibited by reductants, but is catalyzed by common β -substituted alcohols such as alkanolamines, ethyleneglycol, and carbohydrates (sugars).

Organic nitroso and nitro compounds, which release either nitric oxide or nitrogen dioxide upon heating or nitrosonium ion upon treatment with acid, react with secondary amines to produce nitrosamines.

Under certain circumstances, primary, tertiary, and quaternary amino compounds (including amine oxides) produce nitrosamines upon reaction with nitrous acid and nitrogen oxides. Generally, these reactions are less extensive than those with secondary amines.

The extent of nitrosamine formation in foods is affected by the type of matrix (hydrophilic or hydrophobic), the presence of natural antioxidants, and methods of processing and cooking. For example, nitrogen oxides in smoke may produce nitrosamines in smoked products. These reactions may be mediated by other carbonyl and phenolic constituents of smoke.

CONCLUSIONS AND RECOMMENDATIONS

It is now clear that N-nitroso compounds form from nitrous acid and nitrite salts during the processing, cooking, and digestion of food. Thus, attention should be directed toward the conditions that prevail during the preparation, processing, and storage of foods to identify those that could lead to the generation of N-nitroso compounds. For example, the production of nitrite salts by the oxidation of amino compounds, the reduction of nitrate salts, and the hydrolysis of gaseous nitrogen oxides may all occur. Further research is also needed (1) to determine the rate at which N-nitroso compounds form at low nitrite concentrations, which approximate those present in the digestive tract; (2) to study the ability of dietary antioxidants other than ascorbic acid and α -tocopherol to inhibit the formation of N-nitroso compounds; and (3) to characterize the conditions that determine the formation of N-nitroso compounds in complex matrices that mimic food itself. It is also clear that N-nitroso compounds form readily from nitrogen oxides, but much more remains to be learned about the scope and extent of these reactions in the environment, during the cooking of food, and in vivo. Nitrogen oxides also generate N-nitro compounds from amino substrates, a reaction that warrants further attention in view of the carcinogenic properties of one of these compounds (dimethyl nitramine), which is the only one that has been tested.

REFERENCES

- Abou-Jaoudé, A., M.-C. Pascal, and M. Chippaux. 1979. Formate-nitrite reduction in Escherichia coli K12. 2. Identification of components involved in the electron transfer. Eur. J. Biochem. 95:315-321.
- Aldred, S. E., and D. L. H. Williams. 1981. Alkyl nitrites as nitrosating agents. Kinetics and mechanism of the reactions of propyl nitrite in propan-1-ol. J. Chem. Soc. Perkin Trans. II. (7):1021-1024.
- Allen, A. D. 1954. Allen: Studies in the hydrolysis and studies in the hydrolysis and alcoholysis of some organic nitrites. J. Chem. Soc.:1968-1974.
- Al-Mallah, K., P. Collings, and G. Stedman. 1974. Electrophilic nitrosation at sulphur and nitrogen in thiourea. J. Chem. Soc. Dalton Trans.:2469-2472.
- Archer, M. C. In press. Hazards of nitrate, nitrite, and N-nitroso compounds in human nutrition. In J. N. Hatchcock, ed. Nutritional Toxicology, Vol. I. Academic Press, New York.
- Archer, M. C., S. R. Tannenbaum, T.-Y. Fan, and M. Weisman. 1975. Reaction of nitrite with ascorbate and its relation to nitrosamine formation. J. Natl. Cancer Inst. 54:1203-1205.
- Archer, M. C., S. R. Tannenbaum, and J. S. Wishnok. 1976. Nitrosamine formation in the presence of carbonyl compounds. Pp. 141-145 in E. A. Walker, P. Bogovski, L. Gričiute, and W. Davis, eds. Environmental N-Nitroso Compounds: Analysis and Formation, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.
- Atkinson, R., R. A. Perry, and J. N. Pitts, Jr. 1978. Rate constants for the reactions of the OH radical with $(\text{CH}_3)_2\text{NH}$, $(\text{CH}_3)_3\text{N}$, and $\text{C}_2\text{H}_5\text{NH}_2$ over the temperature range 298-425°K. J. Chem. Phys. 68:1850-1853.
- Bayliss, N. S., and R. B. Bucat. 1975. The photolysis of aqueous nitrate solutions. Aust. J. Chem. 28:1865-1878.
- Berry, C. N., and B. C. Challis. 1974. The chemistry of nitroso-compounds. Part VIII. Denitrosation and deamination of N-n-butyl-N-nitrosacetamide in aqueous acids. J. Chem. Soc. Perkin Trans. II:1638-1644.

- Boyland, E., and S. A. Walker. 1974. Effect of thiocyanate on nitrosation of amines. *Nature* 248:601-602.
- Boyland, E., E. Nice, and K. Williams. 1971. The catalysis of nitrosation of thiocyanate from saliva. *Food Cosmet. Toxicol.* 9:639-643.
- Bretschneider, K., and J. Matz. 1973. [In German; English Summary.] [Nitrosamines (NA) in the atmospheric air and in the air at the places of employment.] *Arch. Geschwulstforsch.* 42:36-41.
- Buglass, A. J., B. C. Challis, and M. R. Osborne. 1975. Transnitrosation and decomposition of nitrosamines. Pp. 94-100 in P. Bogovski, E. A. Walker, and W. Davis, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Butt, W. D., and H. Lees. 1958. Cytochromes of Nitrobacter. *Nature* 182:732-733.
- Cardy, R. H., W. Lijinsky, and P. K. Hildebrandt. 1979. Neoplastic and nonneoplastic urinary bladder lesions induced in Fischer 344 rats and B6C3F hybrid mice by N-nitrosodiphenylamine. *Ecotoxicol. Environ. Saf.* 3:29-35.
- Challis, B. C. 1973. Rapid nitrosation of phenols and its implications for health hazards from dietary nitrites. *Nature* 244:466.
- Challis, B. C. 1981. The chemistry of formation of N-nitroso compounds. Pp. 16-55 in G. G. Gibson and C. Ioannides, eds. *Safety Evaluation of Nitrosatable Drugs and Chemicals*. Taylor and Francis Ltd., London, England.
- Challis, B. C., and C. D. Bartlett. 1975. Possible carcinogenic effects of coffee constituents. *Nature* 254:532-533.
- Challis, B. C., and J. Challis. 1970. Reactions of the carboxamide group. Pp. 731-848 in J. Zabicky, ed. *The Chemistry of Amines*. Interscience Publishers, London, New York, Sidney, and Toronto.
- Challis, B. C., and R. J. Higgins. 1972. The chemistry of nitroso-compounds. Part IV. Acid-catalysed nitrosation of para-substituted phenols. *J. Chem. Soc. Perkin Trans. II*:2365-2368.

- Challis, B. C., and S. P. Jones. 1975. The chemistry of nitroso-compounds. Part IX. General acid catalysed decomposition of N-nitroso-2-pyrrolidone, an example of amide hydrolysis via \overline{S}_N^2 displacement on the N-conjugate acid. J. Chem. Soc. Perkin Trans. II:153-160.
- Challis, B. C., and S. A. Kyrtopoulos. 1978. The chemistry of nitroso compounds. Part 12. The mechanism of nitrosation and nitration of aqueous piperidine by gaseous dinitrogen tetroxide and dinitrogen trioxide in aqueous alkaline solutions. Evidence for the existence of molecular isomers of dinitrogen tetroxide and dinitrogen trioxide. J. Chem. Soc. Perkin Trans. II:1296-1302.
- Challis, B. C., and S. A. Kyrtopoulos. 1979. The chemistry of nitroso-compounds. Part 11. Nitrosation of amines by the two-phase interaction of amines in solution with gaseous oxides of nitrogen. J. Chem. Soc. Perkin Trans. I:299-304.
- Challis, B. C., and A. J. Lawson. 1971. The chemistry of nitroso-compounds. Part II. The nitrosation of phenol and anisole. J. Chem. Soc. B:770-775.
- Challis, B. C., and B. F-L. Li. In press. Formation of N-nitrosamines and N-nitramines by photolysis. Paper presented at the Seventh International Meeting on Analysis and Formation of N-Nitroso Compounds, September 28 - October 1, 1981, Tokyo, Japan.
- Challis, B. C., and M. R. Osborne. 1973. The chemistry of nitroso-compounds. VI. Direct and indirect transnitrosation reactions of N-nitrosodiphenylamine. J. Chem. Soc. Perkin Trans. II: 1526-1533.
- Challis, B. C., and J. R. Outram. 1978. Rapid formation of N-nitrosamines from nitric oxide in the presence of silver(I) salts. J. Chem. Soc. Chem. Commun.:707-708.
- Challis, B. C., and J. R. Outram. 1979. The chemistry of nitroso-compounds. Part 15. Formation of N-nitrosoamines in solution from gaseous nitric oxides in the presence of iodine. J. Chem. Soc. Perkin Trans I:2768-2775.
- Challis, B. C., and D. E. G. Shuker. 1979. Rapid nitrosation of amines in aqueous alkaline solutions by β -substituted alkyl nitrites. J. Chem. Soc. Chem. Commun.:315-316.

- Challis, B. C., and D. E. G. Shuker. 1980. Chemistry of nitroso-compounds. Part 16. Formation of N-nitrosamines from dissolved NOCl in the presence of alkanolamines and related compounds. *Food Cosmet. Toxicol.* 18:283-288.
- Challis, B. C., A. Edwards, R. R. Hunma, S. A. Kyrtopoulos, and J. R. Outram. 1978. Rapid formation of N-nitrosamines from nitrogen oxides under neutral and alkaline conditions. Pp. 127-142 in E. A. Walker, L. Gričute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*. IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Challis, B. C., J. R. Outram, and D. E. G. Shuker. 1980. New pathways for the rapid formation of N-nitrosamines under neutral and alkaline conditions. Pp. 43-58 in E. A. Walker, L. Gričute, M. Castegnaro, and M. Börzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Clifford, M. N., S. L. Tang, and A. A. Eyo. 1980. Smoking of foods. *Process Biochem.* 15:8-11, 17, 26.
- Cohen, B. S., and S. Weinhouse. 1971. Reduction of nitrate to nitrite in tissues of the rat. In *Abstracts of Papers, 162nd National Meeting of the American Chemical Society, Sept. 12-17, 1971*. American Chemical Society, Washington, D.C. Abstract 179.
- Coombs, R. G. 1979. Nitrosamines. Pp. 363-370 in D. Barton and W. D. Ollis, eds. *Comprehensive Organic Chemistry: The Synthesis and Reactions of Organic Compounds*. Vol. 2. Nitrogen Compounds, Carboxylic Acids, Phosphorous Compounds. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, and Frankfurt.
- Croisy, A. F., J. C. Fanning, L. K. Keefer, B. W. Slavin, and S.-J. Uhm. 1980. Metal complexes as promoters of N-nitrosation reactions: A progress report. Pp. 83-93 in E. A. Walker, L. Gričute, M. Castegnaro, and M. Börzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Cruickshank, J., and J. M. Moyes. 1914. The presence and significance of nitrites in urine. *Br. Med. J.* 2:712-713.
- Dahn, H., and L. Loewe. 1960. [In German; English Summary.] Über die Oxydation von Ascorbinsäure durch salpetrige Säure Teil II: Die säurekatalysierte Reaktion. *Helv. Chim. Acta* 43:294-303.

- Dainton, F. S., and S. R. Logan. 1965. Radiolysis of aqueous solutions containing nitrite ions and nitrous oxide. *Trans. Faraday Soc.* 61:715-722.
- Davies, R., and D. J. McWeeny. 1977. Catalytic effect of nitroso-phenols and N-nitrosamine formation. *Nature* 266:657-658.
- Davies, R., M. J. Dennis, R. C. Massey, and D. J. McWeeny. 1978a. Some effects of phenol- and thiol-nitrosation reactions on N-nitrosamine formation. Pp. 183-197 in E. A. Walker, L. Griciute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Davies, R., R. C. Massey, and D. J. McWeeny. 1978b. A study of the rate of the competitive nitrosations of pyrrolidine, p-cresol and L-cysteine hydrochloride. *J. Sci. Food Agric.* 29:62-70.
- Dennis, M. J., R. Davies, and D. J. McWeeny. 1979. The transnitrosation of secondary amines by S-nitrosocysteine in relation to N-nitrosamine formation in cured meats. *J. Sci. Food Agric.* 30:639-645.
- Douglass, M. L., B. L. Kabacoff, G. A. Anderson, and M. C. Cheng. 1978. The chemistry of nitrosamine formation, inhibition and destruction. *J. Soc. Cosmet. Chem.* 29:581-606.
- Edwards, J. O. 1954. Correlation of relative rates and equilibria with a double basicity scale. *J. Am. Chem. Soc.* 76:1540-1547.
- Eisenbrand, G., O. Ungerer, and R. Preussmann. 1975. Formation of N-nitroso compounds from agricultural chemicals and nitrite. Pp. 71-74 in P. Bogovski, E. A. Walker, and W. Davis, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Elespuru, R. K., and W. Lijinsky. 1973. The formation of carcinogenic nitroso compounds from nitrite and some types of agricultural chemicals. *Food Cosmet. Toxicol.* 11:807-817.
- Fan, T.-Y., and S. R. Tannenbaum. 1973. Factors influencing the rate of formation of nitrosomorpholine from morpholine and nitrite: Acceleration by thiocyanate and other anions. *J. Agric. Food Chem.* 21:237-240.
- Fan, T. Y., R. Vita, and D. H. Fine. 1978. C-Nitro compounds: A new class of nitrosating agents. *Toxicol. Lett.* 2:5-10.

- Fiddler, W., J. W. Pensabene, R. C. Doerr, and A. E. Wasserman. 1972. Formation of N-nitrosodimethylamine from naturally occurring quaternary ammonium compounds and tertiary amines. *Nature* 236:307.
- Fiddler, W., J. W. Pensabene, E. G. Piotrowski, J. G. Phillips, J. Keating, W. J. Mergens, and H. L. Newmark. 1978. Inhibition of formation of volatile nitrosamines in fried bacon by the use of cure-solubilized α -tocopherol. *J. Agric. Food Chem.* 26:653-656.
- Flournoy, J. M. 1962. Letter to the Editor: Thermal decomposition of gaseous dimethylnitramine. *J. Chem. Phys.* 36:1106-1107.
- Focht, D. D., and W. Verstraete. 1977. Biochemical ecology of nitrification and dinitrification. *Adv. Microb. Ecol.* 1: 135-214.
- Forrest, D., B. G. Gowenlock, and J. Pfab. 1978. Photochemistry of C-nitroso-compounds. Part 6. Quantum yields for the solution-phase photolysis of some geminal chloro-nitroso-compounds. *J. Chem. Soc. Perkin Trans. II*:12-15.
- Foster, W. W., and T. H. Simpson. 1961. Studies of the smoking process for foods. I.--The importance of vapours. *J. Sci. Food Agric.* 12:363-374.
- Fridman, A. L., F. M. Mukhametshin, and S. S. Novikov. 1971. Advances in the chemistry of aliphatic N-nitrosamines. *Russ. Chem. Rev.* 40:34-50.
- Gehlert, P., and W. Rolle. 1977. [In German; English summary.] [Formation of diethylnitrosamine by reaction of diethylamine with nitrogen dioxide in the gas phase.] *Experientia* 33:579-581.
- Gilbert, J., and M. E. Knowles. 1975. The chemistry of smoked foods: A review. *J. Food Technol.* 10:245-261.
- Goaz, P. W., and H. A. Biswell. 1961. Nitrate reduction in whole saliva. *J. Dent. Res.* 40:355-365.
- Gowenlock, B. G., R. J. Hutchison, J. Little, and J. Pfab. 1979. Nitrosative dealkylation of some symmetrical tertiary amines. *J. Chem. Soc. Perkin Trans. II*:1110-1114.
- Grätzel, M., A. Henglein, J. Lilie, and G. Beck. 1969. [In German.] Pulsradiolytische Untersuchung einiger Elementarprozesse der Oxydation und Reduktion des Nitritions. *Ber. Bunsenges. Phys. Chem.* 73:646-653.

- Grätzel, M., S. Taniguchi, and A. Henglein. 1970. [In German.] Pulsradiolytische Untersuchung der NO-Oxidation und des Gleichgewichts $N_2O_3 = NO + NO_2$ in wässriger Lösung. Ber. Bunsenges. Phys. Chem. 74:488-492.
- Green, L., D. Ralt, and S. R. Tannenbaum. In press. Nitrate, nitrite and N-nitroso compounds: Biochemistry, metabolism, toxicity and carcinogenicity. In A. Neuberger and T. H. Jukes, eds. Biochemistry of Nutrition, Vol. 2. University Park Press, Baltimore, Maryland.
- Groenen, P. J. 1976. A new type of N-nitrosation inhibitor. Pp. 171-173 in B. J. Tinbergen and B. Krol, eds. Proceedings of the second International Symposium on Meat Products, September 7-10, 1976, Zeist, the Netherlands. Centre For Agricultural Publishing Documentation, Wageningen, the Netherlands.
- Guignard, J. P., and A. Torrado. 1978. Nitrite indicator strip test for bacteriuria. Lancet 1:47.
- Hallett, G., S. S. Johal, T. A. Meyer, and D. L. H. Williams. 1980. Reactions of nitrosamines with nucleophiles in acid solution. Pp. 31-41 in E. A. Walker, L. Gričiute, M. Castegnaro, and M. Börzsönyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Hamm, R., and K. Hofmann. 1966. Determination of sulphhydryl and disulphide groups in myofibrils and muscle tissue by amperometric titration. Z. Lebensmunters. Forsch. 130:133-145.
- Hanst, P. L., J. W. Spence, and M. Miller. 1977. Atmospheric chemistry of N-nitroso dimethylamine. Environ. Sci. Technol. 11:403-405.
- Hashimoto, S., Y. Kawai, and M. Mutai. 1975. In vitro N-nitroso-dimethylamine formation by some bacteria. Infect. Immun. 11:1405-1406.
- Hecht, S. S., C.-h. B. Chen, R. M. Ornaf, E. Jacobs, J. D. Adams, and D. Hoffmann. 1978. Reaction of nicotine and sodium nitrite: Formation of nitrosamines and fragmentation of the pyrrolidine ring. J. Org. Chem. 43:72-76.
- Hein, G. E. 1963. The reaction of tertiary amines with nitrous acid. J. Chem. Educ. 40:181-184.

- Hewitt, E. J. 1974. Aspects of trace element requirements in plants and micro-organisms: The metallo enzymes of nitrate and nitrite reduction. *MTP Int. Rev. Sci. Biochem. Ser. 1*, 11:199-245.
- Hewitt, E. J. 1975. Assimilatory nitrate-nitrite reduction. *Ann. Rev. Plant Physiol.* 26:73-100.
- Hill, M. J., and G. Hawksworth. 1972. Bacterial production of nitrosamines in vitro and in vivo. Pp. 116-121 in P. Bogovski, R. Preussmann, E. A. Walker, and W. Davis, eds. N-nitroso compounds, Analysis and Formation, IARC Scientific Publication No. 3. International Agency for Research on Cancer, Lyon, France.
- Hisatsune, I. C. 1961. Thermodynamic properties of some oxides of nitrogen. *J. Phys. Chem.* 65:2249-2253.
- Hoffman, A., A. Barranco, B. J. Francis, and J. G. Disney. 1977. The effect of processing and storage upon the nutritive value of smoked fish from Africa. *Trop. Sci.* 19:41-53.
- Hucklesby, D. P., and E. J. Hewitt. 1970. Nitrite and hydroxylamine reduction in higher plants. Fractionation, electron donor and substrate specificity of leaf enzymes, principally from vegetable marrow (Cucurbita pepo L.). *Biochem. J.* 119: 615-627.
- Hughes, M. N., and G. Stedman. 1963. Kinetics and mechanism of the reaction between nitrous acid and hydroxylamine. Part I. *J. Chem. Soc.:*2824-2830.
- Hussain, M. A., G. Stedman, and M. N. Hughes. 1968. Kinetics and mechanism of the reaction between nitrous acid and hydroxylamine. Part III. The formation of hyponitrous acid. *J. Chem. Soc. B:*597-603.
- Iqbal, Z. M., K. Dahl, and S. S. Epstein. 1980. Role of nitrogen dioxide in the biosynthesis of nitrosamines in mice. *Science* 207:1475-1477.
- Ishidate, M., M. Harada, H. Ishiwata, Y. Nakamura, and A. Tanimura. 1974. Studies on in vivo formation of nitrite. Pp. 66 in *Proc. Japan Cancer Assoc., 33rd Annual Meeting, October, 1974, Sendai, Japan. Abstract 254.*
- Jóárt, Gy. 1978. Screening for bacteriuria of schoolchildren by the nitrite reaction. *Int. Urol. Nephrol.* 10:33-40.

- Jones, S. M., P. W. Davies, and A. Savage. 1978. Letter to the Editor: Gastric-juice nitrite and gastric cancer. *Lancet* 1: 1355.
- Kalatzis, E., and J. H. Ridd. 1966. Nitrosation, diazotisation, and deamination. Part XII. The kinetics of N-nitrosation of N-methylaniline. *J. Chem. Soc. B*:529-533.
- Keefer, L. K. 1979. α -Amino nitrite esters and their analogues: Possible reactive intermediates in N-nitrosamine formation. Pp. 91-108 in J-P. Anselme, ed. N-Nitrosamines, ACS Symposium Series No. 101. American Chemical Society, Washington, D.C.
- Keefer, L. K., and P. P. Roller. 1973. N-Nitrosation by nitrite ion in neutral and basic medium. *Science* 181:1245-1247.
- Keith, N. M., M. Whelan, and E. G. Bannick. 1930. The action and excretion of nitrates. *Arch. Intern. Med.* 46:797-832.
- Kim, Y. K., S. R. Tannenbaum, and J. S. Wishnok. 1980. Nitrosation of dialkylamines in the presence of bile acid conjugates. Pp. 207-214 in E. A. Walker, L. Gričute, M. Castegnaro, M. Bőrzsönyi, and W. Davis, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Kiwi, J. T., and M. Daniels. 1978. On the radiolysis of concentrated alkaline and calcium-nitrate solutions. *J. Inorg. Nucl. Chem.* 40:576-579.
- Knowles, M. E., J. Gilbert, and D. J. McWeeny. 1975. Phenols in smoked, cured meats: Nitrosation of phenols in liquid smoke and in smoked bacon. *J. Sci. Food. Agric.* 26:267-276.
- Kosaka, H., K. Imaizumi, K. Imai, and I. Tyuma. 1979. Stoichiometry of the reaction of oxyhemoglobin with nitrite. *Biochim. Biophys. Acta* 581:184-188.
- Kunin, C. M., and J. E. DeGroot. 1977. Sensitivity of a nitrite indicator strip method in detecting bacteriuria in preschool girls. *Pediatrics* 60:244-245.
- Li, B. F. 1981. Formation and Reaction of Some N-Nitroso Compounds. Ph.D. Thesis, University of London, London, England.
- Lijinsky, W., and G. M. Singer. 1975. Formation of nitrosamines from tertiary amines and nitrous acid. Pp. 111-114 in P. Bogovski, E. A. Walker, and W. Davis, eds. N-Nitroso Compounds in the Environment, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.

- Lijinsky, W., E. Conrad, and R. Van de Bogart. 1972. Carcinogenic nitrosamines formed by drug/nitrite interactions. *Nature* 239: 165-167.
- Love, L. A., W. Lijinsky, L. K. Keefer, and H. Garcia. 1977. Chronic oral administration of 1-nitrosopiperazine at high doses to MRC rats. *Z. Krebsforsch.* 89:69-73.
- Lovejoy, D. J., and A. J. Vosper. 1968. Dinitrogen trioxide. Part VI. The reactions of dinitrogen trioxide with primary and secondary amines. *J. Chem. Soc. A*:2325-2328.
- Lustre, O. A., and P. Issenberg. 1970. Phenolic components of smoked meat products. *J. Agric. Food Chem.* 18:1056-1060.
- Maltz, H., M. A. Grant, and M. C. Navaroli. 1971. Reaction of nitroprusside with amines. *J. Org. Chem.* 36:363-364.
- Mandel, M., D. Ichinotsubo, and H. Mower. 1977. Nitroso group exchange as a way of activation of nitrosamines by bacteria. *Nature* 267:248-249.
- Massey, R. C., M. J. Dennis, C. Crews, D. J. McWeeny, and R. Davies. 1980. Model system studies of N-nitrosamine formation in relation to cured meat: The non-polar phase and S-nitroso peptides. Pp. 291-303 in E. A. Walker, L. Gričiute, M. Castegnaro, and M. Bőrzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Masui, M., C. Ueda, T. Yasuoka, and H. Ohmori. 1979. Thioureas as effective catalysts for N-nitrosodimethylamine formation. *Chem. Pharm. Bull.* 27:1274-1275.
- Mauron, J. 1970. [In French.] Le comportement chimique des protéines lors de la préparation des aliments et ses incidences biologiques. *Int. J. Vitam. Res.* 40:209-227.
- McQuinn, R. L., Y.-C. Cheng, and G. A. Digenis. 1979. Convenient preparations of several N-nitroso compounds. *Synth. Commun.* 9:25-30.
- Mellor, J. W. 1928. *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, Vol. VIII. Longmans, Green and Co., New York, London, Toronto, Calcutta, Bombay, and Madras. 1110 pp.

- Mergens, W. J., J. J. Kamm, H. L. Newmark, W. Fiddler, and J. Pensabene. 1978. Alpha-tocopherol: Uses in preventing nitrosamine formation. Pp. 199-212 in E. A. Walker, L. Griciute, M. Castegnaro, and R. E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Michejda, C. J., T. J. Tipton, and D. H. Campbell. 1976. Nitrosative dealkylation under oxidative conditions as a possible source of nitrosamines. Pp. 255-260 in E. A. Walker, P. Bogovski, L. Griciute, and W. Davis, eds. Environmental N-Nitroso Compounds: Analysis and Formation, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.
- Mirvish, S. S. 1970. Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. J. Natl. Cancer Inst. 44:633-639.
- Mirvish, S. S. 1971. Kinetics of nitrosamide formation from alkylureas, N-alkylurethans, and alkylguanidines: Possible implications for the etiology of human gastric cancer. J. Natl. Cancer Inst. 46:1183-1193.
- Mirvish, S. S. 1972. Studies on N-nitrosation reactions: Kinetics of nitrosation, correlation with mouse feeding experiments and natural occurrence of nitrosatable compounds (ureides and guanidines). Pp. 279-295 in W. Nakahara, S. Takayama, T. Sugimura, and S. Odashima, eds. Topics in Chemical Carcinogenesis. University of Tokyo Press, Tokyo, Japan.
- Mirvish, S. S. 1975. Formation of N-nitroso compounds: Chemistry, kinetics, and in vivo occurrence. Toxicol. Appl. Pharmacol. 31: 325-351.
- Mirvish, S. S. 1981. Inhibition of the formation of carcinogenic N-nitroso compounds by ascorbic acid and other compounds. Pp. 557-587 in J. H. Burchenal and H. F. Oettgen, eds. Cancer: Achievements, Challenges and Prospects for the 1980s. Grune and Stratton, New York, London, Toronto, Sydney, and San Francisco.
- Mirvish, S. S. In press. Ascorbic acid inhibition of N-nitroso compound formation in chemical, food, and biological systems. In M. S. Zedeck and M. Lipkin, eds. Inhibition of Tumor Induction and Development. Plenum Press, New York, New York.
- Mirvish, S. S., J. Sams, T. Y. Fan, and S. R. Tannenbaum. 1973. Kinetics of nitrosation of the amino acids proline, hydroxyproline, and sarcosine. J. Natl. Cancer Inst. 51:1833-1839.

- Mirvish, S. S., B. Gold, M. Eagen, and S. Arnold. 1974. Kinetics of the nitrosation of aminopyrine to give dimethylnitrosamine. *Z. Krebsforsch.* 82:259-268.
- Mirvish, S. S., P. Issenberg, and J. P. Sams. In press. A study of N-nitrosomorpholine synthesis in rodents exposed to nitrogen dioxide and morpholine. In R. A. Scanlan and S. R. Tannenbaum, eds. *N-Nitroso Compounds*, ACS Symposium Series. American Chemical Society, Washington, D.C.
- Moeller, T. 1952. Oxy acids of nitrogen. Pp. 613-620 in *Inorganic Chemistry: An Advanced Textbook*. John Wiley & Sons, Inc., New York; Chapman & Hall, Ltd., London.
- Morgan, T. D. B., G. Stedman, and M. N. Hughes. 1968. Kinetics and mechanism of the reaction between nitrous acid and hydroxylamine. Part II. The alkyl hydroxylamines. *J. Chem. Soc. B*:344-349.
- Muhammad, D., and A. G. Maddock. 1978. Radiolysis of the alkali nitrates. *J. Chem. Soc. Faraday Trans. I* 74:919-932.
- National Academy of Sciences. 1978. Nitrates: An Environmental Assessment. A report prepared for the U.S. Environmental Protection Agency by the Panel on Nitrates of the Coordinating Committee for Scientific and Technical Assessments of Environmental Pollutants, Environmental Studies Board, Commission on Natural Resources, National Research Council. National Academy of Sciences, Washington, D.C. 723 pp.
- Neurath, G. B., M. Dünker, and F. G. Pein. 1976. Interaction of nitrogen oxides, oxygen and amines in gaseous mixtures. Pp. 215-225 in E. A. Walker, P. Bogovski, L. Gričiute, and W. Davis, eds. *Environmental N-Nitroso Compounds: Analysis and Formation*, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.
- Newmark, H. L., and W. J. Mergens. In press. Alpha-tocopherol (vitamin E) and its relationship to tumor induction and development. In M. S. Zedeck and M. Lipkin, eds. *Inhibition of Tumor Induction and Development*. Plenum Press, New York.
- Nicholas, D. J. D. 1978. Intermediary metabolism of nitrifying bacteria, with particular reference to nitrogen, carbon, and sulfur compounds. Pp. 305-309 in D. Schlessinger, ed. *Microbiology--1978*. American Society for Microbiology, Washington, D.C.

- Oae, S., Y. H. Kim, D. Fukushima, and K. Shinhama. 1978. New syntheses of thionitrites and their chemical reactivities. *J. Chem. Soc. Perkin Trans. I*:913-917.
- Ohshima, H., and T. Kawabata. 1978. Mechanism of N-nitroso-dimethylamine formation from trimethylamine and trimethylaminoxide. Pp. 143-153 in E. A. Walker, L. Gričiute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Okum, J. D., and M. C. Archer. 1977. Kinetics of nitrosamine formation in the presence of micelle-forming surfactants. *J. Natl. Cancer Inst.* 58:409-411.
- Olsman, W. J. 1977. Chemical behavior of nitrite in meat products. 1. The stability of proteinbound nitrite during storage. Pp. 101-109 in B. J. Tinbergen and B. Krol, eds. *Proceedings of the Second International Symposium on Nitrite in Meat Products*, September 7-10, 1976, Zeist, the Netherlands. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Orii, Y., and H. Shimada. 1978. Reaction of cytochrome *c* with nitrite and nitric oxide. A model of dissimilatory nitrite reductase. *J. Biochem.* 84:1543-1552.
- Osawa, T., Y. Kito, M. Namiki, and K. Tsuji. 1979. A new furoxan derivative and its precursors formed by the reaction of sorbic acid with sodium nitrite. *Tetrahedron Lett.* (45):4399-4402.
- Outram, T. R. 1979. N-Nitrosation by Nitric Oxide. Ph.D. Thesis, University of London, London, England.
- Parks, N. J., K. A. Krohn, C. A. Mathis, J. H. Chasko, K. R. Geiger, M. E. Gregor, and N. F. Peek. 1981. Nitrogen-13-labeled nitrite and nitrate: Distribution and metabolism after intratracheal administration. *Science* 212:58-61.
- Perrott, J. R., G. Stedman, and N. Uysal. 1976. Kinetic and product study of the reaction between nitrous acid and hydrazine. *J. Chem. Soc. Dalton Trans.* 2058-2064.
- Petriconi, G. L., E. G. Gori, and H. M. Papée. 1967. Photolysis of ammonium nitrate solutions by sunlight. *Ric. Sci.* 37:979-983.

- Pignatelli, B., M. Friesen, and E. A. Walker. 1980. The role of phenols in catalysis of nitrosamine formation. Pp. 95-109 in E. A. Walker, L. Gričute, M. Castegnaro, and M. Borzsonyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Pitts, J. N., Jr., D. Grosjean, K. Van Cauwenberghe, J. P. Schmid, and D. R. Fitz. 1978. Photooxidation of aliphatic amines under simulated atmospheric conditions: Formation of nitrosoamines, nitramines, amides, and photochemical oxidant. *Environ. Sci. Technol.* 12:946-953.
- Prabhakararao, K., and D. J. D. Nicholas. 1970. The reduction of sulphite, nitrite and hydroxylamine by an enzymes from baker's yeast. *Biochim. Biophys. Acta* 216:122-129.
- Raisfeld, I. H., C. Lin, J. Cheng, and J. Brandys. 1979. Nitrosamine formation from interaction of cardiovascular drugs. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 38:680. Abstract 2385.
- Rajagopalan, K. V., I. Fridovich, and P. Handler. 1962. Hepatic aldehyde oxidase. *J. Biol. Chem.* 237:922-928.
- Rapp, C., and T. Rydström. 1980. Occupational exposure to N-nitroso compounds. Pp. 565-574 in E. A. Walker, L. Gričute, M. Castegnaro, and M. Borzsonyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Redmond, T. F., and B. B. Wayland. 1968. Dimerization of nitrogen dioxide in solution: A comparison of solution thermodynamics with the gas phase. *J. Phys. Chem.* 72:1626-1629.
- Ridd, J. H. 1961. Nitrosation, diazotisation, and deamination. *Quart. Rev. Chem. Soc.* 15:418-441.
- Roller, P. P., L. K. Keefer, and B. W. Slavin. 1980. Inhibitory agents and chemical mechanisms in the dihalomethane-mediated nitrosation of amines with solid nitrite. Pp. 119-128 in E. A. Walker, L. Gričute, M. Castegnaro, and M. Borzsonyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Ruddell, W. S. J., E. S. Bone, M. J. Hill, L. M. Blendis, and C. L. Walters. 1976. Gastric-juice nitrite: A risk factor for cancer in the hypochlorhydric stomach? *Lancet* 2:1037-1039.

- Ruddell, W. S. J., E. S. Bone, M. J. Hill, and C. L. Waters. 1978. Pathogenesis of gastric cancer in pernicious anaemia. *Lancet* 1:521-523.
- Sander, J., and F. Seif. 1969. [In German; English summary.] (Bacterial reduction of nitrate in the human stomach as a cause for nitrosamine formation.) *Arzneim. Forsch.* 19:1091-1093.
- Scheifele, D. W., and A. L. Smith. 1978. Home-testing for recurrent bacteriuria, using nitrite strips. *Am. J. Dis. Child.* 132:46-48.
- Schlag, P., H. Ulrich, P. Merkle, R. Böckler, M. Peter, and Ch. Herfarth. 1980. Are nitrite and N-nitroso compounds in gastric juice risk factors for carcinoma in the operated stomach? *Lancet* 1:727-729.
- Schmeltz, I., and A. Wenger. 1979. 2-Bromo-2-nitropropane-1,3-diol as a nitrosating agent for diethanolamine: A model study. *Food Cosmet. Toxicol.* 17:105-109.
- Schmidt, E. L. 1978. Nitrifying microorganisms and their methodology. Pp. 288-291 in D. Schlessinger, ed. *Microbiology--1978*. American Society for Microbiology, Washington, D.C.
- Shaw, A. W., and A. J. Vosper. 1971. Dinitrogen trioxide. Part IX. Stability of dinitrogen trioxide in solution. *J. Chem. Soc. A*:1592-1595.
- Shuali, U., M. Ottolenghi, J. Rabani, and Z. Yelin. 1969. On the photochemistry of aqueous nitrate solutions excited in the 195-nm band. *J. Phys. Chem.* 73:3445-3451.
- Sinaniotis, C. A., M. N. Haratsaris, and C. J. Papadatos. 1978. Letter to the Editor: Nitrite indicator strip test for bacteriuria. *Lancet* 1:776-777.
- Singer, S. S. 1980. Transnitrosation by nitrosamines and nitroso-ureas. Pp. 111-117 in E. A. Walker, L. Gričute, M. Castegnaro, and M. Bőrszőnyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Singer, S. S., G. M. Singer, and B. B. Cole. 1980. Alicyclic nitrosamines and nitrosamino acids as transnitrosating agents. *J. Org. Chem.* 45:4931-4935.
- Smith, P. A. S., and R. N. Loeppky. 1967. Nitrosative cleavage of tertiary amines. *J. Am. Chem. Soc.* 89:1147-1157.

- Spiegelhalder, B., G. Eisenbrand, and R. Preussmann. 1976. Influence of dietary nitrate on nitrite content of human saliva: Possible relevance to *in vivo* formation of *N*-nitroso compounds. *Food Cosmet. Toxicol.* 14:545-548.
- Spincer, D., and D. T. Westcott. 1976. Formation of nitroso-dimethylamine in smoke from cigarettes manufactured from different tobacco types. Pp. 133-139 in E. A. Walker, P. Bogovski, L. Gričute, and W. Davis, eds. *Environmental N-Nitroso Compounds: Analysis and Formation*, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.
- Stedman, G. 1959a. Mechanism of the azide-nitrite reaction. Part I. *J. Chem. Soc.* 2943-2949.
- Stedman, G. 1959b. Mechanism of the azide-nitrite reduction. Part II. *J. Chem. Soc.*:2949-2954.
- Suryanarayanan, K., and S. Bulusu. 1972. Photolysis of solid dimethylnitramine: Nitrogen-15 study and evidence for nitrosamine rearrangement. *J. Phys. Chem.* 76:496-500.
- Tannenbaum, S. R., J. S. Wishnok, J. S. Hovis, and W. W. Bishop. 1978. *N*-Nitroso compounds from the reaction of primary amines with nitrite and thiocyanate. Pp. 155-159 in E. A. Walker, L. Gričute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Tannenbaum, S. R., D. Moran, W. Rand, C. Cuello, and P. Correa. 1979. Gastric cancer in Colombia. IV. Nitrite and other ions in gastric contents of residents from a high-risk region. *J. Natl. Cancer Inst.* 62:9-12.
- Thompson, J. T., and D. L. H. Williams. 1977. Direct nitrosation of aniline derivatives and other nucleophilic species by *N*-nitrosodiphenylamine. *J. Chem. Soc. Perkin Trans. II*:1932-1937.
- Treinin, A., and E. Hayon. 1970. Absorption spectra and reaction kinetics of NO_2 , N_2O_3 , and N_2O_4 in aqueous solution. *J. Am. Chem. Soc.* 92:5821-5828.
- Turney, T. A. 1960. The nitrous acid-dinitrogen trioxide equilibrium in aqueous perchloric acid. *J. Chem. Soc.* 4263-4265.
- Turney, T. A., and G. A. Wright. 1959. Nitrous acid and nitrosation. *Chem. Rev.* 59:497-513.

- Van Stee, E. W., G. A. Boorman, and J. K. Haseman. 1980. Pulmonary adenomas in mice exposed to NO₂ by inhalation and morpholine by ingestion. *The Pharmacologist* 22:158.
- Ville, J., and W. Mestrezat. 1907. [In French.] Origine des nitrites contenus dans la salive; leur formation par reduction microbienne des nitrates elimines par ce liquide. *C. R. Soc. Biol.* 73:231-233.
- Walker, E. A., B. Pignatelli, and M. Castegnaro. 1975. Effects of gallic acid on nitrosamine formation. *Nature* 258:176.
- Walker, E. A., B. Pignatelli, and M. Castegnaro. 1979. Catalytic effect of *p*-nitrosophenol on the nitrosation of diethylamine. *J. Agric. Food Chem.* 27:393-396.
- Walters, C. L., and A. McM. Taylor. 1964. Nitrite metabolism by muscle in vitro. *Biochim. Biophys. Acta* 86:448-458.
- Walters, C. L., R. J. Hart, and S. Perse. 1979. The possible role of lipid pseudonitrosites in nitrosamine formation in fried bacon. *Z. Lebensm. Unters. Forsch.* 168:177-180.
- Warthesen, J. J., R. A. Scanlan, D. D. Bills, and L. M. Libbey. 1975. Formation of heterocyclic *N*-nitrosamines from the reaction of nitrite and selected primary diamines and amino acids. *J. Agric. Food Chem.* 23:898-902.
- White, E. H. 1955. The chemistry of the *N*-alkyl-*N*-nitrosamides. I. Methods of preparation. *J. Am. Chem. Soc.* 77:6008-6010.
- White, E. H., and W. R. Feldman. 1957. Letter to the Editor: The nitrosation and nitration of amines and alcohols with nitrogen tetroxide. *J. Am. Chem.* 79:5832-5833.
- White, J. W., Jr. 1975. Relative significance of dietary sources of nitrate and nitrite. *J. Agric. Food Chem.* 23:886-891.
- White, J. W., Jr. 1976. Correction: Relative significance of dietary sources of nitrate and nitrite. *J. Agric. Food Chem.* 24:202.
- Williams, D. L. H. 1976. Denitrosation of *N*-methyl-*N*-nitroso-toluene-*p*-sulphonamide in acid solution. *J. Chem. Soc. Perkin Trans. II*:1838-1841.
- Williams, D. L. H. 1977. *S*-Nitrosation of thiourea and thiocyanate ion. Nitrosyl thiocyanate and the *S*-nitroso-adduct of thiourea as nitrosating agents. *J. Chem. Soc. Perkin Trans. II*:128-132.

Yang, H. S., J. D. Okun, and M. C. Archer. 1977. Nonenzymatic microbial acceleration of nitrosamine formation. *J. Agric. Food Chem.* 25:1181-1183.

Ziebarth, D., and B. Teichmann. 1980. Nitrosation of orally administered drugs under simulated stomach conditions. Pp. 231-244 in E. A. Walker, L. Griciute, M. Castegnaro, and M. Börzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.

CHAPTER 5

NITRATE, NITRITE, AND NITROGEN OXIDES: ENVIRONMENTAL DISTRIBUTION AND EXPOSURE TO HUMANS

| | <u>Page</u> |
|---|-------------|
| ANALYTICAL METHODS..... | 5-3 |
| Analysis of Nitrate..... | 5-3 |
| Analysis of Nitrite..... | 5-4 |
| Analysis of Nitrogen Oxides..... | 5-6 |
| Summary..... | 5-8 |
| ENVIRONMENTAL DISTRIBUTION..... | 5-9 |
| Food..... | 5-9 |
| Water..... | 5-29 |
| Tobacco..... | 5-30 |
| Air..... | 5-31 |
| Summary..... | 5-39 |
| EXPOSURE OF HUMANS TO NITRATE, NITRITE, AND NITROGEN OXIDES..... | 5-41 |
| Exposures from Food..... | 5-42 |
| Exposures from Water..... | 5-52 |
| Exposures from Air..... | 5-53 |
| Summary..... | 5-54 |
| OVERALL SUMMARY AND CONCLUSIONS..... | 5-56 |
| RECOMMENDATIONS..... | 5-57 |
| REFERENCES..... | 5-59 |

CHAPTER 5

NITRATE, NITRITE, AND NITROGEN OXIDES: ENVIRONMENTAL DISTRIBUTION AND EXPOSURE OF HUMANS

This chapter is a review of the environmental distribution, average concentrations, and ranges of concentrations of nitrate, nitrite, and nitrogen oxides. The data are used to estimate the exposure of humans to these substances. The accuracy of the estimates generated by the committee is limited by a number of weaknesses and uncertainties of current methods used to assay nitrate, nitrite, and nitrogen oxides. Moreover, in many cases, data on concentrations in the environmental sources surveyed are meager or outdated. The estimates of exposure from various foods are further limited by the possible inaccuracies in the data on average consumption. As more accurate and comprehensive data on environmental concentrations and consumption levels become available, the estimates developed by the committee can be refined and updated. In the meantime, the committee recommends that the estimates reported herein be used to determine the relative importance of various sources to the total exposure of the human population.

ANALYTICAL METHODS

A number of methods are used to analyze nitrate, nitrite, and nitrogen oxides. Each of them has limitations that should be considered when evaluating the data on the environmental concentrations.

Analysis of Nitrate

Methods for determining nitrate generally entail the use of spectrophotometry (Usher and Telling, 1975) based on the nitration of a phenolic compound, the oxidation of an organic compound by nitrate, or the reduction of nitrate to nitrite or ammonia. The most commonly used procedure is based on the reduction of nitrate to nitrite and subsequent colorimetric analysis of nitrite by the Griess reaction. (For a description of the Griess reaction, see the next section on analysis of nitrite.)

Several techniques have been developed to reduce nitrate. In the Follett and Ratcliff (1963) method, the substance under test is passed through spongy cadmium in a glass column with capillary inlet and outlet tubes. This technique results in essentially 100% reduction, and it can be used for the simultaneous determination of both the nitrate and nitrite content of the substance (Hamilton, 1976; Usher and Telling, 1975). Among the variations of this procedure is one

developed by Elliott and Porter (1971) for meat extracts, in which nitrate is rapidly reduced by shaking the extract with spongy cadmium at a pH of 9.6 for 5 minutes. Other investigators have found that this technique results in a low recovery of nitrate, however, indicating inefficient reduction. Usher and Telling (1975) attributed the decreased reduction efficiency to the presence of polyphosphate in meat. This problem can be overcome by using a buffer with a buffering capacity twice as great as that used by Follett and Ratcliff (1963).

Usher and Telling (1975) have discussed several other general problems with the nitrate reduction technique -- dilution effect, interference from ascorbate, and interference from sulfur dioxide. These authors have concluded that, although this method is generally favored, it may not provide accurate determination of nitrate and nitrite at levels below 10 mg/kg.

Several investigators have described methods in which ion-selective electrodes are used to measure nitrate in extracts of various foods, including spinach (Barker *et al.*, 1971; Voogst, 1969), sugar beets (McCaslin *et al.*, 1970), and baby foods (Liedtke and Meloan, 1976; Pfeiffer and Smith, 1975). Unfortunately, since the nitrate electrode is nonspecific, other ions interfere in the analysis, and steps must be taken to compensate for, or eliminate, their influence (Usher and Telling, 1975). For example, chloride can interfere in nitrate measurements when it is present in quantities 10 or more times greater than the quantity of the nitrate, a situation that exists in cured meats and certain cheeses (Comer, 1978). But there are several methods for removing the chloride ion. For example, the silver resin method proposed by Paul and Carlson (1968) eliminates chloride interference in the analysis of baby foods, including fruits, vegetables, formulated foods, and meats. Pfeiffer and Smith (1975) compared the nitrate electrode method with the xylenol method of the Association of Official Analytical Chemists (AOAC). They reported that the standard error when measuring nitrate at 100 mg/kg was 4.3 mg/kg.

A rapid method for the simultaneous determination of nitrate and nitrite has been developed using high-pressure ion-exchange liquid chromatography (HPLC) to separate the ions and ultraviolet absorption at 210 nm for their detection (Gerritse, 1979; Thayer and Huffaker, 1980). The method can determine as little as 50 pmol of nitrate in samples ranging from 5 to 500 μ l, and has recently been applied to food samples (Leuenberger *et al.*, 1980).

Analysis of Nitrite

Methods commonly used to determine nitrite in foods and other substances have been reviewed by Usher and Telling (1975). These methods are usually based on the interaction of nitrite with a

primary aromatic amine in acid solution to form a diazonium salt. The diazonium salt is then treated with an aromatic compound bearing reactive amino or hydroxyl groups to form an azo color that can be measured spectrophotometrically (Hamilton, 1976; Nicholas and Fox, 1973). In the Greiss reaction, which was adopted by the AOAC as the official method for assaying for nitrite (Horwitz, 1975), sulfanilamide is nitrosated to form an azo compound, which is coupled with 1-naphthylamine to yield a pink dye.

In a modification of the official AOAC method, nitrite is detected by the diazotization of sulfanilamide and subsequent formation of the azo dye after coupling with N-(1-naphthyl)ethylenediamine (Fiddler, 1977). However, since the coupling reagent is a primary amine, it can react with nitrite under the acidic conditions present during analysis and compete with sulfanilamide for the available nitrite. Therefore, if the sulfanilamide and the coupling agent are added together, this competing reaction can lead to the destruction of some nitrite, loss of color, and inaccurate determinations of nitrite (Sen and McPherson, 1978). For this reason, the modified official AOAC method calls for the separate addition of the two color reagents (Fiddler, 1977). Sulfanilamide is added first, and after 5 minutes (when the diazotization reaction is complete), N-(1-naphthyl)ethylenediamine is added to form the pink azo dye. Sen and McPherson (1978) have demonstrated that ascorbate present in food samples can destroy some of the nitrite during preincubation with sulfanilamide. They have suggested an alternative method of analysis in which ascorbic acid, in the amounts normally found in foods and cured meat products, does not interfere. Another problem with this method is that breakdown products of other chemicals may be included in the measurements of nitrite. For example, Pyper and Hartman (personal communication) found that oxyhyponitrite behaves as a mixture of nitrate and nitrite when assayed by the modified AOAC procedure.

Several new procedures have greatly increased the sensitivity of determinations for nitrite in foods and other substances. Walters et al. (1978) acidified food samples with acetic acid and then measured the resulting nitric oxide with a chemiluminescence detector. Although this method does have the potential of being at least one order of magnitude more sensitive than the colorimetric methods, it has certain limitations (Doerr et al., 1981). For example, water was found to decrease the response of the method, thereby limiting its applicability. Furthermore, only acidification of the sample is used to produce nitric oxide from nitrite. However, nitrous acid decomposes by mono-, bi-, and trimolecular reactions to form a number of N-nitrosating species, including the nitrosonium ion (NO^+), as well as the nonnitrosating nitric oxide (Doerr et al., 1981). The amount of nitric oxide that can be produced is dependent upon a balance among competing reactions that can be altered by various compounds present in foods. For example, the presence of nitrosatable compounds such as primary and secondary amines or of reducing agents in samples could conceivably react with NO^+ . As a result, less nitric oxide would be produced and an inaccurate nitrite value would be obtained. Thus, it would be desirable to have a more homogeneous reaction such as that reported by Cox (1980) who added

sodium iodide as a reductant to the sample, thereby driving the nitrous acid decomposition reaction to nitric oxide. This method for measuring nitrite is more than 600 times more sensitive than the colorimetric procedure.

Doerr *et al.* (1981) have also assessed the effectiveness of the chemiluminescence procedure for determining nitrite and compared it with the Griess colorimetric method. The test medium was a meat slurry containing either ascorbate or cysteine, both of which can cause loss of nitrite in meat (Fox and Nicholas, 1974) or interfere in the analysis. To facilitate a comparison of the effectiveness of these analytical procedures when they are free of interfering substances, the investigators added charcoal to the test system to eliminate the ascorbate interference. In both methods sodium nitrite values were comparable in samples without an added reductant, regardless of whether they had been treated with charcoal or not. Unlike the Griess method, the addition of a reductant in the chemiluminescence method did not reduce the amount of nitrite measured. The nitrite values obtained with the two methods were equivalent after treatment with charcoal.

A new method for determining nitrite by HPLC (Gerritse, 1979; Thayer and Huffaker, 1980) was discussed above in the section on nitrate. Concentrations of nitrite as low as 200 pmol can be detected in samples ranging from 5 to 500 μ l.

Special difficulties are encountered when measuring nitrite levels in meat because nitrite combines with some constituents and may not be measured in the assays just described (Cassens *et al.*, 1974). Thus, for each of the techniques described, it is important to know which nitrite species are measured when meat samples are being analyzed. Currently, it is not clear whether all nitrite present in meat is actually measured. However, it appears that similar species may be measured by the colorimetric and chemiluminescence methods since results from these methods are comparable.

Analysis of Nitrogen Oxides

Because of the complex chemistry of the nitrogen oxides, any of the following gases may exist in ambient air: nitrous oxide, nitric oxide, nitrogen dioxide, nitric acid, and nitrous acid. Both nitric oxide and nitrogen dioxide are stable free radicals, and they typically participate in rapid simultaneous reactions. For example, they may combine to form dinitrogen trioxide and dinitrogen tetroxide. It has been extremely difficult to prepare accurately known concentrations of nitrogen dioxide because of its high reactivity with impurities, other pollutants, and the walls of the system. This section briefly reviews common manual and continuous methods as well as calibration procedures.

A precise method for establishing primary nitrogen concentrations is the Griess-Saltzman method (Saltzman, 1954), which is based on the colorimetric Griess reaction described previously. In this method, nitrogen dioxide is reacted with diazotizing-coupling reagents to form a deeply colored azo dye. Prior to the establishment of air quality standards, this method was adopted by the Inter-society Committee (1972). The original U.S. Environmental Protection Agency (EPA) reference method for nitrogen dioxide, which was published along with the air quality standard (U.S. Environmental Protection Agency, 1971b), was based on the adaptation of the Griess-Saltzman method to a 24-hour integrated manual method (Hochheiser, 1965; Jacobs and Hochheiser, 1958). In 1972, the EPA published a notice in the Federal Register indicating that this modified method was unreliable (U.S. Environmental Protection Agency, 1972). As a result, an extensive effort was undertaken to develop and validate more reliable procedures.

After extensive interlaboratory testing, the EPA finally selected chemiluminescence as the reference method for nitric oxide and nitrogen dioxide, but it listed the sodium arsenite and TGS-ANSA (triethanolamine-guaiacol-sulfite/8-amino-1-naphthalenesulfonic acid-ammonium salt) methods as being equally reliable (U.S. Environmental Protection Agency, 1976a,b).

Chemiluminescence Method. Atmospheric nitrogen dioxide is determined indirectly by photometric measurements of the light intensity at wavelengths greater than 600 nm, which result from the chemiluminescent reaction of nitric oxide with ozone. Nitrogen dioxide is first quantitatively reduced to nitric oxide by a thermal converter. Since nitric oxide, which commonly exists in ambient air with nitrogen dioxide, passes through the converter unchanged, the resultant total NO_x concentration is equal to nitric oxide plus nitrogen dioxide. A sample of input air is also measured without having passed through the converter. The latter measurement (background nitric oxide) is then subtracted from the measurement of total NO_x to determine the concentration of nitrogen dioxide.

This method requires dynamic calibration with known quantities of nitric oxide and nitrogen dioxide. Nitric oxide samples of known concentrations, which are stored in high-pressure cylinders to ensure stability, are commercially available, whereas nitrogen dioxide samples of known concentrations can be generated either by a gas phase titration (GPT) technique or by nitrogen dioxide permeation devices.

Sodium Arsenite Method. The sodium arsenite method is a 24-hour integrated manual method similar to the original EPA reference method (Christie et al., 1970). Nitrogen dioxide is collected by bubbling air through an orifice-type bubbler into a sodium hydroxide-sodium arsenite solution to form a stable solution of sodium nitrite. The

nitrite ion produced during sampling is reacted with phosphoric acid, sulfanilamide, and N-(1-naphthyl)-ethylenediamine dihydrochloride to form an azo dye, which is then measured colorimetrically.

TGS-ANSA Method. The TGS-ANSA method is also a 24-hour integrated manual method. Nitrogen dioxide is collected by bubbling air through an orifice-type bubbler into a solution of triethanolamine, *o*-methoxyphenol (guaiacol), and sodium metabisulfite. The nitrite ion produced during sampling is determined colorimetrically by reacting the exposed absorbing reagent with sulfanilamide and 8-amino-1-naphthalenesulfonic acid-ammonium salt (ANSA).

Summary

Classical methods for the determination of nitrate are generally unreliable since their success is based on the degree to which nitrate is reduced to nitrite -- a reaction that is difficult to control and reproduce. Other techniques to measure nitrate, such as the ion-selective electrode techniques, are subject to many interferences, which necessitate further clean-up steps.

The successful determination of nitrite is not as difficult as it is for nitrate. Once in solution, free nitrite is readily determined by diazotization and coupling reactions or, more recently, by chemiluminescence techniques, which are 600 times more sensitive than the colorimetric procedures. In addition, the colorimetric methods give incorrect, lower nitrite values than the chemiluminescence methods in the presence of reductants such as ascorbate. However, the addition of charcoal removes these reductants, whereupon the nitrite values measured by the colorimetric methods are equivalent to those measured by chemiluminescence techniques.

Based on its accuracy and freedom from many known interferences, the chemiluminescence method is recommended as a reference technique, not only for cured meats but also for other samples of biological origin; however, the high cost of the required instrumentation (e.g., a thermal energy analyzer) will probably preclude its wide acceptance as a routine method for determination of nitrite.

In the following section on environmental distribution, the values used for nitrate and nitrite have been taken from the published literature. A variety of test methods were used to arrive at these data, and not all investigators described the recovery from control samples to which varying concentrations of nitrate or nitrite were added to measure any oxidation or reduction that may have occurred in the sample prior to testing. Even when the procedures are properly controlled, incorrect readings can result from the presence of reducing agents (e.g., when reductants are present during the use of colorimetric methods to measure nitrite and no charcoal is added).

Methods for measuring nitrogen oxides have recently been standardized. Three of them -- the chemiluminescence, sodium arsenite, and TGS-ANSA methods -- are regarded by the EPA as being equally reliable.

ENVIRONMENTAL DISTRIBUTION

Major sources of nitrate and nitrite intake are food and water. Nitrogen oxides are found primarily in polluted air (in the ambient atmosphere, in indoor environments, and in the workplace) and in tobacco smoke. In this section, data on the amounts of nitrate, nitrite, and nitrogen oxides in various environmental sources are reviewed. Despite the limitations of the various methods for measuring these chemicals (especially nitrate and nitrite) and the limited amount of data available on their concentrations in the environment, the committee has assumed that published measurements are a reasonably accurate reflection of the relative levels present in environmental media to which the human population is exposed.

Food

The committee recognizes that the concentrations of nitrate and nitrite vary considerably within each food category. Nonetheless, it has estimated average concentrations and has used these estimates later in the chapter to project the average intake of these ions from food. These estimates have been developed to compare the relative contribution of various foods to the total intake of nitrate and nitrite and to compare exposure from these sources with exposure from other environmental media, such as water and air.

Considerable confusion has occurred in the past when the nitrate and nitrite contents of various foods were compared on the basis of published data that were expressed in several different molecular forms (Phillips, 1968b). For example, the nitrate or nitrite content of foods is commonly expressed in terms of the sodium salt, the nitrate or nitrite ion, or as nitrate- or nitrite-nitrogen. In this chapter, the concentrations of nitrate and nitrite are expressed in terms of the two ions (NO_3^- and NO_2^-). If the data taken from the literature were expressed in different terms, appropriate conversions were made.

In many cases, the committee relied on the same data base as that used by White (1975, 1976) to estimate relative exposure to dietary sources of nitrate and nitrite. When data from other comparable surveys were available, the committee used them as a supplement to this data base in an attempt to refine and update the figures used by White.

Cured Meat Products: Nitrate and Nitrite. Selected data concerning the nitrate and nitrite content of cured meat products from the United States and Canada are summarized in Table 5-1.

TABLE 5-1

The Content of Nitrate and Nitrite in Some Cured Meats

NOTE: Many of these values have been converted from concentrations of the sodium salt to concentrations of the ion, and some have been rounded off to two significant figures.

| Product | No. of Samples Tested ^a | Nitrate Content, mg/kg | | Nitrite Content, mg/kg | | Year | Reference |
|--|------------------------------------|------------------------|---------|------------------------|-------------------|---------|---|
| | | Range | Average | Range | Average | | |
| Bacon, uncooked (nitrate-cured) ^b | 12 | | | 37-430 | 140 | 1926 | Kerr et al., 1926 |
| Bacon, uncooked (nitrite-cured) | 12 | | | 8-63 | 28 | 1972 | Sen et al., 1974 |
| | 14 | | | 7-68 | 35 | 1974 | Sen et al., 1975 |
| | 12 | ND ^c -64 | 33 | ND-88 | 42 | 1975 | Sen et al., 1977 |
| | 20 | 7-320 | 96 | 3-170 | 25 | 1972 | Panalaks et al., 1973 |
| | 12 | | | 4-32 | 17 | 1974 | Greenberg, 1977 |
| | 316 ^d | | | | 40 ^d | 1977 | American Meat Institute, 1977 |
| | 82 ^e | | | | 21 ^e | 1978-79 | American Meat Institute, personal communication |
| | 2,476 ^{e,f} | | | | 12 ^{e,f} | 1978-79 | N. Nelson, U.S. Department of Agriculture, personal communication, 1981 |
| Bacon, fried | 14 | | | 7-68 | 35 | 1976 | Greenberg, 1977 |
| Bologna | 12 | 4-98 | 32 | 5-18 | 7 | 1973 | Panalaks et al., 1974 |
| Bologna | 21 | 9-220 | 87 | <0.7-150 | 29 | 1972 | Panalaks et al., 1973 |
| | 3 | 26-60 | 42 | <0.7-7 | 5 | 1973 | Panalaks et al., 1974 |
| | 20 | | | 3-55 ^g | 31 ^g | 1977-78 | Buege et al., 1978 |
| Hams (nitrate-cured) ^b | 12 | | | 24-640 | 280 | 1926 | Kerr et al., 1926 |
| Hams (nitrite-cured) | 23 | 0.7-1,400 | 150 | <0.7-140 | 29 | 1972 | Panalaks et al., 1973 |
| | 19 | | | | 16 | 1979-80 | Birdsall, 1981 |
| Ham, fried | 20 | | | 4-220 | 37 | 1974 | Greenberg, 1977 |
| Ham, pasteurized, canned, cured | 1 | 140 | 140 | 99 | 99 | 1972 | Panalaks et al., 1973 |
| Salami, European-type sausages | 25 | 4-270 | 89 | <0.7-66 | 17 | 1972 | Panalaks et al., 1973 |
| | 63 | 4-540 | 78 | 5-97 | 13 | 1973 | Panalaks et al., 1974 |
| Sausages, fermented | 25 | | | | 6 ^g | 1977 | American Meat Institute, 1977 |
| Sausages, smoked and unsmoked (nitrate-cured) ^g | 13 | | | 13-940 | 190 | 1926 | Kerr et al., 1926 |
| Sausages, smoked and unsmoked (wieners and sausages) | 18 | 17-240 | 110 | <0.7-52 | 9.6 | 1972 | Panalaks et al., 1973 |
| Sausages, smoked and unsmoked (wieners) | 2 | 66, 130 | 96 | 10, 10 | 10 | 1973 | Panalaks et al., 1974 |
| Sausages, smoked and unsmoked | 10 | | | 0-50 | 31 | 1975 | Coppola et al., 1976 |
| | 20 | | | 5-43 ^g | 24 ^g | 1977-78 | Buege et al., 1978 |
| | 152 | | | | 19 ^g | 1979-80 | Birdsall, 1981 |
| Sausages, pasteurized, canned, cured | 2 | 15, 18 | 16 | 5, 7 | 6 | 1973 | Panalaks et al., 1974 |
| Sausage, summer | 20 | | | 0-29 ^g | 6 ^g | 1977-78 | Buege et al., 1978 |
| Shelf-stable canned cured meat | 16 | <0.7-840 | 100 | <0.7-17 | 6 | 1972 | Panalaks et al., 1973 |
| | 7 | <0.7-110 | 26 | 5-8 | 6 | 1973 | Panalaks et al., 1974 |
| | 3,944 | | | | 19 ^g | 1977 | American Meat Institute, 1977 |
| Refrigerated canned meats | 242 | | | | 44 ^g | 1977 | American Meat Institute, 1977 |

^aConcentrations, expressed as the nitrate and nitrite ion, were measured in products at the retail level, unless otherwise designated.

^bRepresentative of nitrite concentrations found after curing in nitrate pickle, in common practice before USDA regulations became effective in 1926 (U.S. Department of Agriculture, 1926). In earlier times, however, many meat products were dry-cured and aged at ambient temperatures without any nitrate or nitrite additions (Kemp et al., 1974, 1975).

There appear to be no data on the nitrate or nitrite contents of this class of product.

^cND = Not detected.

^d1-3 days after processing. Before USDA final ruling limiting sodium nitrite addition to bacon to 120 mg/kg, eliminating addition of nitrate to bacon, requiring addition of ascorbate or isoascorbate to bacon, and limiting nitrosamine levels in fried bacon to less than 10 ppb (U.S. Department of Agriculture, 1978b).

^e1-3 days after processing. After USDA final ruling (see footnote d).

^f1,764 samples from large, and 302 samples from small, federally inspected plants each contained nitrite at a median of 12 mg/kg; 127 samples from large, and 283 samples from small, state-inspected plants contained nitrite at a median of 11 mg/kg.

^gConcentrations were measured shortly after processing (1-14 days).

Nitrate and/or nitrite had been added to most, if not all, of these products during the curing process. All data were taken from literature published during the past decade except for the data pertaining to "nitrate-cured" bacon, sausages, and ham, which illustrate the high nitrite content of cured meats in the United States during the mid-1920's. The large decline in the average nitrite content of these products since that time reflects both the impact of federal regulations and the changes engendered by the meat-packing industry.

One of the most striking aspects of the data presented in Table 5-1 is the wide range of nitrate and nitrite concentrations found within certain product categories. In some cases, average concentrations have been considerably influenced by only a few measurements of high concentrations. Data from many of the surveys cited in this table indicate that a significant percentage of the products tested contained low levels of residual nitrite. For example, among the 297 products analyzed by Panalaks *et al.* (1973, 1974), 193 (65%) contained residual nitrite at approximately 10 mg/kg or less.

In addition, 127 products (43%) contained nitrite at levels of 7 mg/kg or less. Thus, nearly one-half of the Canadian products surveyed by Panalaks in 1972 and 1973 contained the same level as the average in cured meat products from Norway (Table 5-2), where the total amount of nitrate and nitrite used as food additives has decreased by more than 80% since 1973 (Ringen, personal communication, 1981).

White (1975, 1976) estimated the intake of nitrate from cured meats for the U. S. population. His estimate was based on a survey by Fudge and Truman (1973), who reported levels of nitrate in 171 samples of predominantly European meat products. The average concentration of nitrate in these samples was approximately 200 mg/kg (Table 5-2). Since there has been no comparable survey of U. S. products, the committee has used White's data as a basis for estimating the average concentration of nitrate in cured meats. These data were modified to reflect some changes made since White's survey. For example, a ban on the addition of nitrate to all but a few products was enacted in Canada (Health and Welfare Canada, 1975). As a result, there has been a fivefold decrease in the nitrate content of Canadian products between 1971 and 1978 (Table 5-2). The trend toward decreasing residual nitrate concentrations in cured meats can be discerned by comparing the average concentrations measured in several surveys of Canadian products conducted over the last decade. Although similar regulations have not been adopted in the United States, the meat industry has taken internal action to reduce the nitrate content of cured meats. However, there has been no close monitoring of such concentrations to determine how effective this voluntary program has been. In order to develop an estimate for U.S. products, the committee has assumed that the nitrate content of U.S. products has decreased as it has in Canada and that additional decreases have occurred since 1978 and has reduced reducing by nearly sixfold the average concentration reported by Fudge and Truman. In this manner,

TABLE 5-2

Average Residual Nitrate and Nitrite Concentrations in Cured Meats^a

NOTE: Some of these values have been converted from concentrations of the sodium salt to concentrations of the ion, and some have been rounded off to two significant figures.

| <u>Source of Data</u> | <u>Nitrate, mg/kg</u> | <u>Nitrite, mg/kg</u> | <u>References</u> |
|--|---------------------------|---------------------------|--|
| 1971 Survey of Canadian products ^b | 130 | 19 | Panalaks <u>et al.</u> , 1973 |
| 1973 Survey of Canadian products ^b | 64 | 11 | Panalaks <u>et al.</u> , 1974 |
| 1978 Survey of Canadian products ^b | 28 | 15 | C. J. Randall, personal communication, 1979 |
| 1936 Survey of U.S. products ^b | NR ^c | 30 | Kolari and Aunan, 1972 |
| 1937 Survey of U.S. products ^b | NR | 42 | Kolari and Aunan, 1972 |
| 1971 Survey of U.S. products ^d | NR | 23 | Kolari and Aunan, 1972 |
| 1972 Survey of U.S. products ^e | NR | 32 | Kolari and Aunan, 1972 |
| 1978 Survey of U.S. products ^b | 28 | 11 | C. J. Randall, personal communication, 1979 |
| 1972 Survey of European Products | 210 | 14 | Fudge and Truman, 1973 |
| 1976 Survey of Norwegian products | 25 | 8 | Lyng, 1978 |

^aThese averages do not take consumption patterns into consideration.

^bProducts examined at the retail level.

^cNR = no data reported.

^dProducts examined within 1 to 14 days after production.

^eProducts examined within 1 to 2 days after production.

the committee has estimated the nitrate content of cured meat to be approximately 40 mg/kg (Table 5-3). This is approximately 40% higher than the only value for the nitrate content of U.S. products identified by the committee (Randall, personal communication, 1979).

In contrast to the clear trend of a decrease in residual nitrate in cured meats seen in the Canadian data, no distinct pattern has been observed for residual nitrite (Table 5-2). In 1978, an apparent decrease in residual nitrite was detected in bacon (American Meat Institute, personal communication). The significance of the observed drop, however, is unclear because ascorbate is known to interfere with the detection of nitrite, and, since 1978, bacon samples have contained substantial amounts of ascorbate and isoascorbate. Moreover, if the nitrite was not measured immediately after processing, the low levels could be attributed to decreases in residual nitrite that occur over time after processing. However, these explanations are merely conjectural since the details of this study have not yet been published. In contrast, some investigators have reported an increase in the nitrite content of some cured meat products (Sen *et al.*, 1977; U.S. Department of Agriculture, 1976); however, these findings were not statistically significant. Moreover, differences detected in the study by the U. S. Department of Agriculture (USDA) may have resulted from sampling the products at different time intervals after processing (U.S. Department of Agriculture, 1978a).

The decrease in detectable nitrite levels in cured meats after processing is due primarily to its reactivity (Cassens *et al.*, 1979). Because of this reactivity with various components in meat, less than 50% of the nitrite added can be analyzed chemically shortly after processing (Cassens *et al.*, 1974). Before 1974, little was known about the fate of added nitrite, except for the cured meat pigment (nitric oxide myoglobin) and the residual nitrite. Although Pivnik *et al.* (1967), Nordin (1969), and Herring (1973) demonstrated rapid decreases in nitrite in model systems or in commercially processed meat products, these investigators did not account for the nitrite lost. Since then, Fujimaki *et al.* (1975), Sebranek (1974), and Cassens *et al.* (1977) have demonstrated that nitrite combines with both the water- and salt-soluble meat fractions. Although much of the protein-bound nitrite exists as S-nitrosothiols, which can participate in nitrosation reactions (Davies *et al.*, 1978), Massey *et al.* (1980) have reported that the rate of nitrosation by S-nitrosocysteine is considerably reduced when the nitrosocysteine is incorporated into simulated peptide chains. Thus, the importance of bound nitrite in nitrosation reactions remains unclear.

The committee's estimates of the nitrite content of cured meats and subsequent exposure of humans from this source (see next section) rely exclusively on data concerning residual nitrite, and, because it is not clear whether bound nitrite is measured in current assay methods, the estimates may not reflect the possible contribution of bound nitrite. These estimates have also been adjusted to reflect residual nitrite levels that would be present in products at the time of consumption.

TABLE 5-3

Average Concentrations of Nitrate, Nitrite, and Nitrogen Oxides
Used by the Committee to Estimate Exposure of Humans

| <u>Source</u> | <u>Nitrate</u> | <u>Nitrite</u> | <u>Nitrogen Oxides</u> |
|----------------------------|-----------------|-----------------|-------------------------|
| Cured meats | 40 mg/kg | 10 mg/kg | |
| Fresh meats | 10 mg/kg | 1 mg/kg | |
| Vegetables | NA ^a | NA ^a | |
| Fruits | 20 mg/kg | 0 ^b | |
| Baked goods and cereals | 12 mg/kg | 2.6 mg/kg | |
| Milk and milk products | 0.5 mg/liter | 0 ^b | |
| Water | 1.3 mg/liter | 0 ^b | |
| Ambient atmosphere | | | 0.058 mg/m ³ |
| Tobacco smoke | | | 0.51 mg/cigarette |

^aNot applicable. Overall average concentrations in vegetables were not used in exposure calculations. See Table 5-8 for individual averages for 35 vegetables.

^bNitrite concentration was considered to be negligible.

In 1975, White estimated the nitrite content of cured meats by using the broad data base developed by Kolar and Aunan (1972), which included data collected from more than 950 samples of meat products tested at the retail level in 1936 and 1937 and 1 to 14 days after processing in 1971 and 1972 (Table 5-2). The committee has used the average nitrite value for the most recent year of this survey -- 32 mg/kg in 1972 -- to estimate the average residual nitrite in cured meat products. However, because nitrite was measured shortly after processing, and the committee wished to develop an estimate indicative of the nitrite content at the time of consumption, it has reduced this estimate based on average decreases in residual nitrite occurring between processing and consumption.

Birdsall (1981) estimated the average nitrite content of cured meats at the time of consumption to be approximately 7 mg/kg. This is approximately a 70% decrease from the 24 mg/kg reported as the average amount detected in products tested shortly after processing (American Meat Institute, 1977). The lower figure (7 mg/kg) is the average of measurements taken at 15 to 28 days after processing for noncanned items, at 29 or more days after processing for refrigerated canned products, and at 60 or more days after processing for shelf-stable canned products (Table 5-4).

TABLE 5-4

Average Residual Nitrite (mg/kg) in Cured Meats at Specified Times After Packaging when Stored at 4.4°C to 7.2°C^a

NOTE: Concentrations of sodium nitrite used in the original estimates have been converted to nitrite ion.

| Product Category | Nitrite Concentration, mg/kg (and Number of Samples) at Different Stages (No. of Days) after Packaging | | | | | |
|--|---|-----------------|------------------|-------------------|-------------------|------------------|
| | <u>≤ 3 Days</u> | <u>4-6 Days</u> | <u>7-14 Days</u> | <u>15-28 Days</u> | <u>29-59 Days</u> | <u>≥ 60 Days</u> |
| Bacon | 40 (N = 316) | 13 (N = 45) | 12 (N = 19) | 9 (N = 116) | 6 (N = 31) | 6 (N = 30) |
| Fermented sausage | 6 (N = 25) | 7 (N = 5) | 7 (N = 8) | 6 (N = 40) | 6 (N = 27) | 4 (N = 6) |
| Loaves | 9 (N = 32) | 9 (N = 17) | 15 (N = 18) | 8 (N = 42) | 6 (N = 25) | 3 (N = 12) |
| Cooked sausage | 27 (N = 604) | 13 (N = 60) | 13 (N = 112) | 7 (N = 91) | 5 (N = 113) | 3 (N = 62) |
| Canned meats, refrigerated | 44 (N = 242) | 40 (N = 98) | 34 (N = 8) | 15 (N = 22) | 9 (N = 64) | 7 (N = 64) |
| Canned meats, shelf stable ^b | [-----19-----] [----- (N = 3,944) -----] | | | | | 6 (N = 814) |

^aFrom Birdsall, 1981.

^bHeld at ambient temperatures.

The approximately 70% decrease in detected residual nitrite reported by Birdsall compares well with the approximately 80% nitrite loss in products between processing and consumption reported by Buege et al. (1978) and the 85-98% loss reported by Cassens et al. (1979).

If a reduction factor of 70% is assumed for Kolari and Aunan's 1972 average of 32 mg/kg, the residual nitrite concentration in products at the consumer level would be 10 mg/kg. For the purpose of estimating the residual nitrite content of cured meats at the time of consumption, the committee has used this amount -- 10 mg/kg (Table 5-3).

Fresh Meat Products: Nitrate and Nitrite. Fresh and unprocessed meat products, which constitute more than half of the meat sold in the United States, are generally not regarded as major contributors to the nitrate and nitrite ingested by humans. For example, White (1975) did not include fresh meat in his estimates of dietary sources of these ions because "no definitive information on the amount of nitrate or nitrite in fresh meat was located."

The committee has reviewed several studies of the nitrate content of fresh meat products. In one of these, Kačmár and Bartik (1965) found nitrate concentrations of approximately 170 mg/kg in fresh pork muscle; however, most other investigators who have studied nitrate in the fresh muscle of various animal species have reported much lower concentrations. For example, Whelan (1935a,b) found nitrate concentrations of approximately 6 mg/kg and 33 mg/kg in the tissues of dogs fed diets containing moderate and high concentrations of nitrate, respectively.

Wright and Davison (1964) reported nitrate concentrations of 0.9 mg/kg in the meat of dairy cows used as controls in a sodium nitrate feeding study and 11 mg/kg in the meat of cattle fed hay supplemented with close to lethal levels of sodium nitrate. Usher and Telling (1975) reviewed the data for meat blanks (controls without added nitrate or nitrite) given in a number of independent studies and found that the reported nitrate concentrations ranged from 0 to 49 mg/kg. They estimated that the level of sensitivity of the assay method(s) used in the studies was 6 mg/kg. Skovgaard (1980) reported a nitrate concentration of 19 mg/kg in bacon to which no nitrate or nitrite had been added, and Christiansen et al. (1973) found 55 mg/kg in comminuted hams after cooking.

There is little information on the nitrite content of fresh meats, but there are data indicating that free natural nitrite levels in meats processed without added nitrite are generally low. In 1979, approximately 3 billion kilograms of such products was processed without added nitrite (American Meat Institute, 1980; U.S. Department of Agriculture, 1980). Kemp et al. (1975) reported that dry-cured hams treated only with salt and sugar contained nitrite at approximately 5 mg/kg and that this value did not change appreciably with

storage. However, experimental error and cross-contamination from nitrate- and nitrite-treated hams were not excluded as sources of the nitrite. Christiansen et al. (1973) found concentrations of nitrite ranging from 1 to 8 mg/kg in ground fresh hams stored from 7 to 168 days at 7°C.

To develop an estimate of human intake from this source, the committee has assumed that meat products to which no nitrate or nitrite has been added contain nitrate concentrations of 10 mg/kg and that the nitrite content is only sporadic, averaging 1 mg/kg (Table 5-3).

Vegetables: Nitrate and Nitrite. Nitrate concentrations of vegetables are listed in Tables 5-5A and 5-5B. The tremendously wide ranges in the nitrate levels of certain vegetables are not due merely to diversity among extraction and other assay procedures. To a great extent, they reflect true variations in the nitrate content of different samples of the same type of vegetable. It is important to recognize that information on the nitrate content of vegetables without information on the content of various nitrosation inhibitors, especially ascorbic acid, may be misleading. Thus, later in this section, average concentrations of nitrate and ascorbate in various vegetables are compared.

During growth, the nitrate content of vegetables is affected most by nitrogen supply and light conditions (Corré and Breimer, 1979), although a number of factors may be involved (Corré and Breimer, 1979; Maynard, 1978; Maynard et al., 1976).

For example:

- Related plant strains (cultivars) systematically differ in nitrate content.
- Different levels and timing of nitrogen fertilizer application affect the nitrate content. Generally speaking, nitrate accumulation increases as the amount of nitrogen fertilizer used increases and if the fertilizer is applied shortly before harvest.
- Nitrate levels tend to increase as daytime temperatures drop below an optimal temperature. Thus, geographic region and season of harvest affect nitrate content.
- Greenhouse plants tend to accumulate higher levels of nitrate than do plants grown outdoors, perhaps because nitrogen fertilizers are used more heavily indoors.
- Plants grown in shade, at high latitudes with limited sunlight, and during a drought accumulate higher levels of nitrate than do plants grown under optimal conditions of water and light supply.
- Leafy plants harvested on a sunny afternoon often contain less nitrate than those harvested in the morning or during inclement weather.

TABLE 5-5A

Nitrate Content of Vegetables from Local Retail Markets

NOTE: Some of the concentrations have been rounded off to two significant figures.

| Vegetable | Average Concentration of Nitrate, mg/kg Fresh Weight, Except for Wilson, 1949 ^a | | | | | | Frozen (individual studies) | |
|----------------------|---|------------------------------|----------------------------|--------------------------------|---------------------|------------------------------|--------------------------------|------------------------------|
| | Fresh or Canned (individual studies) | | | | | | Jackson | Siciliano |
| | Richardson, 1907 | Wilson, 1949 ^a | Jackson et al., 1967 | Maynard and Barker, 1972 | Lee et al., 1972 | Siciliano et al., 1975 | Jackson et al., 1967 | Siciliano et al., 1975 |
| Asparagus | | 50 | | | | 3 | | 16 |
| Bean: dry (navy) | 68 | | | | | | | |
| green | 440 | | 250 | 150 | | 100 | 200 | 270 |
| lima | 310 | | 130 | | | | 88 | 27 |
| Beet | 2,600 | 1,300 | 1,700 | 2,600 | 2,400 | 3,000 | | |
| Broccoli | | 2,300 | | | 940 | | 550 | 510 |
| Brussels sprouts | | | | | | | | 84 |
| Cabbage | 200 | 1,200 | 320 | 720 | 910 | 780 | | |
| Carrot | 66 | 320 | 18 | 140 | 330 | 72 | 200 | 97 |
| Cauliflower | 230 | 2,000 | 53 | | 1,000 | | | 250 |
| Celery | 1,500 | 2,200 | 2,800 | 2,400 | 1,000 | 2,200 | | |
| Corn | 37 | | | | | | | 45 |
| Cucumber | 160 | | | | | 24 | | |
| Eggplant | 1,500 | | | | | 300 | | |
| Endive | | | | | | 60 | | |
| Kale/collard | | | 1,900 | | | 1,600 | 1,500 | 2,800 |
| Leek | 440 | | | | | | | |
| Lettuce ^b | 1,700 | 1,100 | 660 | 750 | 280 | 1,200 | | |
| Melon | 38 | 500 | | | | | | |
| Mushroom | | | | | | 63 | | |
| Okra | | | | | | 2 | | 70 |
| Onion | 230 | | 180 | 60 | | | | 80 |
| Parsley | 1,100 | | 1,700 | | | | | |
| Peas | 25 | | 40 | | | | 62 | 20 |
| Pepper: sweet | | | 200 | | | 62 | 130 | 50 |
| Potato: white | 77 | 63 | 57 | 190 | | 120 ^c | 130 | 150 |
| sweet | 66 | | 53 | 0 | | | | |
| Pumpkin or squash | 690 | | 300 | | | 460 | | 410 |
| Radish | 1,800 | 740 | 1,500 | 1,800 | | 2,700 | | |
| Rhubarb | | 3,200 | | | | | 390 | |
| Spinach ^b | 1,900 | 1,600 | 240 | 2,300 | 2,100 | 2,200 | 670 | 2,100 |
| Tomato | 120 | 0 | 72 | 89 | | | | |
| Turnip | 1,000 | | | | | | | |
| Turnip greens | | | | | | 2,200 | 1,600 | 3,500 |

^aExpressed as mg/liter (ppm) in juice, not as mg/kg fresh weight.^bLettuce and spinach varieties whose leaves have wrinkled edges often contain higher nitrate levels than smooth-edged varieties (Barker et al., 1974; Maynard and Barker, 1972, 1974; Olday et al., 1976), but exceptions occur (Maynard et al., 1976).^cData from Heisler et al., 1973.

TABLE 5-5B

Nitrate Content of Vegetables from Local Retail Markets

NOTE: Some of the concentrations have been rounded off to two significant figures

| Vegetable | Concentration of Nitrate, mg/kg Fresh Weight, Except for Column Entitled "Class" ^a | | | | | | |
|----------------------|---|--|------------------|---------------------------|---|--|---|
| | Averages | | | | Ranges | | |
| | Fresh, Canned and/or Frozen (literature surveys) | | | | Class ^a Corr ^e and Breimer, 1979 | Fresh, Canned, and/or Frozen (literature surveys) | |
| White, 1975 | FDA, 1979 | Corr ^e and Breimer, 1979 ^b | White, 1975 | Lee, 1970 ^c | | White, 1975 | Corr ^e and Breimer, 1979 |
| Asparagus | 21 | 200 | 66 | 1 | | 16-50 | 13-700 |
| Bean: dry (navy) | 13 ^d | 69 | | | | 13 ^d | |
| green | 250 | 330 | 430 | 3 | | 200-270 | 44-1,100 |
| lima | 54 | 220 | | | | 88-130 | |
| Beet | 2,800 | 2,700 | 2,100 | 5 | 600-4,500 | 1,300-3,000 | 100-8,100 |
| Broccoli | 780 | 1,900 | 700 | 2 | 400-860 | 510-2,300 | 140-1,300 |
| Brussels sprouts | | | 118 | | | | 0-170 |
| Cabbage | 640 | 550 | 400 ^e | 3 | 150-1,700 | 310-900 | 0-2,700 |
| Carrot | 120 | 130 | 280 | 3 | 50-500 | 18-340 | 0-2,800 |
| Cauliflower | 550 | 600 | 420 | 2 | 100-1,200 | 53-2,000 | 0-4,500 |
| Celery | 2,300 | 1,800 | 2,300 | 5 | 50-3,200 | 1,000-2,800 | 50-5,300 |
| Corn | 45 | | | | | 45 | |
| Cucumber | 24 | 160 | 190 | 2 | | 24 | 17-570 |
| Eggplant | 300 | 300 | 240 | 2 | | 300 | 180-300 |
| Endive | | 1,400 | 1,300 | 4 | | | 10-3,800 |
| Kale/collard | | 1,900 | 790 | 3 | 650-4,800 | | 30-5,500 |
| Leek | | | 510 | 4 | | | 36-4,500 |
| Lettuce ^f | 850 | 1,100 | 2,600 | 5 | 300-6,000 | 280-1,200 | 90-13,000 |
| Melon | 430 | 430 | 290 | 2 | | 430 | 40-600 |
| Mushroom | | 110 | 160 | 1 | | | 40-400 |
| Okra | | | | | | | |
| Onion | 130 | 160 | 200 | 2 | | 62-180 | 0-2,300 |
| Parsley | | 1,300 | 1,000 | 4 | | | 0-4,100 |
| Peas | 28 | | 27 | 1 | | 20-62 | 0-110 |
| Pepper: sweet | 120 | 170 | 120 | 1 | | 50-200 | 0-350 |
| Potato: white | 120 | 110 | 110 | 1 | | 57-190 | 0-1,000 |
| sweet | 53 | 64 | 39 | 1 | | 53 | 0-130 |
| Pumpkin or squash | 410 | 300 | 380 | 3 | 400-2,300 | 300-460 | 34-2,200 |
| Radish | | 1,700 | 1,900 | 5 | 850-6,500 | | 60-9,000 |
| Rhubarb | | 3,200 | 2,100 | 4 | | | 400-5,500 |
| Spinach ^g | 1,900 | 1,800 | 1,700 | 5 | 2,300-4,500 | 240-2,400 | 2-6,700 |
| Tomato | 62 | 64 | 53 | 1 | | 0-89 | 0-170 |
| Turnip | | 1,100 | 390 | 2 | 50-3,500 | | 10-2,900 |
| Turnip greens | | | 6,600 | 5 | | | 6,600 |

^aClassification of vegetables according to nitrate content of the fresh product: 1 = most values lower than 200 mg/kg; 2 = most values lower than 500 mg/kg; 3 = most values lower than 1,000 mg/kg; 4 = most values lower than 2,500 mg/kg; 5 = values frequently higher than 2,500 mg/kg. From Table 7 in Corr^e and Breimer, 1979.

^bAverages of average values given in Table 6 of Corr^e and Breimer (1979), fresh vegetables only.

^cData are from Smith, 1966 and Phillips, 1968a, as cited in Lee, 1970.

^dData from McNamara et al., 1971; see White, 1975.

^eExcluding Oxheart cabbage, which averages 1,529 mg/kg.

^fLettuce and spinach varieties whose leaves have wrinkled edges often contain higher nitrate levels than smooth-edged varieties (Barker et al., 1974; Maynard and Barker, 1972, 1974; Olday et al., 1976), but exceptions occur (Maynard et al., 1976).

- Some plant diseases, insect damage, or exposure to herbicides, such as those used in weed control, often increase nitrate accumulation.
- Soil deficiencies, such as insufficient molybdenum or potassium, or acidic, organically rich (peat) soils lead to elevated nitrate content.

The above factors increase nitrate levels in produce by affecting one or more processes. Most importantly, they affect nitrogen uptake, nitrogen transport, and nitrate reduction and assimilation. Although nitrate uptake by the roots is not strongly affected by photosynthesis, the reduction and assimilation of nitrate are closely coupled with photosynthesis, which occurs predominantly in the leaves of the plant. Photosynthesis is also required for the transfer of nitrate from its predominant location in storage vacuoles to an active metabolic pool capable of maintaining nitrate reductase levels (Aslam *et al.*, 1976; Ferrari *et al.*, 1973; Heimer and Filner, 1971; Martinoia *et al.*, 1981; Shaner and Boyer, 1976). Nitrate reductase is a molybdenum-containing, intricately regulated enzyme complex that affects nitrate assimilation (Beevers and Hageman, 1969; Haynes and Goh, 1978; Wright and Davison, 1964). Thus, perturbations in nitrate reductase activity, in photosynthesis, or in nitrogen uptake can lead to nitrate accumulation.

Factors affecting nitrate accumulation are not mutually exclusive and often operate in concert to increase nitrate levels. An extreme example of interactions, involving tomatoes grown under artificial circumstances, has been reported by Luh *et al.* (1973). Tomatoes from plants grown at daytime temperatures of 35°C stored nitrate at approximately 0.4 mg/kg fresh weight whether nitrogen fertilizer was added or not. Tomatoes from plants grown at daytime temperatures of 20°C stored nitrate at approximately 0.7 mg/kg when denied heavy fertilization, but stored about 73 mg/kg when supplied with excess nitrogen nutrition. Although such high levels may not be found in the field, this example illustrates the importance of multiple factors in producing wide variations among field-grown crops (Corré and Breimer, 1979; Maynard *et al.*, 1976). Thus, knowledge of how nitrate content is influenced by the above-mentioned factors can be used to lower the content of this ion in vegetables (Table 5-6). Other reviews also contain suggestions on how this may be accomplished (Hartman, 1981; National Academy of Sciences, 1978).

Li *et al.* (1980) and Schuphan (1974) have observed an inverse correlation between nitrate and ascorbate concentrations in vegetables. These observations support the earlier findings of Kilgore *et al.* (1964) who reported that turnip greens from unshaded plants exposed to normal levels of nitrogen-containing fertilizer contained nitrate at 1,593 mg/kg and ascorbate at 1,351 mg/kg, whereas shaded plants fertilized with an excess amount of nitrogen-containing fertilizer and sodium nitrate, contained nitrate at 4,707 mg/kg and ascorbate at 833 mg/kg (Kilgore *et al.*, 1964), resulting in nitrate: ascorbate ratios of 0.30 and 0.06, respectively.

TABLE 5-6

Procedures for Reducing Nitrate Concentrations in Spinach^a

NOTE: These concentrations have been converted from nitrate-nitrogen to nitrate ion and some have been rounded to two significant figures.

| Condition | Specific Adjustment | Nitrate Concentration, mg/kg | | | Reference |
|-------------|---|------------------------------|----------------------|---------------------------------------|---------------------------|
| | | Original Condition | Following Adjustment | Reduction in Nitrate Concentration, % | |
| | | <u>Dry-Weight Basis:</u> | | | |
| Cultivar | Use of smooth-leaved (cv. Tuftegard) instead of savoyed-leaved (cv. Bloomsdale) | 7,400 | 2,000 | 73 | Cantliffe, 1972 |
| Fertilizer | 50% NH ₄ -N and 50% NO ₃ -N, instead of 100% NO ₃ -N | 90,000 | 60,000 | 34 | Mills et al., 1976 |
| | Nitrapyrin used with 1:1 NH ₄ -N:NO ₃ -N fertilizer | 60,000 | 40,000 | 34 | Mills et al., 1976 |
| Light | Harvest after 12 hours of light instead of 0 hours | 5,100 | 3,700 | 27 | Minotti and Stankey, 1973 |
| | | <u>Fresh-Weight Basis:</u> | | | |
| Preparation | Petiole removal cv. America | 2,400 | 1,700 | 29 | Olday et al., 1976 |
| | cv. Hybrid 424 | 1,000 | 530 | 47 | Olday et al., 1976 |

^aData adapted from Maynard, 1978.

Maynard and Barker (1972) studied possible trends in the nitrate content of vegetables from 1907 to 1970, and concluded that their data "do not reflect any tendency for substantial change in the nitrate-nitrogen concentration of vegetables over the period studied." Although there are other possible explanations for the observed differences (e.g., differences in assay methods), Table 5-7, based on data from Corr  and Breimer, indicates that there may have been increases in the nitrate content of some vegetables, including carrots, lettuce, and spinach, during the past decade. If, in fact, such increases in the nitrate content have occurred, heavy use of nitrogen fertilizers, as well as the timing of application of the fertilizer, may have been a contributing factor.

TABLE 5-7

Comparison of the Nitrate Content of Fresh Market Vegetables
in the 1960's and the 1970's

NOTE: Some of these concentrations have been rounded off to two significant figures.

| Vegetable | Average Concentration (mg/kg) and Number (N) of Values That were Averaged | | | |
|-----------------|--|---------------|--------|---------------|
| | Corr  and Breimer (1979) | | 1970's | |
| | 1960's | | 1970's | |
| | N | Concentration | N | Concentration |
| Bean: green | 4 | 400 | 5 | 450 |
| Beet | 5 | 1,500 | 12 | 2,300 |
| Brussels sprout | 2 | 25 | 4 | 190 |
| Cabbage | 7 | 385 | 23 | 420 |
| Celery | 1 | 2,800 | 11 | 2,300 |
| Carrot | 5 | 130 | 19 | 330 |
| Cucumber | 3 | 240 | 8 | 180 |
| Endive | 3 | 1,100 | 18 | 1,300 |
| Leek | 3 | 450 | 6 | 560 |
| Lettuce | 5 | 1,100 | 33 | 2,800 |
| Pea | 2 | <5 | 3 | 41 |
| Pepper: sweet | 3 | 140 | 4 | 110 |
| Potato: white | 5 | 88 | 12 | 120 |
| Radish | 4 | 1,300 | 14 | 2,100 |
| Spinach | 11 | 1,100 | 34 | 1,900 |
| Tomato | 4 | 38 | 10 | 59 |

Despite the wide variations in the nitrate content of vegetables, the average levels reported by White (1975), which are based solely on the data presented in papers published between 1949 and 1975 (Table 5-5), are in accord with the averages from more than 400 sets of data compiled by Corré and Breimer (1979), which are also presented in Table 5-5, permitting classification of vegetables into five categories, according to nitrate accumulation.

To develop an estimate of nitrate in vegetables (Table 5-8), the committee averaged the estimates of White (1975) and Corré and Breimer (1979) given in Table 5-5. Thus, the committee's estimates reflect the large data base used by Corré and Breimer while giving some bias to U.S. products, which were surveyed by White. When one of the data bases failed to provide a figure, the committee used the average value from the other survey.

The estimates of White and those of Corré and Breimer rely primarily on nitrate levels measured in fresh vegetables (although White did include some processed vegetables in his averages). In general, the nitrate content of processed vegetables (canned or frozen) and of fresh, cooked vegetables is lower because nitrate stored in vacuoles is released during boiling into the surrounding fluid, resulting in reductions as high as 50% (Kenny and Walshe, 1975; Kilgore *et al.*, 1963; Krehl and Winters, 1950; Sohler *et al.*, 1976). The extent of these reductions depend on the amount of water used. Similar losses of vitamin C can also occur (Krehl and Winters, 1950). In industrial processing, the most important procedure affecting nitrate content is blanching, which usually decreases the nitrate (Corré and Breimer, 1979). Blanching of spinach can lead to a 30% decrease in nitrate content; however, more than 90% of the ascorbate content can be lost during this process (Schuphan, 1974). Additional industrial processing may decrease the nitrate content further. For example, there is a 40% to 50% reduction of nitrate in spinach following canning (Lee *et al.*, 1971; Sohler *et al.*, 1976). Freezing also appears to decrease the level of nitrate (Corré and Breimer, 1979). For nitrate-rich vegetables, Corré and Breimer (1979) estimated that a nitrate loss of 20% to 25% on a fresh weight basis was a reasonable average.

Quantitative determination of nitrite in vegetables is difficult because some of the nitrite is lost during extraction (Klepper, 1979). As a result, the reported data may underestimate the true nitrite content by a factor (approximately 2) that varies with each vegetable (Klepper, 1979). Although reports of the nitrite content of vegetables are sparse (Corré and Breimer, 1979), it is known that the concentration of nitrite in fresh market vegetables is generally low and usually does not exceed 1 to 2 mg/kg (Corré and Breimer, 1979). Older data reflect higher levels of nitrite -- from 4.3 to 76 mg/kg (Richardson, 1907), although the reasons for this are unknown.

The nitrite content of vegetables is known to increase with storage, especially at higher temperatures, due to reduction of nitrate to nitrite by nitrite reductase present in plant tissue and in contaminating bacteria (Corré and Breimer, 1979; Lee, 1970; Lin and Yen, 1980; Phillips, 1968a,b, 1971). This is especially true

TABLE 5-8

An Estimate of the Average Concentrations
of Nitrate and Nitrite in Vegetables

| Vegetable | Concentration, mg/kg (fresh weight) | |
|--------------------|-------------------------------------|----------------------|
| | Nitrate ^a | Nitrite ^b |
| Artichoke | 12 ^c | 0.4 |
| Asparagus | 44 | 0.6 |
| Bean: green | 340 | 0.6 |
| lima | 54 | 1.1 |
| dry (navy) | 13 | NR ^d |
| Beet | 2,400 | 4.0 ^e |
| Broccoli | 740 | 1.0 |
| Brussels sprouts | 120 | 1.0 |
| Cabbage | 520 | 0.5 |
| Carrot | 200 | 0.8 ^e |
| Cauliflower | 480 | 1.1 |
| Celery | 2,300 | 0.5 |
| Corn | 45 | 2.0 |
| Cucumber | 110 | 0.5 |
| Eggplant | 270 | 0.5 |
| Endive | 1,300 | 0.5 |
| Kale/collard | 800 | 1.0 |
| Leek | 510 | NR ^d |
| Lettuce | 1,700 | 0.4 |
| Melon | 360 | NR ^d |
| Mushroom | 160 | 0.5 ^e |
| Okra | 38 ^c | 0.7 |
| Onion | 170 | 0.7 |
| Parsley | 1,010 | NR ^d |
| Peas | 28 | 0.6 |
| Pepper: sweet | 120 | 0.4 |
| Potato: white | 110 | 0.6 |
| sweet | 46 | 0.7 |
| Pumpkin and squash | 400 | 0.5 |
| Radish | 1,900 | 0.2 |
| Rhubarb | 2,100 | NR ^d |
| Spinach | 1,800 | 2.5 ^e |
| Tomato | 58 | NR ^d |
| Turnip | 390 | NR ^d |
| Turnip greens | 6,600 | 2.3 |

^aData are primarily from fresh vegetables and are derived by averaging the data of White (1975, 1976) and Corr  and Breimer (1979).

^bData are primarily from processed vegetables, adapted from Siciliano et al. (1975).

^cData from Siciliano et al., 1975.

^dNR = No data reported.

^eAverage includes values for both fresh and processed vegetables.

of processed vegetables, probably since nitrate is released from vacuoles where it is normally sequestered (Martinoia *et al.*, 1981). The soluble nitrate is then available for reduction to nitrite. Thus, the nitrite content of processed and even frozen vegetables is often from two- to threefold higher than that of their unprocessed counterparts (Corré and Breimer, 1979; Siciliano *et al.*, 1975). Elevated levels are also characteristic of processed infant foods (Kamm *et al.*, 1965).

Average amounts of nitrite in fresh and/or processed vegetables are given in Table 5-8. On the basis of these data, Siciliano *et al.* (1975) concluded that the nitrite content of commercial fresh, frozen, or canned vegetables, as available to the consumer, is generally low (1.0 mg/kg or less). However, these investigators also pointed out that prolonged storage of open, thawed, cooked, or uncooked vegetables, or their storage under improper conditions, may lead to higher nitrite levels through the conversion of nitrate to nitrite.

The nitrite content is drastically increased, exceeding 100 mg/kg and reaching approximately 400 mg/kg, in vegetables pickled by fermentation, which is traditional in some areas of Japan and China (Matsui, 1944; Yanagihara *et al.*, 1963). Such foods do not constitute a significant source of nitrate or nitrite intake *per capita* in the United States, although they could be a substantial component of the diets of some ethnic groups within this country. The nitrite content of U.S.-style pickles does not seem to have been measured.

Another important factor affecting nitrite concentration is the level of ascorbate in vegetables since ascorbate can react with and eliminate nitrite (Lemoigne *et al.*, 1937; Mirvish *et al.*, 1972) (also see discussion in Chapters 4 and 6). Table 5-9 presents the ratio of ascorbate to nitrate, on a molar basis, for those vegetables listed in Table 5-9. The data in Table 5-9 indicate that the ratio of ascorbate to nitrate for one class of vegetables may vary by at least fivefold, depending upon plant growth conditions, and may vary more than 30-fold from one vegetable to another (e.g., celery *versus* artichoke). The low ascorbate concentration in carrots may explain why nitrite accumulates so readily in temperature-abused carrot juice (Hall *et al.*, 1977; Keating *et al.*, 1973), even though the nitrate content of carrots is generally not high (Table 5-9).

Plants also contain antioxidants other than ascorbate. For example, polyphenols occur in especially large amounts in many plants and plant products. Although most phenols inhibit nitrosation, some may enhance it (see Chapter 4).

Fruits and Fruit Juices: Nitrate and Nitrite. White (1975, 1976) reviewed the very limited data provided by three surveys of the nitrate and nitrite content of fruits and fruit juices (Achtzehn and Hawat, 1969; Richardson, 1907; Rooma, 1971). These data indicate that the nitrate content was low (10 mg/kg of fruit or 2 mg/liter of juice) and that the nitrite content was negligible.

TABLE 5-9

Ratios of Ascorbate to Nitrate for Selected Vegetables^a

NOTE: Some of the concentrations have been rounded off to two significant figures

| Vegetable | Nitrate, mg/kg ^b | Ascorbate, mg/kg ^c | Ascorbate, mg/kg ^d | Ascorbate, mg/kg ^e | Ratio of Ascorbate ^e to Nitrate, ^b mol/mol |
|--------------------|--------------------------------|----------------------------------|----------------------------------|----------------------------------|---|
| Artichoke | 12 | | 90 | 120 | 3.5 |
| Asparagus | 44 | | 840 | 330 | 2.7 |
| Bean: green | 340 | 90 | 760 | 190 | 0.20 |
| lima | 54 | | 290 | 290 | 1.9 |
| dry (navy) | 13 | | 60 | 0 | — |
| Beet | 2,400 | | 100 | 100 | 0.02 |
| Broccoli | 750 | | 1,100 | 1,100 | 0.50 |
| Brussels sprouts | 120 | 1,020 | 4,200 | 1,000 | 3.1 |
| Cabbage | 520 | | | 470 ^f | 0.32 |
| Carrot | 200 | 26 | 220 | 80 | 0.14 |
| Cauliflower | 480 | 520 | 780 | 780 | 0.57 |
| Celery | 2,300 | | 90 | 100 | 0.02 |
| Corn | 45 | | 120 | 120 | 0.94 |
| Cucumber | 110 | | 340 | 120 | 0.39 |
| Eggplant | 270 | | 50 | 50 | 0.06 |
| Endive | 1,300 | | 100 | 100 | 0.03 |
| Kale/collard | 800 | | 1,200 | 1,200 | 0.56 |
| Leek | 510 | | 180 | 170 | 0.12 |
| Lettuce | 1,700 | | 80 | 130 | 0.03 |
| Melon | 360 | | | 320 | 0.31 |
| Mushroom | 160 | | 50 | 30 | 0.07 |
| Okra | 38 | | | 310 | 3.0 |
| Onion | 170 | 90 | 100 | 100 | 0.21 |
| Parsley | 1,000 | 2,700 | 1,700 | 1,700 | 0.60 |
| Peas | 28 | | 220 | 270 | 3.4 |
| Pepper: sweet | 120 | 1,200 | 1,300 | 1,300 | 3.7 |
| Potato: white | 110 | 110 | 730 | 200 ^h | 0.62 |
| sweet | 46 | | 210 | 210 | 1.6 |
| Pumpkin and squash | 400 | 105 | 140 | 150 | 0.13 |
| Radish | 1,900 | | 260 | 260 | 0.05 |
| Rhubarb | 2,100 | | 90 | 90 | 0.02 |
| Spinach | 1,800 | 840 | 510 | 510 | 0.10 |
| Tomatoes | 60 | | 200 | 230 | 1.4 |
| Turnip | 390 | | 360 | 360 | 0.33 |
| Turnip greens | 6,600 | | 1,400 | 1,400 | 0.07 |

^aAscorbate levels are for fresh, uncooked produce as purchased. Ascorbate loss during cooking varies with the method, but generally ranges from 15% to 60% (Diem and Lentner, 1970; Pennington and Church, 1980).

^bData from Table 5-8.

^cData from Kelly and Latsko, 1980.

^dData from Diem and Lentner, 1970.

^eData from Pennington and Church, 1980.

^fAscorbate concentrations average 510 mg/kg for freshly harvested, 420 mg/kg for stored, cabbage.

^gAverage of canteloupe, honeydew, and watermelon.

^hValues range from about 260 mg/kg in recently harvested potatoes to about 120 mg/kg after 3 months storage and about 80 mg/kg after 6 months storage (Pennington and Church, 1980).

Herrmann (1972) summarized data from five other surveys of the nitrate content of fruit and fruit juices. He reported that strawberries occasionally contained more than 100 mg/kg and grapes occasionally reached 17 mg/kg, but most concentrations in oranges, apples, pears, strawberries, sweet and sour cherries, and grapes ranged from 0 to less than 10 mg/kg.

Huguet *et al.* (1976) found pears to contain nitrate at 34 mg/kg (or mg/liter of juice), whereas cherries and apples contained 24 mg/kg. These levels were essentially unaffected by fertilizer usage. Different varieties may have different nitrate levels, but the significance of these differences is difficult to assess from the limited data presented (Huguet *et al.*, 1976). Kenny and Walshe (1975) detected no nitrate in Golden Delicious apples. Nitrite has been measured less frequently. In one report, Harada *et al.* (1972) detected less than 1 mg/kg of nitrite in apples, oranges, and other fruits.

Because of the higher levels of nitrate in fruit reported in studies that were not included in White's survey, the committee has used an estimate of twice the amount used by White--20 mg/kg nitrate--and assumed as did White that the nitrite content of fruits is negligible (Table 5-3).

Baked Goods and Cereals: Nitrate and Nitrite. The accumulation of nitrate in grains is increased by many of the same factors that influence the accumulation of nitrate in vegetables (see section on vegetables above and the detailed discussion in Corr e and Breimer, 1979); however, accumulation is generally less in grains than in stems and in leaves (Hanway *et al.*, 1963; Wu and McDonald, 1976). The nitrate and nitrite content of cereals can also increase during drying if internal-combustion-type dryers are used (Fornal *et al.*, 1975). White (1975) drew attention to the absence of reliable data concerning nitrate and nitrite contents of U.S. baked goods, a situation that prevails today. He did report that bread contained nitrate at approximately 20 mg/kg and nitrite at approximately 0.17 mg/kg, based on two limited surveys (Richardson, 1907; Rooma, 1971).

Studies by McNamara *et al.* (1971) indicate that the nitrate content of winter wheat seeds varies with strain and growth conditions (0.4 to 11 mg/kg), indicating that a reasonable median for nitrate in winter wheat might be approximately 2 mg/kg. Similar data were obtained in studies of a variety of wheat cultivars (Wu and McDonald, 1976). These investigators reported nitrate concentrations of approximately 9 to 15 mg/kg for wheat and 4 to 14 mg/kg for wheat flour. Selenka and Brand-Grimm (1976) found that wheat flour contained nitrate at approximately 1 mg/kg and nitrite at 1.2 mg/kg, but white bread and flour after baking contained nitrate at an average of 13 mg/kg and nitrite at 3.4 mg/kg. Darker breads formulated with rye (e.g., rye bread and pumpernickel) averaged nitrate concentrations of 20 mg/kg and nitrite at 4.3 mg/kg. Harada *et al.* (1972) reported that flours contain approximately 3 mg/kg nitrite, whereas bread crumbs, noodles, and macaroni contain concentrations between 10 and 16 mg/kg. Kamm *et al.* (1965) has reported the nitrate concentration in graham crackers to be 211 mg/kg.

To estimate the nitrate and nitrite content of baked goods and cereals, the committee averaged the data presented above for nitrate (excluding graham crackers) to obtain an average of 12 mg/kg. For nitrite, the value for white and dark breads and White's earlier estimate were averaged to obtain a concentration of 2.6 mg/kg (Table 5-3).

Milk and Milk Products: Nitrate and Nitrite. When White (1975) estimated the nitrate intake from milk, he used data from Hänni (1954), Davis and MacDonald (1953), and Sander (1967), which indicated that milk contained nitrate in concentrations less than 1 mg/liter. In 1964, Wright and Davison reported that milk from control cows in a nitrate feeding study contained 4.8 mg/liter. Recent data from Denmark further suggest that levels of nitrate in milk may be higher than previously believed, and the State Food Institute (Statens Levnedsmiddelinstitut, 1981) has estimated that the average nitrate concentration of milk and milk products is 8 mg/liter.

The concentrations of both nitrate and nitrite ions may reflect the nitrate content of feed and forage available to the ruminant or the nitrate intake of the nursing mother. There is a trend toward the use of increasing amounts of nitrogen fertilizer, which is capable of increasing the nitrate load of forage crops (ap Griffith, 1958, 1960; Whitehead et al., 1978). Very high nitrate/nitrite levels can be reached under certain conditions, sometimes leading to nitrite intoxication in livestock (Case, 1957; Hanway et al., 1963; Lorenz, 1978; Whitehead et al., 1978; Wright and Davison, 1964). Although the nitrate content of milk generally does not increase drastically when cows eat fodder or drink water containing high levels of nitrate (Cefalu, 1954; Hänni, 1954; Wright and Davison, 1964), a fourfold increase of nitrate, from 4.8 mg/liter to approximately 20 mg/liter, was observed in one case when nitrate intake was high (Wright and Davison, 1964). In addition, nitrate levels in dried milk increase when direct-firing processes are used (Manning et al., 1968; Rammell and Joerin, 1972).

In some cheeses, nitrate concentrations averaged from 7 to 28 mg/kg (Pedersen et al., 1980; Woerner and Fricker, 1960) and nitrite concentrations averaged from approximately 1.2 to 1.6 mg/kg (Harada et al., 1972; Woerner and Fricker, 1960) after potassium nitrate had been added during processing to prevent bacterial growth. However, a recent report on the nitrate and nitrite content of Danish cheeses indicates that comparable levels (10 mg/kg nitrate; 0.2 mg/kg nitrite) are found in cheese regardless of whether nitrate has been added (Statens Levnedsmiddelinstitut, 1981).

In the absence of more definitive data on U.S. products, the committee has used the same estimate for nitrate (0.5 mg/liter) and nitrite (negligible levels) as White (1975) did to calculate the contribution of milk and milk products to human exposure (Table 5-3).

Water

Nitrate and Nitrite. The nitrate content of water is an important factor in determining the exposure of humans to nitrate because of the large quantity consumed daily and the apparently increasing concentrations of nitrate in U.S. drinking water. Concentrations of nitrate and nitrite in surface water and groundwater are dependent upon geochemical conditions and agricultural run-off as well as upon management practices in the treatment of waste from humans and animals (World Health Organization, 1978), including municipal and industrial wastewaters, refuse dumps, animal feedlots, and septic tanks (Emerick, 1974; National Academy of Sciences, 1977a).

Gradual increases in nitrate levels in many surface and groundwater sources of drinking water have been reported. Concentrations of nitrate in major U.S. rivers, recorded by the U.S. Geological Survey, indicate trends toward increasing nitrate concentrations in the Delaware, San Joaquin, Ohio, Mississippi, and Wabash Rivers (National Academy of Sciences, 1977a). Figure 5-1 shows nitrate concentrations in the Sangamon River, a source of drinking water in central Illinois. At times during 4 months of 1971, Sangamon River water contained nitrate concentrations close to 200 mg/liter. This high level probably resulted from fertilizer run-off (Commoner, 1977; Klepper, 1978; Kohl et al., 1971).

The amount of fertilizer used is not the sole factor affecting nitrate run-off from agricultural practices. In some areas, irrigation practices play an important role as well (Saffigna and Keeney, 1977; Spalding et al., 1980). Furthermore, an estimated 1.6 billion metric tons of animal excreta annually contribute nitrate to soils and waters in the United States (Garman, 1969).

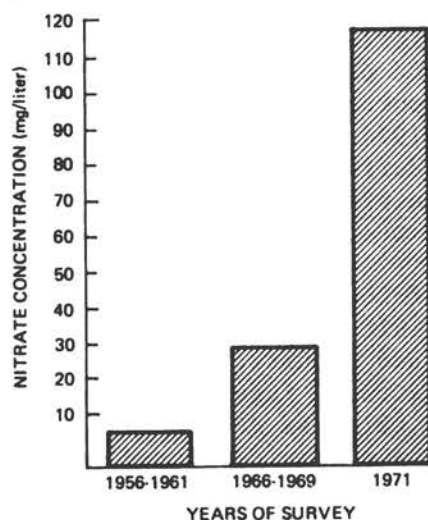


FIGURE 5-1. Nitrate concentration in the Sangamon River from 1956 to 1971. Based on data from Kohl et al., 1971.

The nitrate content of U.S. drinking water supplies varies greatly. White (1975) obtained his estimates from a 1962 survey of the water supplies of the 100 largest cities in the United States (Durfor and Becker, 1964), in which the average concentration was 0.66 mg/liter. However, many sources of drinking water have been reported to contain higher concentrations of nitrate. The Safe Drinking Water Committee (National Academy of Sciences, 1977a) cited several examples of water supplies containing nitrate in concentrations exceeding 44 mg/liter. The source of most of these supplies was well water, although municipal supplies were also implicated. Table 5-10 presents data from additional surveys of water with high nitrate content.

In estimating the intake from water, the committee has used the 1962 survey of Durfor and Becker (1964), as did White (1975). However, it has increased the average to 1.3 mg/liter because of the data just presented, which demonstrate increases in nitrate concentration since 1962 and higher concentrations in smaller water supplies. The committee recognizes that this average concentration underestimates the exposure of persons living in a region with high-nitrate drinking water. Therefore, it has also estimated the intake from water with a nitrate concentration of 100 mg/liter (comparable to the average amount measured in the Sangamon River). Nitrite content is considered to be negligible (Table 5-3); however, this may not be true in regions of other countries where water with high levels of nitrate is stored, resulting in reduction of nitrate to nitrite and subsequent ingestion of high nitrite levels (Li et al., 1980).

Tobacco

Nitrate. Tobacco is rich in nitrate, the amount depending on the strain. The highest concentrations of nitrate are found in the Burley and Maryland varieties. The amount and type of fertilizer, location of leaves on the plant, and curing process (air-cured versus flue-cured) are also important determinants of nitrate content of tobacco (Fuqua et al., 1974; Mizusaki et al., 1977a,b; Sims et al., 1970, 1979; Wynder and Hoffmann, 1968). Many other factors that influence the accumulation of nitrate in vegetables (discussed earlier) probably also influence the nitrate content of tobacco leaves.

The contribution of tobacco to the exposure of humans to nitrate and/or nitrite is difficult to determine because tobacco is used in many different ways and amounts. For example, individuals who chew tobacco probably extract, ingest, and retain large amounts of nitrate. Exposure from this use does not seem to have been investigated and no estimates have been developed by the committee. On the other hand, although cigarette smokers do not directly ingest tobacco nitrate as nitrate, reliable data on intake indicate that nitrogen oxides formed during tobacco combustion are inhaled. See section below on nitrogen oxides from tobacco.

TABLE 5-10

**Examples of High Nitrate Concentrations Found in
U.S. Drinking Water Supplies**

| <u>Year(s)</u> | <u>Nitrate Concentration, mg/liter</u> | <u>No. of Water Supply Systems Containing These Concentrations/ Total</u> | <u>Reference</u> |
|----------------|--|---|-------------------------------|
| 1942-1974 | > 44 | 79/1467 ^a | Woll, 1978 |
| 1945-1951 | 4-40 | 11 ^b | Harmeson <u>et al.</u> , 1971 |
| 1950 | > 44 | 183/389 ^c | Bosch <u>et al.</u> , 1950 |
| 1950 | >440 | 51/389 ^c | Bosch <u>et al.</u> , 1950 |
| 1950 | > 22 | 28/514 ^d | Bosch <u>et al.</u> , 1950 |
| 1950 | > 44 | 16/514 ^d | Bosch <u>et al.</u> , 1950 |
| 1960-1961 | > 21 | 182/800 ^e | Anonymous, 1969 |
| 1960-1961 | > 45 | 88/800 ^e | Anonymous, 1969 |
| 1963 | > 45 | 4/789 ^f | Larson, 1963; |
| 1965 | > 45 | 37 ^g | Ridder and Oehme, 1974 |
| 1965 | > 90 | 3 ^g | Ridder and Oehme, 1974 |
| 1966 | > 44 | 481/8844 ^b | Harmeson <u>et al.</u> , 1971 |
| 1966-1970 | 11-90 | 30 ^h | Larson and Henley, 1966 |
| 1969 | > 45 | 19/969 ⁱ | McCabe <u>et al.</u> , 1970 |

^aSurvey of groundwater samples in Maryland.

^bMaximum nitrate runoff from selected watersheds in Illinois.

^cSurvey of wells in Minnesota, including 139 wells supplying families for which cases of methemoglobinemia had been reported.

^dSurvey of municipal water supplies in Minnesota.

^eSurvey of wells in southern California.

^fSurvey of public groundwater supplies in Illinois.

^gSurvey of municipal water supplies in Kansas.

^hSurvey of wells in Illinois.

ⁱSurvey of public water supplies throughout the United States.

Air

Atmospheric Concentrations of Nitrogen Oxides. The most complete and authentic source of data on nitrogen oxide concentrations is the National Aerometric Data Bank of the U.S. Environmental Protection Agency (EPA). This data bank receives inputs from the National Air Sampling Network (NASN) as well as from other state and local sources. The NASN is comprised of approximately 100 sites at which nitrogen dioxide and sulfur dioxide are monitored. Until recently, it also included six Continuous Air Monitoring Project (CAMP) stations. Additional data have been provided by more localized studies, such as the Chattanooga Study (Helms et al., 1970) and the California Air Resources Board (1974), which issued a 10-year summary of data gathered through its large network of monitoring stations.

The distribution of nitrogen oxides in the atmosphere is by no means uniform. Localized concentrations often exceed the "average" concentration by a factor as high as 100 (U.S. Environmental Protec-

tion Agency, 1971a). These concentrations are located in urban areas where automotive traffic is heavy and in major industrial areas where nitric acid plants and uncontrolled stationary combustion sources produce oxides of nitrogen. The effect of these sources on pollutant concentrations is largely determined by the movement of the air mass containing the pollutants.

Geographic and meteorologic factors can combine to amplify the effect of man-made emissions. For example, in the calm air mass of the Los Angeles basin, both horizontal and vertical movements of the air mass are minimal. Under these conditions, nitrogen oxides build up, and nitric oxide is converted to the more harmful nitrogen dioxide.

Because a large portion of urban nitrogen oxides is generated by human activity, variations in oxide concentrations correlate directly with such activities. The major variable is vehicular traffic. Periods of heavy traffic, such as morning and evening rush hours, produce correspondingly high concentrations of nitric oxide. During slack periods of traffic, such agents as breezes and sunlight disperse, convert, or otherwise reduce these high concentrations. These competing factors create typical daily patterns in nitrogen oxide concentrations.

Tables 5-11 and 5-12 show typical nitric oxide and NO_x (nitric oxide plus nitrogen dioxide) levels in California from 1963 to 1972. Virtually all of the data were collected with the original Greiss-Saltzman procedure (Saltzman, 1954) (see methods section of this chapter). Table 5-13 provides the nitrogen oxide concentrations in 47 U.S. cities. These data were accumulated with the chemiluminescence and sodium arsenite methods. Although the results of the two methods are similar, they cannot be compared closely because sampling locations and analysis periods were different. Nonetheless, the data are probably typical of the nitrogen dioxide concentrations in the various cities. Thus, nitrogen dioxide levels are generally below the national air quality standard: an annual arithmetic mean of 0.05 ppm ($\approx 90 \mu\text{g}/\text{m}^3$) (U.S. Environmental Protection Agency, 1971b). However, at peak periods, urban air can contain nitrogen oxides in concentrations as high as 1 ppm. To estimate average exposure, the committee averaged the concentrations for nitrogen dioxide given in Table 5-13 for chemiluminescence assays to give a value of $58 \mu\text{g}/\text{m}^3$. For high nitrogen oxide intakes, the committee has used $118 \mu\text{g}/\text{m}^3$, the value for Los Angeles.

Concentrations of Nitrogen Oxides in Indoor Air. Indoor combustion sources give rise to high concentrations of a number of pollutants, especially carbon monoxide and nitrogen dioxide. Space heaters and water heaters appear to emit pollutants into building interiors only when their design is faulty or when the appliance has not been maintained properly. On the other hand, gas stoves almost always contribute significantly to indoor carbon monoxide and NO_x levels that sometimes exceed the federal ambient air quality standard.

TABLE 5-11

Hourly Average Concentration of Nitric Oxide in California^a

| Location | Average Concentration, $\mu\text{g}/\text{m}^3$, 25° C | | | | | | | | | | | | | | | | | | | |
|-------------------|---|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|
| | 1963 | | 1964 | | 1965 | | 1966 | | 1967 | | 1968 | | 1969 | | 1970 | | 1971 | | 1972 | |
| | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max |
| Anaheim | 26 | 360 | 31 | 370 | 31 | 860 | 26 | 490 | 50 | 810 | 31 | 1,600 | 74 | 1,190 | 59 | 1,090 | 80 | 1,000 | 91 | 1,050 |
| Azusa | 25 | 520 | 23 | 260 | 20 | 290 | 21 | 320 | 25 | 490 | 37 | 360 | 26 | 430 | 42 | 500 | 43 | 500 | 42 | 790 |
| Burbank | 124 | 1,400 | 105 | 890 | 123 | 1,260 | 115 | 1,110 | 127 | 1,110 | 131 | 1,620 | 156 | 1,780 | 167 | 1,350 | 160 | 1,370 | 150 | 1,130 |
| Fresno | 13 | 1,070 | 23 | 580 | 18 | 640 | 16 | 660 | 21 | 730 | 21 | 780 | 23 | 1,050 | — | — | — | — | — | — |
| La Habra | — | — | — | — | — | — | — | — | — | — | 62 | 1,130 | 41 | 570 | 42 | 620 | 58 | 583 | 68 | 980 |
| Lennox | — | — | — | — | 127 | 1,250 | 148 | 2,160 | 181 | 2,220 | 166 | 1,660 | 157 | 2,050 | 176 | 2,270 | 181 | 2,340 | 172 | 1,870 |
| Long Beach | 134 | 1,140 | 140 | 1,540 | 117 | 920 | 123 | 970 | 140 | 1,280 | 143 | 1,170 | 130 | 1,160 | 148 | 1,960 | 118 | 1,070 | 128 | 1,520 |
| Los Angeles | | | | | | | | | | | | | | | | | | | | |
| Downtown | 121 | 1,050 | 98 | 1,600 | 101 | 1,410 | 112 | 1,020 | 119 | 1,410 | 120 | 1,360 | 119 | 1,490 | 130 | 1,600 | 139 | 1,410 | 122 | 1,130 |
| USC | 69 | 700 | 71 | 1,000 | 70 | 740 | 73 | 850 | 74 | 750 | — | — | — | — | — | — | — | — | — | — |
| West LA | 101 | 1,050 | 89 | 1,170 | 92 | 1,290 | 83 | 1,300 | 88 | 1,330 | 92 | 1,050 | 89 | 1,840 | 108 | 1,360 | 111 | 1,720 | 91 | 1,120 |
| Oakland | 58 | 1,140 | 90 | 1,140 | 58 | 810 | 60 | 840 | 60 | 1,120 | 55 | 910 | — | — | — | — | — | — | — | — |
| Oakland-Jackson | — | — | — | — | — | — | — | — | — | — | — | — | 43 | 850 | 40 | 710 | 44 | 1,050 | 45 | 660 |
| Pasadena | — | — | — | — | — | — | — | — | — | — | 81 | 820 | 71 | 820 | 84 | 980 | 69 | 690 | 89 | 860 |
| Villa Street | 70 | 850 | 63 | 760 | 50 | 520 | 60 | 610 | 66 | 650 | 57 | 550 | — | — | — | — | — | — | — | — |
| Pomona | — | — | — | — | 68 | 580 | 75 | 550 | 95 | 760 | 105 | 910 | 103 | 900 | 118 | 810 | 121 | 780 | 116 | 820 |
| Redlands | — | — | — | — | — | — | — | — | — | — | 22 | 460 | 20 | 210 | 22 | 1,170 | 32 | 1,760 | 43 | 650 |
| Redwood City | — | — | — | — | — | — | — | — | 42 | 530 | 54 | 590 | 47 | 630 | 42 | 580 | 41 | 780 | 48 | 590 |
| Richmond | — | — | — | — | — | 62 | 910 | 46 | 710 | 60 | 500 | 52 | 870 | 32 | 820 | 31 | 730 | 65 | 490 | — |
| Riverside | — | — | 57 | 1,350 | 46 | 700 | 46 | 530 | 53 | 640 | — | — | — | — | — | — | — | — | 41 | 680 |
| Sacramento | 48 | 1,330 | 49 | 1,330 | 41 | 1,190 | 44 | 920 | 42 | 1,110 | 33 | 1,140 | 33 | 1,140 | 35 | 750 | 33 | 780 | — | — |
| San Bernardino | 11 | 310 | 11 | 310 | 27 | 420 | 39 | 620 | 41 | 440 | 42 | 360 | 26 | 260 | 39 | 280 | 52 | 490 | 41 | 630 |
| San Diego | 44 | 910 | 30 | 1,350 | 38 | 1,110 | 62 | 1,480 | 43 | 980 | 57 | 1,350 | 38 | 1,110 | 36 | 860 | 81 | 2,150 | 66 | 1,300 |
| San Francisco | | | | | | | | | | | | | | | | | | | | |
| Ellis Street | — | — | — | — | — | — | — | — | — | — | 70 | 1,600 | 38 | 740 | 58 | 630 | 48 | 690 | 68 | 620 |
| Union Square | 102 | 1,590 | 103 | 950 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Mission Street | — | — | — | — | 52 | 760 | — | — | 49 | 1,090 | 63 | 1,670 | — | — | — | — | — | — | — | — |
| Santa Cruz County | — | — | — | — | — | 9 | 160 | 6 | 160 | 7 | 200 | 7 | 250 | — | — | — | — | — | — | — |
| Stockton | — | — | 39 | 620 | 32 | 590 | 41 | 1,070 | 16 | 440 | 25 | 620 | 26 | 640 | 22 | 700 | 25 | 700 | 30 | 570 |

^aTable from National Academy of Sciences, 1977b. Based on data from State of California Air Resources Board, 1966.

TABLE 5-12

Hourly Average Concentrations of NO_x in California^a

Average Concentration, ppm

| Location | 1963 | | 1964 | | 1965 | | 1966 | | 1967 | | 1968 | | 1969 | | 1970 | | 1971 | | 1972 | |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max | Mean | Max |
| Anaheim | 0.06 | 0.42 | 0.06 | 0.35 | 0.06 | 0.80 | 0.06 | 0.53 | 0.08 | 0.76 | 0.12 | 1.45 | 0.11 | 1.10 | 0.10 | 1.05 | 0.12 | 1.18 | 0.12 | 1.02 |
| Azusa | 0.06 | 0.48 | 0.07 | 0.28 | 0.06 | 0.35 | 0.06 | 0.48 | 0.07 | 0.52 | 0.08 | 0.45 | 0.07 | 0.53 | 0.10 | 0.56 | 0.10 | 0.54 | 0.10 | 0.77 |
| Burbank | 0.16 | 1.20 | 0.14 | 0.83 | 0.17 | 1.18 | 0.16 | 1.02 | 0.19 | 1.08 | 0.25 | 1.72 | 0.22 | 1.73 | 0.23 | 1.30 | 0.22 | 1.22 | 0.20 | 1.16 |
| Fresno | 0.03 | 0.93 | 0.04 | 0.52 | 0.04 | 0.55 | 0.04 | 0.60 | 0.04 | 0.66 | 0.05 | 0.70 | 0.05 | 0.94 | — | — | — | — | — | — |
| La Habra | — | — | — | — | — | — | — | — | — | — | 0.10 | 0.96 | 0.07 | 0.52 | 0.06 | 0.60 | 0.10 | 0.65 | 0.11 | 1.05 |
| Lennox | — | — | — | — | 0.17 | 1.21 | 0.18 | 1.95 | 0.23 | 2.10 | 0.21 | 1.66 | 0.19 | 1.86 | 0.21 | 2.15 | 0.22 | 2.21 | 0.21 | 1.71 |
| Long Beach | 0.17 | 1.14 | 0.18 | 1.36 | 0.15 | 0.85 | 0.16 | 0.85 | 0.19 | 1.24 | 0.20 | 1.20 | 0.18 | 1.15 | 0.20 | 1.94 | 0.16 | 1.05 | 0.17 | 1.50 |
| Los Angeles | | | | | | | | | | | | | | | | | | | | |
| Downtown | 0.16 | 1.11 | 0.14 | 1.46 | 0.16 | 1.35 | 0.17 | 0.98 | 0.17 | 1.34 | 0.17 | 1.27 | 0.16 | 1.41 | 0.18 | 1.50 | 0.20 | 1.42 | 0.18 | 1.16 |
| USC | 0.12 | 0.88 | 0.12 | 0.89 | 0.13 | 0.82 | 0.12 | 0.86 | 0.13 | 0.84 | — | — | — | — | — | — | — | — | — | — |
| Oakland | 0.10 | 0.95 | 0.11 | 1.26 | 0.08 | 0.74 | 0.08 | 0.79 | 0.09 | 1.06 | 0.08 | 0.85 | — | — | — | — | — | — | — | — |
| Oakland-Jackson | — | — | — | — | — | — | — | — | — | — | — | — | 0.07 | 0.70 | 0.07 | 0.66 | 0.08 | 0.96 | 0.07 | 0.67 |
| Pasadena | — | — | — | — | — | — | — | — | — | — | 0.15 | 1.03 | 0.14 | 1.19 | 0.16 | 1.20 | 0.13 | 0.86 | 0.16 | 0.94 |
| Villa St. | 0.11 | 0.79 | 0.11 | 0.70 | 0.10 | 0.60 | 0.11 | 0.78 | 0.12 | 0.71 | 0.10 | 0.58 | — | — | — | — | — | — | — | — |
| Pomona | — | — | — | — | 0.12 | 0.59 | 0.12 | 0.59 | 0.15 | 0.67 | 0.16 | 0.84 | 0.16 | 0.89 | 0.18 | 0.81 | 0.18 | 0.80 | 0.17 | 0.94 |
| Redlands | — | — | — | — | — | — | — | — | — | — | 0.04 | 0.43 | 0.03 | 0.22 | 0.05 | 1.00 | 0.07 | 1.51 | 0.07 | 0.60 |
| Redwood City | — | — | — | — | — | — | — | — | 0.07 | 0.49 | 0.07 | 0.52 | 0.07 | 0.70 | 0.07 | 0.58 | 0.06 | 0.74 | 0.07 | 0.55 |
| Richmond | — | — | — | — | — | 0.08 | 0.80 | 0.07 | 0.90 | 0.09 | 0.55 | 0.08 | 0.80 | 0.06 | 0.72 | 0.05 | 0.63 | 0.08 | 0.44 | — |
| Riverside | — | — | 0.09 | 1.16 | 0.09 | 0.76 | 0.08 | 0.55 | 0.09 | 0.67 | — | — | — | — | — | — | 0.04 | 0.20 | 0.06 | 0.60 |
| Sacramento | 0.08 | 1.19 | 0.81 | 1.22 | 0.07 | 1.05 | 0.07 | 1.00 | 0.07 | 1.14 | 0.05 | 1.06 | 0.06 | 1.03 | 0.06 | 0.72 | 0.05 | 0.74 | — | — |
| San Bernardino | 0.02 | 0.25 | 0.01 | 0.25 | 0.05 | 0.39 | 0.07 | 0.57 | 0.08 | 0.48 | 0.07 | 0.36 | 0.06 | 0.33 | 0.08 | 0.30 | 0.08 | 0.48 | 0.08 | 0.58 |
| San Diego | 0.06 | 0.96 | 0.04 | 1.18 | 0.05 | 0.98 | 0.08 | 1.42 | 0.05 | 0.84 | 0.07 | 1.66 | 0.05 | 0.94 | 0.05 | 0.73 | 0.10 | 1.82 | 0.09 | 1.14 |
| San Francisco | | | | | | | | | | | | | | | | | | | | |
| Ellis Street | — | — | — | — | — | — | — | — | — | — | 0.10 | 1.42 | 0.07 | 0.64 | 0.09 | 0.62 | 0.07 | 0.70 | 0.09 | 0.58 |
| Union Square | 0.13 | 1.34 | 0.14 | 0.93 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Mission Street | — | — | — | — | 0.08 | 0.71 | — | — | 0.07 | 0.97 | 0.09 | 1.50 | — | — | — | — | — | — | — | — |
| Santa Cruz County | — | — | — | — | — | — | 0.03 | 0.17 | 0.02 | 0.20 | 0.02 | 0.22 | 0.02 | 0.23 | — | — | — | — | — | — |
| Stockton | — | — | 0.06 | 0.68 | 0.05 | 0.53 | 0.05 | 0.90 | 0.04 | 0.42 | 0.05 | 0.60 | 0.06 | 0.62 | 0.04 | 0.62 | 0.04 | 0.68 | 0.05 | 0.55 |

^aTable from National Academy of Sciences, 1977b. Based on data from State of California Air Resources Board, 1966.

TABLE 5-13

Concentrations of Nitrogen Dioxide in Various Cities, Determined by
Sodium Arsenite and Chemiluminescence Methods^a

| Region | Average Concentration of Nitrogen Dioxide for Period of Operation, $\mu\text{g}/\text{m}^3$, 25°C | |
|--|--|-----------------------------|
| | Sodium Arsenite Method ^b | Chemiluminescence Method |
| Atlanta | 80 | 62 |
| Baltimore | 96 | 64 |
| Boston | 74 | — |
| Chattanooga | 53 | 38 |
| Chicago ^c | 117 | 121 |
| Cincinnati | 73 | 61 |
| Cleveland | 57 | 53 |
| Columbus | 68 | 52 |
| Corpus Christi-Victoria | 43 | 43 |
| Dallas-Fort Worth | 76 | 47 |
| Dayton | 64 | 53 |
| Denver ^c | 42 | 110 |
| Detroit-Port Huron | 80 | 60 |
| Dubuque | 30 | 23 |
| Florida, Southeast (Miami) | 55 | 53 |
| Florida, West Central (Tampa) | 56 | 52 |
| Four Corners | 30 | — |
| Genesee-Finger Lakes (Rochester) | 48 | 26 |
| Hampton Roads (Norfolk) | 52 | 39 |
| Hartford-New Haven-Springfield | 82 | 73 |
| Houston-Galveston | 64 | 66 |
| Indianapolis | 61 | 56 |
| Los Angeles | 182 | 118 |
| Louisville | 87 | 68 |
| Massachusetts, Central (Worcester) | 71 | — |
| Memphis | 64 | 31 |
| Michigan, Central (Grand Rapids) | 59 | 44 |
| Minneapolis-St. Paul | 31 | 47 |
| National Capital ^c | 88 | 64 |
| New York-New Jersey-Connecticut | 100 | 65 |
| Niagara Frontier (Buffalo) | 32 | 49 |
| Omaha-Council Bluffs | 60 | 30 |
| Pennsylvania, Central (Johnstown) | 25 | 64 |
| Pennsylvania, South Central (Lancaster) | 60 | 36 |
| Pennsylvania, Southwest (Pittsburgh) | 78 | 64 |
| Pennsylvania-Upper Delaware Valley, Northeast (Reading) | 52 | 60 |
| Philadelphia | 83 | 84 |

TABLE 5-13 (Continued)

| Region | Average Concentration of Nitrogen Dioxide for Period of Operation, $\mu\text{g}/\text{m}^3$, 25°C | |
|----------------------------------|--|--------------------------|
| | Sodium Arsenite Method ^b | Chemiluminescence Method |
| Phoenix-Tucson | 80 | 69 |
| Providence | 45 | -- |
| Puget Sound (Seattle) | 47 | 51 |
| San Diego | 63 | 76 |
| San Francisco Bay Area | 85 | 84 |
| St. Louis | 79 | 58 |
| State Capital (Richmond) | 58 | 37 |
| Toledo | 54 | 38 |
| Wasatch Front (Salt Lake City) | 62 | 114 |
| Wisconsin, Southeast (Milwaukee) | 76 | -- |

^aTable adapted from National Academy of Sciences, 1977b, pp. 86-87. Based on data from U.S. Environmental Protection Agency, 1973.

^bConcentrations corrected to reflect 85% collection efficiency. Data indicate that there is 95% confidence that the corrected measurements are within + 10% of actual nitrogen dioxide concentrations.

^cAll measurements at same site. In other regions, all measurements were not made at same site.

In a 1973-1974 study of indoor sources of air pollutants, Cote *et al.* (1974) determined the emission rates of nitric oxide and nitrogen dioxide from an unvented gas-fired space heater. Under low-flame steady-state conditions, typical pollutant emissions were 214 mg/hour for nitric oxide and 130 mg/hour for nitrogen dioxide. When the flame was high, the emissions were 837 mg/hour for nitric oxide and 272 mg/hour for nitrogen dioxide.

A brief EPA study showed that peak nitrogen dioxide concentrations as high as 1 ppm ($\sim 1,880 \mu\text{g}/\text{m}^3$) and 1-hour averages ranging from 0.25 to 0.50 ppm ($\sim 470-940 \mu\text{g}/\text{m}^3$) are reached in a closed kitchen with no external ventilation (Eaton *et al.*, 1972).

Wade and coworkers (1975) studied four houses equipped with gas stoves to determine concentrations of nitric oxide, nitrogen dioxide, and carbon monoxide and their impact on air quality. During 2-week

periods, samples were collected simultaneously at four sampling locations at each house -- three indoors and one outdoors. The investigators concluded that:

- Emissions from gas stoves contribute nitrogen dioxide, nitric oxide, and carbon monoxide to the indoor atmosphere of houses in which such stoves are used. Concentrations of these gases in kitchens responded rapidly to stove use and, for a given house during a given season, there was a rough correlation between average nitrogen dioxide concentrations and average stove use.

- Nitrogen dioxide and nitric oxide were produced in roughly equal amounts. Indoor concentrations of these pollutants were invariably higher than those outside.

- Normal stove operations frequently resulted in nitrogen dioxide concentrations in the kitchens averaging $100 \mu\text{g}/\text{m}^3$ over the 2-week sampling periods.

- Comparison of samples taken during warm and cold weather during 1973-1974 indicated that pollutant concentrations were more uniformly distributed within the various rooms of the house during cold weather, when the house was closed up more often than during the warmer months.

- A diffusion experiment conducted in one of the houses showed that the half-life of nitrogen dioxide was only one-third of that for carbon monoxide and nitric oxide, indicating that nitrogen dioxide decays through reaction or adsorption in addition to normal dilution from air exchange. The effect was observed in some of the other houses by comparing the relative concentrations of nitrogen dioxide and other pollutants in various parts of the house.

Moschandreas et al. (1978) conducted an extensive study of indoor air quality in houses in five U.S. metropolitan centers. These investigators identified three types of indoor environments based on whether the houses had electric cooking and heating appliances, gas furnaces and electric cooking appliances, or gas cooking and heating equipment. In houses equipped with gas cooking appliances, indoor concentrations of nitric oxide were consistently higher than those observed outdoors. Houses with gas furnaces but electric cooking appliances usually contained concentrations of nitric oxide that were higher than those found outdoors. However, during intervals interspersed throughout the monitoring period, concentrations of nitric oxide measured outdoors surpassed those measured indoors. Indoor nitric oxide concentrations in totally electric homes were usually lower than outdoor concentrations.

The influence of gas stoves on indoor levels of nitrogen dioxide was confirmed by Palmes et al. (1977), who used integrating "personal" monitors. Melia et al. (1978) collected samples with the

same type of monitor in an epidemiological study to show that indoor levels of nitrogen dioxide were significantly higher in homes with gas stoves than in those with electric stoves. Other possible indoor combustion sources of NO_x are gas water heaters, gas dryers, and charcoal broilers, especially if they are faulty.

There is only limited information concerning the effects of combustion sources on indoor air quality in commercial buildings. Nevertheless, the sources mentioned above can presumably affect the quality of indoor air in commercial buildings as they do in residential buildings.

In summary, the highest average concentrations of indoor nitrogen dioxide are found in domestic kitchens. These concentrations can range from 100 to $\sim 940 \mu\text{g}/\text{m}^3$ and may reach peaks of approximately $1,880 \mu\text{g}/\text{m}^3$ when a gas stove is used in a poorly ventilated kitchen.

Concentrations of Nitrogen Oxides in Workplace Air. The presence of nitrogen dioxide in workplace air is fairly common, resulting primarily from the decomposition of nitrate (e.g., during dynamite blasting or silaging), from reactions of nitric acid with metals or other reducing agents (e.g., during acid dipping and dye and aniline manufacturing), from various processes in which air is heated to a high temperature (e.g., in furnaces or during welding and cutting torch operations), or from the exhaust of internal combustion engines. Although nitrogen dioxide (and/or dinitrogen tetroxide) has been used in industry as a nitrating or oxidizing agent and as the oxidizer in hypergolic rocket fuel, it is produced most often as an undesirable by-product from industrial practices and/or processes (Lewis, 1980). The National Institute for Occupational Safety and Health (1976) has estimated that at least 1.5 million workers in the United States are potentially exposed to nitrogen dioxide.

Estimates of concentrations of nitrogen oxides in various occupational settings are difficult to derive because there are generally no data. However, Wade *et al.* (1950) reported that 90 deaths prior to 1930 and 47 deaths between 1930 and 1949 were caused by exposure to high concentrations of nitrogen dioxide.

Concentrations of Nitrogen Oxides in Cigarette Smoke. Although originally thought to be a mixture of nitric oxide and nitrogen dioxide, the nitrogen oxides in fresh cigarette smoke, as inhaled, are predominantly, if not almost exclusively, nitric oxide (Adams *et al.*, 1978; Jenkins and Gill, 1980; Norman and Keith, 1965). Nitric oxide in cigarette smoke has a half-life of approximately 10 minutes

in the presence of the oxygen in cigarette smoke (~10 vol %) where it is oxidized to nitrogen dioxide (Adams et al., 1978). Nitric oxide and its derivatives react with other molecules in cigarette smoke to form a variety of mutagens and carcinogens, such as tobacco-specific nitrosamines (Hoffmann et al., in press).

The nitric oxide content of cigarette smoke is positively related to the nitrate content of the tobacco (Broaddus et al., 1965; Fuqua et al., 1976; Sims et al., 1979). In view of the factors influencing the nitrate content of tobacco, it is not surprising to find wide variations in the nitric oxide content of mainstream smoke from different cigarettes (Table 5-14).

Several methods have been used to determine the nitric oxide content of cigarette smoke (Adams et al., 1978). Jenkins and Gill (1980) reported a procedure that yields nitric oxide values that are higher than those determined by other methods: for smoke with a high nitric oxide content they were approximately 50% higher, for smoke with a low nitric oxide content they were about 170% higher. Their method involves rapid dilution to minimize interference in the assay by other smoke components. This rapid dilution might also minimize side reactions that may occur in cigarette smoke during normal inhalation. Based on the values given by these investigators for nine experimental cigarettes, the average concentration of nitric oxide per cigarette is approximately 510 μg . The committee has used 0.51 mg nitric oxide per cigarette to estimate the possible exposure of humans from this source (Table 5-3).

Summary

Wide ranges of nitrate and nitrite concentrations can be found in all food categories surveyed by the committee. For this reason, the committee has often based its estimates of the concentrations of these substances on data from broad surveys and, when possible, has averaged data from a number of studies. Canadian surveys of cured meat products have shown that the concentration of nitrate has been decreasing for the past decade, presumably because of the elimination of nitrate salts in all but a few products in Canada. Although there are no published data for the nitrate content of cured meat products in the United States, a similar decline is assumed to have occurred in U.S. cured meat products as well because a voluntary program to reduce the use of nitrate has been adopted by the meat industry. Current average nitrate concentrations in U.S. cured meat products have been estimated by the committee to be approximately 40 mg/kg (expressed as the nitrate ion). The committee estimated that the average concentration of residual nitrite in cured meats was 10 mg/kg (expressed as the nitrite ion). It arrived at this value by decreasing the average reported by Kolari and Aunan (1972) by 70% to reflect residual nitrite levels at the time of consumption. It did not consider the amounts of bound nitrite that may be present, and may not be measured, in

TABLE 5-14

Estimates of the Nitrogen Oxide Content of Cigarette Smoke

NOTE: Some numbers presented in this table have been rounded off to two significant figures.

| Nitrogen Oxides, $\mu\text{g}/\text{Cigarette}$ | | References |
|---|------------------|-------------------------------|
| Range | Average | |
| -- | 150 | Bokhoven and Niessen, 1961 |
| -- | 270 ^a | Gori, 1976 |
| 260-420 | 340 | Adams <i>et al.</i> , 1978 |
| -- | 280 ^b | Hoffmann <i>et al.</i> , 1980 |
| -- | 100 ^c | Hoffmann <i>et al.</i> , 1980 |
| 90-1,400 | 510 ^d | Jenkins and Gill, 1980 |

^aNo data are available for average nitrogen oxide content of cigarettes before 1960; however, an experimental cigarette considered to be representative of cigarettes marketed at that time contained 270 μg .

^bAverage amount in smoke of regular retail brands of cigarettes available in 1980.

^cAverage amount in smoke of low-tar retail brands of cigarettes available in 1978-1979.

^dAverage amount in smoke of nine types of experimental cigarettes. Higher recovery method gave higher values for nitrogen oxides.

cured meat products. Based on a limited number of studies of nitrate and nitrite content in fresh meat, 10 mg/kg nitrate and 1 mg/kg nitrite were assumed to be the average concentrations in these products.

Vegetables also vary in nitrate and nitrite content. Nitrate content can be modified by growing conditions, by time of harvest, by certain genetic factors, and by the amount and kind of nitrogen fertilizer used and the timing of its application. An overall average concentration of nitrate and nitrite in vegetables was not estimated. Instead, the committee developed averages for 35 different vegetables. The nitrate values, which are primarily for fresh vegetables, are based on literature surveys conducted by White (1975) and Corré and Breimer (1979). Most of the concentrations of nitrite in vegetables used by the committee were derived from the study by Siciliano *et al.* (1975), which was based primarily on concentrations in processed vegetables.

Average concentrations developed by the committee for nitrate in other food sources are: fruit, 20 mg/kg; baked goods and cereals, 12 mg/kg; and milk products, 0.5 mg/liter. The average nitrite content was estimated to be 2.6 mg/kg in baked goods and cereals and negligible in the other two categories.

The concentration of nitrite is also considered to be negligible in water; however, an estimated average concentration of nitrate in U.S. drinking water is 1.3 mg/liter, based primarily on a 1962 survey of the 100 largest U.S. municipal drinking water supplies and adjusted to reflect reports of increases in water supplies since 1966 and the higher nitrate content of smaller drinking water supplies.

Varying concentrations of nitrogen oxides are found in air outdoors, indoors, and in the workplace. In general, atmospheric levels of nitrogen dioxide are less than 0.05 ppm ($\sim 90 \mu\text{g}/\text{m}^3$), although as much as 1 ppm of nitrogen oxides during peak periods. In domestic kitchens, peak levels of nitrogen dioxide may also reach concentrations of 1 ppm ($\sim 1,880 \mu\text{g}/\text{m}^3$). Estimates of average concentrations in occupational settings are difficult to derive because of the lack of data.

Cigarette smoke also contains nitrogen oxides -- primarily nitric oxide. The committee has concluded that 0.51 mg of nitric oxide per cigarette, an average based on the measurements of Jenkins and Gill (1980), is a reasonable estimate of exposure from this source.

A summary of the estimated average concentrations (with the exception of vegetables) is given in Table 5-3. These values are used in the following section to estimate the relative importance of each environmental source to total exposure of humans to nitrate and nitrite and are not intended to represent absolute amounts contained in the individual sources.

EXPOSURE OF HUMANS TO NITRATE, NITRITE, AND NITROGEN OXIDES

The following discussion of the ingestion of nitrate and nitrite takes into consideration the relative contributions of a variety of exogenous sources such as air, water, and food, including cured and fresh meats. However, daily intake of nitrate and nitrite (especially when expressed as averages for an entire population) may not be as relevant to the determination of in vivo effects as peak concentrations that may occur at certain body sites immediately after ingestion. In addition, estimates of nitrite ingestion do not include

the conversion of nitrate to nitrite in the saliva — a factor that is considered in detail in Chapter 8. This section presents estimates of the mean exogenous exposure of the U.S. population to nitrate and nitrite as well as estimates for certain population groups whose exposure may deviate significantly from the mean.

Exposures from Food

Two methods are generally used to estimate the ingestion of nitrate and nitrite in food. One method depends on direct assays of nitrate and nitrite in duplicate portions of representative meals. The other method involves the use of published tables of average consumption, e.g., the Household Consumption Survey of the U. S. Department of Agriculture (1980), and the nitrate and nitrite content of various dietary constituents reported in the literature.

The direct assay method affords several advantages over the method using food consumption tables: The nitrate and nitrite content in food is measured after it has been prepared, thereby providing a more accurate assessment of the amounts actually ingested. Food consumption is also measured directly, thus eliminating the need for food consumption tables that can over- or underestimate actual intake (Selenka and Brand-Grimm, 1976).

One weakness in the assessment of intake by the direct assay method is the possibility of biasing the data through selection of meals to be tested, since individual variations in dietary habits occur on a daily and seasonal basis. As shown in Table 5-15, more

TABLE 5-15

Per Capita Ingestion of Nitrate and Nitrite Measured By Direct Assay of Meals

NOTE: Some of the values have been rounded off to two significant figures.

| Nitrate | | Nitrite | | Country | References |
|---------|---------|---------|---------|-------------|---|
| Average | Range | Average | Range | | |
| 50 | 26-81 | 3.7 | 0.6-7.3 | Sweden | Jägerstad and Nilsson, 1976; Jägerstad <u>et al.</u> , 1976 |
| 63 | 6.1-270 | 2.3 | 0.7-14 | Netherlands | Stephany and Schuller, 1978 |

than a tenfold range of nitrite ingestion and a fourfold range in nitrate ingestion were observed by Jägerstad and colleagues in a study conducted in Sweden (Jägerstad and Nilsson, 1976; Jägerstad et al., 1976). In contrast, Stephany and Schuller (1978) recorded a twentyfold range in nitrite ingestion and a fortyfold range in nitrate ingestion in the Netherlands. The wide ranges in the latter study were attributed to seasonal variations in the diets. Thus, intake figures from a direct assay could be misleading.

In this report, data on exposure to nitrate and nitrite from food were obtained exclusively from food consumption/production tables and averages developed from published reports of nitrate and nitrite content, primarily because direct assay data for meals consumed by the U.S. population are not available. The committee has used consumption data from the U.S. surveys listed in Table 5-16 to estimate the average per capita consumption of various categories of foods (Table 5-17). In an attempt to compensate for the wide ranges in consumption, it has also estimated the intake for population groups with high (4 times the average) consumption of cured meat and high (4 times the average) consumption of vegetables.

Despite the limitations of the data on average consumption as well as those described for environmental concentrations in the preceding section, the committee believes that its estimates are a useful indication of relative exposures of the human population to nitrate and nitrite from the various environmental sources.

Cured Meats. Four estimates of average individual daily intake of nitrate and nitrite from cured meats are presented in Tables 5-18 and 5-19. Some of these data differ by approximately elevenfold. Such discrepancies are due to different estimates of residual nitrate and nitrite in products as they are consumed, coupled with different estimates of average consumption.

In Table 5-18, White's estimate of 9.4 mg daily nitrate intake from cured meats reflects the nitrate content of European meat products in 1972 (White, 1975, 1976). This estimate is also based on production figures for 1972, rather than on consumption data, and it may overestimate intake. More recently, Birdsall (1981) also used White's value as a conservative (maximal) estimate of nitrate content. The Food and Drug Administration's (1979) estimate of zero nitrate in cured meats does not take into account the fact that even uncured meats contain nitrate.

Hartman (1981) used White's estimate of 9.4 mg/person/day to calculate the intake of nitrate from cured meats (Table 5-18). However, he reduced White's estimate by 20% to account for a drop in the nitrate content of cured meats since 1972 and to account for a decrease in the amount of product consumed. Evidence supporting this assumption is given in Table 5-2. The committee's estimate

TABLE 5-16

Some Estimates of Per Capita Daily Consumption of Vegetables and Meats,
Based on Food Consumption Surveys

NOTE: Some of these data have been rounded off to two significant figures.

| Food | Consumption, g/Person/Day, by Study | | | | | | | | |
|---|-------------------------------------|---|---|-------------------|-----------------|-----------------|----------------|-------------------------------------|----------------|
| | United States | | | | United Kingdom | | Norway | Federal Republic of Germany | Switzerland |
| | White, 1975, 1976 ^a | U.S. Department of Agriculture, 1980 ^b | Food and Drug Administration, 1979 ^c | 1980 ^b | Ashton, 1970 | Walker, 1975 | Høyem, 1974 | Selenka and Brand-Grimm, 1976 | Tremp, 1980 |
| Cured meats | 45 | 20 | 29 | 30 | -- | -- | -- | -- | -- |
| Total meats, including sausages and other proc- essed meats | 180 ^d | 80 ^d | 120 ^d | 120 ^d | 31 | 32 | 120 | 110 | 100 |
| Vegetables, excluding white potatoes | 150 | -- | 55 | -- | 64 | 66 | 100 | 89 | 100 |
| White potatoes | 120 | -- | 27 | -- | -- | 140 | 230 | 130 | 53 |
| Total vegetables | 270 | 200 | 82 | 320 | -- | 200 | 330 | 220 | 150 |
| Fruits and juices | 180 | 170 | -- | 290 | -- | -- | -- | 150 | 150 |
| Baked goods and cereals | 90 | 62 | -- | 83 | -- | -- | 190 | 160 | 110 |
| Milk and milk products | 500 | 310 | -- | 300 | -- | -- | 550 | 12 | 300 |

^aEstimates based on farm production figures correction for estimated losses in food preparation.

^bEstimates based on food consumption surveys.

^cEstimates based the Fourth Natural Household Menu Census Study conducted from July 1972 to June 1973 by the Market Research Corporation of America, Northbrook, Illinois.

^dBased on the assumptions that cured meats constitute roughly one-half of all processed meats and that processed meats constitute roughly one-half of total meat consumption (see text discussion on meats). Therefore, estimates of cured meat consumption have been multiplied by 4 to estimate total meat consumption.

TABLE 5-17

An Estimate of Average Daily Intake

| <u>Source</u> | <u>Amount</u> |
|-------------------------|-------------------|
| Cured meats | 30 g |
| Fresh meats | 60 g |
| Vegetables | 190 g |
| Fruits | 220 g |
| Baked goods and cereals | 100 g |
| Milk and milk products | 370 g |
| Water | 1.6 liters |
| Air | 20 m ³ |
| Cigarettes | 20 cigarettes |

(40 mg/kg) of nitrate in cured meat products also takes into account the decrease in residual nitrate. It averaged the consumption data of White (1975, 1976), the U.S. Department of Agriculture (1980), and the Food and Drug Administration (1980) (Table 5-16) to develop an estimate of approximately 30 g/person/day for cured meats. Thus, average consumption would be 1.2 mg of nitrate per person per day (Table 5-20).

Nitrite ingested in cured meats was estimated to be 0.30 mg/person/day (30 g/person/day x 10 mg/kg nitrite) (Table 5-21). This estimate is close to those generated independently by Birdsall (1981) and Hartman (1981) (Table 5-19).

Although the residual nitrite content of many cured meats is low, cured meats can contribute a substantial percentage of the nitrite ingested by certain subgroups of the population that consume large amounts of these products. Thus, the committee has estimated the intake for a subgroup with a high cured meat diet in addition to estimates for average intake from meats. Assuming that daily consumption of cured meats in this subgroup is 4 times the average consumption (120 g/person/day), daily intake of nitrate would be 4.8 mg and nitrite intake would be 1.2 mg.

Fresh Meat Products. Average consumption of fresh meats has been estimated by averaging the consumption of "total meats" indicated by U.S. surveys in Table 5-16 and dividing by 0.5 (approximately one-half of meat consumed is fresh, unprocessed meat). This figure is approximately 60 g/person/day. Assuming a 10 mg/kg concentration of nitrate in fresh meats, daily intake of nitrate from this source would be 0.6 mg/person/day (Table 5-20). At a nitrite level of 1 mg/kg, intake is estimated to be 0.06 mg/person/day from fresh meat products (Table 5-21).

TABLE 5-18

Four Estimates of Per Capita Daily Dietary
Nitrate Intake by U.S. Residents

NOTE: Some of these data have been rounded off to two significant figures.

| Food | <u>Intake, mg/Person/Day, and (Percentage of Total Daily Intake from All Sources), by Study</u> | | | |
|------------------------|---|---|----------------------|-----------------------|
| | <u>White, 1975, 1976</u> | <u>Food and Drug Administration, 1979</u> | <u>Hartman, 1981</u> | <u>Birdsall, 1981</u> |
| Cured meats | 9.4 (9.4) | 0 ^a (0) | 0.43 (0.6) | 9.4 (9.4) |
| Vegetables | 86 (86) | 37 (95) | 62.0 (91.7) | 86 (86) |
| Fruits and juices | 1.4 (1.4) | NR ^b | 1.2 (1.8) | 1.4 (1.4) |
| Bread | 2.0 (2.0) | NR | 2.0 (2.8) | 2.0 (2.0) |
| Milk and milk products | 0.2 (0.2) | NR | 0.2 (0.3) | 0.2 (0.2) |
| Water | 0.7 (0.7) | 2 (5) | 2.0 (2.8) | 0.7 (0.7) |
| TOTAL | 100 (100) | 39 (100) | 70 (100) | 100 (100) |

^a0 indicates a dietary contribution that is relatively unimportant in comparison with other food categories listed.

^bNR = No data were reported.

TABLE 5-19

Four Estimates of Per Capita Daily Dietary
Nitrite Intake by U.S. Residents

NOTE: Some of these data have been rounded off to two significant figures.

| Food | <u>Intake, mg/Person/Day, and (Percentage of Total Daily Intake from All Sources), by Study</u> | | | |
|------------------------|---|---|----------------------|-----------------------|
| | <u>White, 1975, 1976</u> | <u>Food and Drug Administration, 1979</u> | <u>Hartman, 1981</u> | <u>Birdsall, 1981</u> |
| Cured meats | 2.4 (92) | 0.22 (65) | 0.37 (68) | 0.35 (61.5) |
| Vegetables | 0.2 (7) | 0.12 (35) | 0.16 (29) | 0.2 (35) |
| Fruits and juices | 0 ^a (0) | NR ^b | 0 (0) | 0 (0) |
| Bread | 0.02 (1) | NR | <0.02 (3) | 0.02 (3.5) |
| Milk and milk products | 0 (0) | NR | 0 (0) | 0 (0) |
| Water | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| TOTAL | 2.6 (100) | 0.34 (100) | 0.54 (100) | 0.57 (100) |

^a0 indicates a dietary contribution that is relatively unimportant in comparison with other food categories listed.

^bNR = No data were reported.

TABLE 5-20

Committee's Estimates of Per Capita Daily Nitrate Intake for the U.S. Population (Averaged) and for Three Population Subgroups, Indicating the Spectrum of Ranges of Intake Believed to be Prevalent in the United States Today

| Source | Exposure, mg/Person, and Percentage Contribution from Each Dietary Source ^a | | | | | | | |
|-------------------------|--|-----|------------------------------|-----|-------------------------|-----|---------------------------------|-----|
| | U.S. Average | | High Cured Meat ^b | | Vegetarian ^c | | Nitrate-Rich Water ^d | |
| | mg | % | mg | % | mg | % | mg | % |
| Cured meats | 1.2 | 1.6 | 4.8 | 6 | 0 | 0 | 1.2 | 0.5 |
| Fresh meats | 0.6 | 0.8 | 0.6 | 0.8 | 0 | 0 | 0.6 | 0.2 |
| Vegetables | 65 | 87 | 65 | 83 | 260 | 97 | 65 | 28 |
| Fruits and juices | 4.3 | 6 | 4.3 | 6 | 4.3 | 1.6 | 4.3 | 1.8 |
| Baked goods and cereals | 1.2 | 1.6 | 1.2 | 1.5 | 1.2 | 0.4 | 1.2 | 0.5 |
| Milk and milk products | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 |
| Water | 2 | 2.6 | 2 | 2.5 | 2 | 0.7 | 160 | 68 |
| TOTAL | 75 | | 78 | | 268 | | 233 | |

^aThese data pertain to exposure to exogenous sources (ingestion) only. Analysis of impact of body load of nitrite and nitrate is to be found in Chapter 8. Figures are crude and are given for the purpose of determining relative contributions from various sources (see text).

^bAssuming 4 times the daily consumption of cured meats listed for the "average" daily ingestion.

^cAssuming a vegetarian consumes 4 times the amount of vegetables in the same distribution as does the "average" person.

^dData are for the Sangamon River area in Central Illinois.

TABLE 5-21

Committee Estimates of Per Capita Daily Nitrite Intake for the U.S. Population (Averaged) and for Three Population Subgroups, Indicating the Spectrum of Ranges of Intake Believed to be Prevalent in the United States Today

| Source | Exposure, mg/Person, and Percentage Contribution from Each Dietary Source ^a | | | | | | | |
|-------------------------|--|-----|------------------------------|-----|--------------------------|-----|---------------------------------|-----|
| | U.S. Average | | High Cured Meat ^b | | Vegetarians ^c | | Nitrate-Rich Water ^d | |
| | mg | % | mg | % | mg | % | mg | % |
| Cured meats | 0.30 | 39 | 1.2 | 71 | 0 | 0 | 0.30 | 39 |
| Fresh meats | 0.06 | 7.7 | 0.06 | 3.5 | 0 | 0 | 0.06 | 7.7 |
| Vegetables | 0.12 | 16 | 0.12 | 7 | 0.48 | 62 | 0.12 | 16 |
| Fruits and juices | 0.01 | 1.3 | 0.01 | 0.6 | 0.01 | 1.3 | 0.01 | 1.3 |
| Baked goods and cereals | 0.26 | 34 | 0.26 | 15 | 0.26 | 34 | 0.26 | 34 |
| Milk and milk products | 0.01 | 1.3 | 0.01 | 0.6 | 0.01 | 1.2 | 0.01 | 1.3 |
| Water | 0.01 | 1.3 | 0.01 | 0.6 | 0.01 | 1.3 | 0.01 | 1.3 |
| TOTAL | 0.77 | | 1.7 | | 0.77 | | 0.77 | |

^aThese data pertain to exposure to exogenous sources (ingestion) only. Analysis of impact of body load of nitrite and nitrate is to be found in Chapter 8. Figures are crude and are given for the purpose of determining relative contributions from various sources (see text).

^bAssuming 4 times the daily consumption of cured meats listed for the "average" daily ingestion.

^cAssuming a vegetarian consumes 4 times the amount of vegetables in the same distribution as does the "average" person.

^dData are for the Sangamon River area in Central Illinois.

Vegetables. To estimate the intake of nitrate and nitrite, White (1975) used 1972 production figures, which had been adjusted to approximate weight losses occurring in vegetable preparation, to determine per capita consumption. The Food and Drug Administration (FDA, 1979) based its estimates of average consumption on a "frequency-of-eating" survey coupled with data from the U.S. Department of Agriculture on "average portion sizes." White (1975) estimated the average total vegetable consumption to be approximately 270 g per person daily, whereas the FDA estimated only 80 g per person per day. If white potatoes are excluded from the lists, then the FDA estimate is 55 g and White's estimate of 150 g is nearly 3 times greater. The estimates diverge even more for nitrate-rich vegetables (categories 4 and 5, Table 5-5). The FDA estimated the individual daily intake of these vegetables to be 6.8 g, whereas White estimated 33 g per person per day. This is more than a fivefold difference.

Because there are inherent biases in each method used (e.g., production figures even when corrected for loss often result in overestimates and food consumption surveys may over- or underestimate actual consumption), the committee has averaged these two sets of data (Food and Drug Administration, 1979; White, 1975) to estimate the average consumption listed in Table 5-22. Based on these averages, the contribution of nitrate from vegetables in the average U.S. diet is estimated to be approximately 65 mg nitrate and 0.12 mg nitrite per person per day (Table 5-21). These figures can be compared with previous estimates given in Tables 5-18 and 5-19. If vegetarians consume, on the average, 4 times the amount of the same vegetables as the general population, then vegetarians will ingest 260 mg of nitrate and 0.48 mg of nitrite per day (Tables 5-20 and 5-21).

Fruits and Fruit Juices. The committee estimates that the daily per capita ingestion of fruits and fruit juices is 216 g. This is the average of three estimates (Food and Drug Administration, 1980; U.S. Department of Agriculture, 1980; White, 1975) (Table 5-16). Assuming that these products contain nitrate concentrations of approximately 20 mg/kg, which is double White's 1975 value for fruits, the average ingestion is estimated to be 4.3 mg of nitrate per person per day. Nitrite intake from this source is very low -- .01 mg per person per day (Tables 5-20 and 5-21).

Baked Goods and Cereals. Ingestion of bread, rolls, and other baked goods is estimated to be 78 g per person per day -- the average value of three separate U.S. consumption estimates (Food and Drug Administration, 1980; U.S. Department of Agriculture, 1980; White, 1975) (Table 5-16). Cereal consumption is estimated to be 22 g per person per day (Food and Drug Administration, 1980). Thus, total average consumption of baked goods and cereals is estimated to equal 100 g/person/day. Based on this estimate of consumption and the estimated nitrate and nitrite content of the products in this food category, the committee estimates that 1.2 mg of nitrate and 0.26 mg of nitrite are ingested per person per day from this source (Tables 5-20 and 5-21).

TABLE 5-22

Estimated Average Intakes of Nitrate and Nitrite from Vegetables

| Vegetable | Concentration, mg/kg | | Average Amount of Vegetable Consumed Per Day, g | Average Consumed Per Day, µg | |
|---------------------|----------------------|-----------------|---|---------------------------------|-----------------|
| | Nitrate | Nitrite | | Nitrate | Nitrite |
| Artichoke | 12 | 0.4 | 0.12 | 1 | 0.5 |
| Asparagus | 44 | 0.6 | 1.2 | 53 | 0.7 |
| Bean: green | 340 | 0.6 | 8.1 | 2,700 | 4.8 |
| lima | 54 | 1.1 | 1.4 | 76 | 1.5 |
| dry (navy) | 13 | NR ^a | 3.8 ^b | 49 | NR ^a |
| Beet | 2,400 | 4.0 | 1.3 | 3,100 | 5.2 |
| Broccoli | 740 | 1.0 | 1.5 | 1,100 | 1.5 |
| Brussels sprouts | 120 | 1.0 | 0.2 | 24 | 0.2 |
| Cabbage | 520 | 0.5 | 5.5 ^c | 2,800 | 2.8 |
| Carrot | 200 | 0.8 | 5.8 | 1,200 | 4.6 |
| Cauliflower | 480 | 1.1 | 0.9 | 440 | 1.0 |
| Celery | 2,300 | 0.5 | 3.8 | 8,800 | 1.9 |
| Corn | 45 | 2.0 | 14 | 610 | 27 |
| Cucumber | 110 | 0.5 | 1.9 | 210 | 1.0 |
| Eggplant | 270 | 0.5 | 0.4 | 110 | 0.2 |
| Endive | 1,300 | 0.5 | 0.01 | 18 | 0 |
| Kale/collard | 800 | 1.0 | 0.5 | 400 | 0.5 |
| Leek | 510 | NR ^a | NR ^a | (5) ^d | NR ^a |
| Lettuce | 1,700 | 0.4 | 13 | 22,000 | 5.1 |
| Melon | 360 | NR ^a | 11 ^b | 3,900 | NR ^a |
| Mushroom | 160 | 0.5 | 0.3 | 49 | 0.2 |
| Okra | 38 | 0.7 | NR ^a | 1 | 0 |
| Onion | 170 | 0.7 | 6.8 | 1,100 | 4.8 |
| Parsley | 1,010 | NR ^a | NR ^a | (10) ^d | NR ^a |
| Peas | 28 | 0.6 | 6.2 | 170 | 3.7 |
| Pepper: sweet | 120 | 0.4 | 1.4 | 170 | 0.6 |
| Potato: white | 110 | 0.6 | 73 | 8,300 | 44 |
| sweet | 46 | 0.7 | 3.4 | 160 | 2.4 |
| Pumpkin and squash | 400 | 0.5 | 1.4 | 560 | 0.7 |
| Radish | 1,900 | 0.2 | 0.3 | 580 | 0.1 |
| Rhubarb | 2,100 | NR ^a | NR ^a | (21) ^d | NR ^a |
| Spinach | 1,800 | 2.5 | 1.8 | 3,200 | 4.5 |
| Tomato and products | 58 | NR ^a | 21 | 1,200 | NR ^a |
| Turnip | 390 | NR ^a | 0.2 | 78 | NR ^a |
| Turnip greens | 6,600 | 2.3 | 0.3 | 2,000 | 0.7 |
| TOTAL | | | ~190 | ~65,000 | ~120 |

^aNR = No data reported.^bNot on the FDA (1979) list. Figure is one-half of White's (1975) estimate.^cIncluding sauerkraut.^dNitrate intake per day based on average daily consumption of 0.01 g of vegetable.

Milk and Milk Products. Ingestion of milk and milk products is considered to be 370 g per person per day for U.S. adults. This is an average of three separate U.S. consumption estimates (Food and Drug Administration, 1980; U.S. Department of Agriculture, 1980; White, 1975) (Table 5-16). Based on estimates that these products contain 0.5 mg/liter of nitrate and negligible levels of nitrite, intake would be approximately 0.2 mg of nitrate and less than 0.01 mg of nitrite per person per day from this source (Tables 5-20 and 5-21). However, if the concentration of nitrate in U.S. milk and milk products is similar to that found in Danish products (e.g., an average of 8 mg/liter), intake could be somewhat higher -- approximately 3.0 mg per person per day. Even if this higher concentration were present, the amount ingested would not contribute a significant percentage of daily nitrate intake for the average adult.

For infants, however, milk with a nitrate content of 8 mg/liter can contribute substantially to exposure since consumption of 0.75 liter of such milk by a 4.5-kg infant would be comparable, on a body-weight basis, to daily ingestion of approximately 120 mg of nitrate by an average adult. Turek *et al.* (1980a,b) found that nitrate present in the urine of infants was an accurate reflection of the amount of nitrate ingested. Suckling infants excreted appreciable concentrations of nitrate (averaging 39 mg of nitrate per liter) and bottle-fed infants excreted approximately 3 times as much. Although breast milk or cow's milk could contribute to nitrate intake by infants, extremely high intakes occur most frequently when infant formula is prepared with well water containing high concentrations of nitrate (> 44 mg/liter) (Walton, 1951). (See also discussion of methemoglobinemia in Chapter 9.) For example, Donahoe (1949) reported that the cyanosis of a breast-fed infant with presumed methemoglobinemia cleared when the mother stopped drinking well water suspected of containing a high concentration of nitrate.

Collectively, these observations indicate that levels of nitrate that could be significant to the infant can be accumulated in the milk of both humans and ruminants. Infant formulas made with high-nitrate water may also be an important source of nitrate (see next section).

Exposures from Water

The major ingredient of most beverages (e.g., coffee, tea, and soft drinks) is water. The average daily consumption of water from these sources plus drinking water is approximately 1.6 liters of water for the average adult (National Academy of Sciences, 1977a) (Table 5-17). The committee has estimated that U.S. drinking water supplies contain an average nitrate concentration of 1.3 mg/liter. Thus, daily intake of nitrate from this source would be approximately 2.0 mg/person/day (Table 5-19). Ingestion of nitrite from this source is considered to be negligible (Table 5-21).

To estimate intake for regions with drinking water supplies containing high concentrations of nitrate, the committee used data from studies of the Sangamon River region near Decatur, Illinois, where concentrations of nitrate in water averaged approximately 100 mg/liter in 1971 (Kohl *et al.*, 1971). Assuming that 1.6 liters are ingested daily, intake in such regions would be approximately 160 mg per person per day (Table 5-20)

Exposures from Air

Atmospheric Nitrogen Oxides. Inhaled nitrogen oxides and alkyl nitrites could play an important role in the exposure of humans to nitrate and nitrite (Ehrenberg *et al.*, 1980; Erlandsson, 1981; Hoffmann *et al.*, 1975; Newmark and Mergens, 1981; World Health Organization, 1978). Parks *et al.* (1981) have found that considerable amounts of nitrate and nitrite may be accumulated in the lungs under certain circumstances and that these ions may be rapidly and widely distributed in the body as nitrate. Very recently, Oda (1981) reported that inhalation of nitrogen dioxide leads to the appearance of relatively large amounts of nitrate and nitrite in the blood of rats. Pryor and Lightsey (1981) reported that nitrogen dioxide reacts with unsaturated fats to produce nitrite. This reaction could occur in vivo as well.

An average concentration of nitrogen dioxide in the atmosphere is usually less than the air quality standard of $90 \mu\text{g}/\text{m}^3$, although concentrations of nitrogen oxides in air of smog-laden cities may reach 1 ppm. Estimates of the daily intake of nitrite and nitrate from this source range widely. Erlandsson (1981) estimated that an atmospheric NO_x concentration of $114 \mu\text{g}/\text{m}^3$ in Gothenburg, Sweden, would result in an average daily exposure of 1.2 mg nitrate and 0.9 mg nitrite. At maximal NO_x concentrations of $929 \mu\text{g}/\text{m}^3$, he estimated the average daily exposures to be 9.4 mg nitrate and 7 mg nitrite. In the United States, Newmark and Mergens, 1981, observed that intake can be as high as 1 mmol (average of 54 mg nitrite plus nitrate) in cities during periods of smog formation.

Although the figures pertaining to inhalation exposure are conjectural and in need of confirmation, the committee, for purposes of demonstration, has assumed that all of the nitrogen dioxide is converted to nitrate (this will result in a high estimate since it is likely that less than 100% will be converted) and that the average adult breathes 20 m^3 of air per day. If, the average nitrogen dioxide concentration is $58 \mu\text{g}/\text{m}^3$, the estimated average intake of nitrate from this source is 1.5 mg. High intake can be estimated by using $118 \mu\text{g}/\text{m}^3$ (the average in Los Angeles) to give 3.1 mg nitrate/day. Peak exposures could occur during heavy smog formation and result in much higher intakes. Because these estimates are conjectural, they have not been considered in calculations of total exposure to nitrate. No estimates have been developed for indoor or occupational exposures.

Nitrogen Oxides in Tobacco Smoke. Almost all of the nitrogen oxides inhaled in cigarette smoke are retained in the body (Bokhoven and Niessen, 1961). Thus, the amount inhaled is presumed to equal the amount ingested. Also, for purposes of demonstration, the committee has assumed that 20 cigarettes (one pack) is smoked daily and that 100% of the mainstream smoke is inhaled, although the percent actually inhaled varies from individual to individual.

Under the conditions just described, if all of the nitric oxide inhaled were converted to nitrate, a concentration of 0.51 mg of nitric oxide per cigarette would result in the ingestion of approximately 21 mg of nitrate per 20 cigarettes. This is a high estimate because the conversion of nitric oxide to nitrate is probably less than 100%. Modern, low-tar U.S. cigarettes would contribute one-half to one-third the amount of nitric oxide. Small cigars may produce from 0.2 to 2.0 mg of nitric oxide per cigar (Adams *et al.*, 1978) and could contribute significantly to exposure, even if only a small portion of the smoke is inhaled.

Summary

Tables 5-20 and 5-21 present estimates of the nitrate and nitrite intake for the average U.S. population, for those whose diets contain higher-than-average amounts of vegetables or meats, and for those who are exposed to nitrate-rich drinking water. Actually, the intake of nitrate and nitrite by the average U.S. citizen will probably vary. For example, the amount of meats and vegetables consumed generally varies on a daily and seasonal basis. Additionally, although the committee did not include an estimate of the nitrate intake from the atmosphere in these summary tables, it is likely that inhalation of nitrogen oxides will contribute to the total body burden of nitrate and nitrite and exposure to smog is at least an occasional experience for most Americans. The data in Tables 5-20 and 5-21 can be used to generate further estimates for various population subgroups based on combinations of the types of exposure just mentioned.

These estimates indicate that the average U.S. population is exposed to nitrate primarily from vegetables (87%). Other contributors of nitrate intake are, in descending order of importance: fruits and juices (6%), water (~3%), and cured meats (~2%). Intake of nitrite is provided by cured meats (39%), baked goods and cereals (34%), and vegetables (16%). It is important to point out, however, that these are estimates of the intake and not necessarily the endogenous exposure, since 50% of ingested nitrate is converted to nitrite in vivo. Contributions of various sources to the total body burden of nitrite, which takes this fact into account, are given in Chapter 8.

The committee's estimates must be regarded as tentative because of the limited data bases and the numerous other factors discussed throughout this chapter, not the least of which are limitations inherent in the analytical methods used to measure the nitrate, nitrite, and nitrogen oxides in various media. Also, because estimates of intake from foods were based on food consumption tables rather than on direct assay, the committee's estimates may reflect any inherent errors in these data. Thus, the estimate of 0.12 mg of nitrite (16%) from vegetables as a U.S. average is subject to error of uncertain dimensions because the standard errors cannot be calculated for the nitrite concentration in each vegetable presented in Table 5-8.

These estimates are probably most useful as indicators of the relative contributions made by various sources to the average daily intake. That is, although they are crude, the individual estimates in this section do suggest that some sources of nitrate and nitrite ingestion are more important than others. Additionally, they point out some ranges in exposure that are probably characteristic of large segments of the U.S. population today. For example, they indicate that approximately 39% of ingested nitrite comes from cured meats and that consumption of much larger amounts of cured meats, as in the case of a high cured meat diet, can contribute significantly (71%) to the total nitrite intake from exogenous sources.

Data in Table 5-22 indicate that only a few vegetables contribute most of the nitrate load. In addition, evidence presented earlier in Table 5-7 suggests that heavy use of nitrogen fertilizers during the past decade may have caused significant increases in the nitrate content of carrots, lettuce, and spinach.

Drinking water is an important consideration in determining environmental exposure to nitrate because of the large amount of water ingested daily and because of the reports of high concentrations of nitrate found in certain water supplies, such as private wells. Evidence that the nitrate content of drinking water may be increasing also suggests that this source of nitrate may be even more important in the future. There seems to be general agreement that the nitrite content of drinking water supplies is uniformly low and not an important contributor to human exposure to nitrite in the United States.

The committee's estimates are conjectural because there are no data on actual conversion of nitrogen dioxide to nitrate and nitrite in humans; however, nitrogen oxides may contribute to the exposure of humans to nitrate, especially in polluted atmospheres, and from tobacco smoke, which may contribute up to 21 mg of nitrate daily for persons smoking 20 cigarettes/day.

OVERALL SUMMARY AND CONCLUSIONS

Levels of nitrate and nitrite vary widely among foods and in the environment. In foods, they depend on a number of factors, including agricultural practices and storage conditions. These factors, combined with the limitations in analytical techniques for measuring nitrate and nitrite, in the data on environmental concentrations, and in methods for the accurate determination of food consumption, make it difficult to estimate precisely the exposure of humans to these compounds. Nevertheless, the committee has made rough quantitative estimates of such exposures. These estimates should not be taken at face value; rather, they should be used as a guide to gain an understanding of the relative contributions of different sources to the exposure of humans to nitrate and nitrite.

Differing lifestyles and dietary habits can lead to variations in the amount of nitrate ingested by different groups. In the average diet, vegetables contribute most of the nitrate (87% of total daily intake). Vegetarians may consume substantially higher amounts of nitrate than does the general population. Milk generally contains very low levels of nitrate; however, recent data from Denmark suggest that nitrate may be present in milk at levels higher than those previously reported. Milk is not a significant contributor of nitrate for adults, but it may be an important source of nitrate for infants. Other sources of intake include nitrate-rich drinking water and fruit and fruit juices.

Of the total daily intake of dietary nitrite, 39% is contributed by cured meats, 34% by baked goods and cereals, and 16% by vegetables. The concentration of nitrite in these foods, especially in cured meats, varies widely, and, depending on lifestyle and dietary habits, the fraction of daily nitrite exposure from any one source can vary from 0 to 90%. Thus, there may be considerable variation in the total daily intake of nitrite. (The total gastric exposure to nitrite, which includes nitrite resulting from the reduction of nitrate in the saliva, is discussed in Chapter 8.)

Two additional factors should be considered when determining the significance of exposure to nitrate and nitrite. First, vegetables contain inhibitors of nitrosation, such as ascorbic acid, and catalysts, such as certain phenols. These tend to affect the extent of in vivo nitrosation and, thus, the synthesis of N-nitroso compounds. Second, assays for the residual nitrite content of processed meats do not necessarily indicate the amount that can participate in nitrosation reactions in vivo (e.g., some species of bound nitrite may not be measured).

Although the intake of nitrogen oxides contributes to the daily exposure to nitrite and nitrate, the contribution from this source is relatively small for the average U.S. population. However, peak levels of nitrogen oxides in smog-laden cities may result in more substantial exposures.

RECOMMENDATIONS

1. Methods and Data Availability. The committee recommends that more accurate estimates of exposure to nitrate and nitrite be obtained by improving assay procedures, especially to distinguish between free and bound nitrite in meat products and to examine whether residual nitrite is a true measure of nitrosating capacity. There is also a need for new analyses of nitrate and nitrite content of meats -- cured, fresh, and processed without the addition of nitrite. In addition, a better data base for the nitrate and nitrite content of milk, milk products, and grains is needed.

Furthermore, prepared meals in the United States should be analyzed for their nitrate and nitrite content. (This has already been done for representative meals in other countries.) The results should be compared with those from food consumption tables to cross-check the validity of different methods of estimating intake, as well as to provide a realistic indication of extremes in individual consumption.

To ensure effective dissemination of data regarding the nitrate and nitrite content of U.S. foods and drinking water, research staffs of the FDA, EPA, and USDA should be encouraged to expedite publication of new data in scientific journals.

2. Reduction of Nitrate Intake from Vegetables and Water. Vegetables and water containing high concentrations of nitrate are major contributors to the intake of nitrate. Thus, attention should be directed toward the feasibility of reducing the nitrate content of these sources.

Numerous approaches could be used to decrease nitrate concentrations in vegetables. These are discussed earlier in this chapter (see Table 5-6). Exposure of humans to nitrate and nitrite that can participate in nitrosation reactions can be effectively reduced by ingesting vegetables containing high levels of nitrosation inhibitors such as ascorbate. Thus, the committee recommends that further studies be conducted to develop additional methods of reducing nitrate in vegetables while retaining ascorbic acid and other inhibitors of nitrosation.

Because certain domestic water supplies contain levels of nitrate that are considerably higher than average, the committee recommends that the sources of nitrate be determined and that efforts be made to reduce these concentrations to more acceptable levels.

3. Reduction of Nitrite Intake. To the extent possible, nitrite intake should also be decreased. If the amount of nitrite added to cured meats is reduced, this reduction should not compromise protection against botulism.

REFERENCES

- Achtzehn, M. K., and H. Hawat. 1969. [In German; English summary.] [Nitrate accumulation in vegetables - a possibility of nitrate intoxication in babies?] *Nahrung* 13:667-676.
- Adams, J. D., K. D. Brunnemann, and D. Hoffman. 1978. Determination of nitric oxide in unaged smoke by GSC-TEA. Paper presented at the 32nd Tobacco Chemists' Research Conference, October 30 - November 1, 1978, Montreal, Quebec, Canada.
- American Meat Institute. 1977. Progress Report: Residual Sodium Nitrite in Cured Meat Products. Report prepared for the July 25, 1977 meeting of the USDA Expert Panel on Nitrites and Nitrosamines. American Meat Institute, Arlington, Virginia. [5] pp.
- American Meat Institute. 1980. Meatfacts: A Statistical Summary About America's Largest Food Industry. American Meat Institute, Arlington, Virginia. 27 pp.
- Anonymous. 1969. Poisoning the wells. *Environment* 11:16-27, 45.
- ap Griffith, G. 1958. Nitrate content of some grass species and strains. *Nature* 182:1099-1100.
- ap Griffith, G. 1960. Nitrate content of herbage at different manurial levels. *Nature* 185:627-628.
- Ashton, M. R. 1970. The Occurrence of Nitrates and Nitrites in Foods. Literature Survey No. 7. British Manufacturers Industries Research Association, Leatherhead, Surrey, United Kingdom. 33 pp.
- Aslam, M., A. Oaks, and R. C. Huffaker. 1976. Effect of light and glucose on the induction of nitrate reductase and on the distribution of nitrate in etiolated barley leaves. *Plant Physiol.* 58:588-591.
- Barker, A. V., N. H. Peck, and G. E. MacDonald. 1971. Nitrate accumulation in vegetables. I. Spinach grown in upland soils. *Agron. J.* 63:126-129.
- Barker, A. V., D. N. Maynard, and H. A. Mills. 1974. Variations in nitrate accumulation among spinach cultivars. *J. Am. Soc. Hortic. Sci.* 99:132-134.
- Beevers, L., and R. H. Hageman. 1969. Nitrate reduction in higher plants. *Ann. Rev. Plant. Physiol.* 20:495-522.

- Birdsall, J. J. 1981. Human dietary nitrite intake; and, results of animal feeding studies of nitrite. Paper presented at the Public Meeting of the Committee on Nitrite and Alternative Curing Agents in Food, January 22, 1981, National Research Council, National Academy of Sciences, Washington, D.C. [16] pp.
- Bokhoven, C., and H. J. Niessen. 1961. Amounts of oxides of nitrogen and carbon monoxide in cigarette smoke, with and without inhalation. *Nature* 192:458-459.
- Bosch, H. M., A. B. Rosenfield, R. Huston, H. R. Shipman, and F. L. Woodward. 1950. Methemoglobinemia and Minnesota well supplies. *J. Am. Water Works Assoc.* 42:161-170.
- Broadus, G. M., J. E. York, and J. M. Moseley. 1965. Factors affecting the levels of nitrate nitrogen in cured tobacco leaves. *Tobacco* 161(24):23-32.
- Buege, D. R., M. H. Lee, and R. G. Cassens. 1978. Residual nitrite levels in meat products manufactured by Wisconsin meat processors. Research Division of the College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin. Available from Agricultural Bulletin Building, Madison, Wisconsin as No. A2983. [4] pp.
- California Air Resources Board. 1974. Ten-Year Summary of California Air Quality Data, 1963-1972. Air Analysis Section, Division of Technical Services, California Air Resources Board, Sacramento, California. 211 pp.
- Cantliffe, D. J. 1972. Nitrate accumulation in vegetable crops as affected by photoperiod and light duration [beets, radishes, spinach, beans]. *J. Amer. Soc. Hortic. Sci.* 97:414-418.
- Case, A. A. 1957. Some aspects of nitrate intoxication in livestock. *J. Am. Vet. Med. Assoc.* 130:323-329.
- Cassens, R. G., J. G. Sebranek, G. Kubberod, and G. Woolford. 1974. Where does the nitrite go? *Food Prod. Dev.* 8(10):50-56.
- Cassens, R. G., G. Woolford, S. H. Lee, and R. Goutefongea. 1977. Fate of nitrite in meat. Pp. 95-100 in B. J. Tinbergen and B. Krol, eds. *Proceedings of the Second International Symposium on Nitrite in Meat Products, September 7-10, 1981, Zeist, the Netherlands.* Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Cassens, R. G., M. L. Greaser, T. Ito, and M. Lee. 1979. Reactions of nitrite in meat. *Food Technol. Chicago* 33:46-57.

- Cefalù, M. 1953. [In Italian.] I nitrati nelle acque potabili in rapporto al problema della metemoglobinemia infantile. [Nitrates in potable water and their relation to the problem of methemoglobinemia in infants.] Nuovi Ann. Igiene Microbiol. Roma 4:422-434.
- Christiansen, L. N., R. W. Johnston, D. A. Kautter, J. W. Howard, and W. J. Aunan. 1973. Effect of nitrite and nitrate on toxin production by Clostridium botulinum and on nitrosamine formation in perishable canned comminuted cured meat. Appl. Microbiol. 25:357-362.
- Christie, A. A., R. G. Lidzey, and D. W. F. Radford. 1970. Field methods for determination of nitrogen dioxide in air. Analyst 95:519-524.
- Comer, J. 1978. The application of ion selective electrodes to food analysis. Pp. 197-222 in R. D. King, ed. Developments in Food Analysis Techniques. Applied Science Publishers, Ltd., London, United Kingdom.
- Commoner, B. 1977. Cost-risk-benefit analysis of nitrogen fertilization: A case history. Ambio 6:157-161.
- Coppola, E. D., A. F. Wickroski, and J. G. Hanna. 1976. Nitrite in meat products determined by fluorescence quenching of p-amino-benzoate ion. J. Assoc. Off. Anal. Chem. 59:783-786.
- Corré, W. J., and T. Breimer. 1979. Nitrate and Nitrite in Vegetables. Literature Survey No. 39. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands. 85 pp.
- Cote, W. A., W. A. Wade, III, and J. Yocom. 1974. A Study of Indoor Air Quality. Final Report. U.S. Environmental Protection Agency Report No. EPA-650/4-74-042. U.S. Environmental Protection Agency, Washington, D.C. 282 pp.
- Cox, R. D. 1980. Determination of nitrate and nitrite at the parts per billion level by chemiluminescence. Anal. Chem. 52:332-335.
- Davis, J. G., and F. J. MacDonald. 1953. Richmond's Dairy Chemistry, 5th ed. Chas. Griffin Co. Ltd., London, United Kingdom. 603 pp.
- Davis, R., M. J. Dennis, R. C. Massey, and D. J. McWeeny. 1978. Some effects of phenol- and thiol-nitrosation reactions on N-nitrosamine formation. Pp. 183-197 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.

- Diem, K., and C. Lentner, eds. 1970. Foods: Principal nutrients and minerals. Pp. 498-515 in Scientific Tables, 7th ed. Ciba-Geigy Limited, Basle, Switzerland. Distributed by Geigy Pharmaceuticals, Division of Ciba-Geigy Corporation, Ardsley, New York.
- Doerr, R. C., J. B. Fox, Jr., L. Lakritz, and W. Fiddler. 1981. Determination of nitrite in cured meats by chemiluminescence detection. *Anal. Chem.* 53:381-384.
- Donahoe, W. E. 1949. Cyanosis in infants with nitrates in drinking water as cause. *Pediatrics* 3:308-311.
- Durfor, C. N., and E. Becker. 1964. Public Water Supplies of the 100 Largest Cities in the United States, 1962; Geological Survey Water-Supply Paper 1812. U.S. Government Printing Office, Washington, D. C. Available from U. S. Geological Survey, Reston, Virginia. 364 pp.
- Eaton, W. C., J. N. Howard, Jr., R. M. Burton, F. Benson, and G. H. Ward. 1972. A Preliminary Study of Indoor Air Pollution in a Home Using a Gas Stove. Part I: Oxides of Nitrogen. Human Studies Laboratory, U. S. Environmental Protection Agency, Washington, D. C.
- Ehrenberg, L., S. Hussain, M. N. Saleh, and U. Lundqvist. 1980. Nitrous esters--A genetical hazard from nitrogen oxides (NO_x)? *Hereditas* 92:127-130.
- Elliott, R. J., and A. G. Porter. 1971. A rapid cadmium reduction method for the determination of nitrate in bacon and curing brines. *Analyst London* 96:522-527.
- Emerick, R. J. 1974. Consequences of high nitrate levels in feed and water supplies. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 33:1183-1187.
- Erlandsson, G. B. 1981. Fate of nitrite. Paper submitted to the Committee on Nitrite and Alternative Curing Agents in Food, Executive Office, Assembly of Life Sciences, National Research Council. National Academy of Sciences, Washington, D. C. 4 pp.
- Ferrari, T. E., O. C. Yoder, and P. Filner. 1973. Anaerobic nitrite production by plant cells and tissues: Evidence for two nitrate pools. *Plant Physiol.* 51:423-431.
- Fiddler, R. N. 1977. Collaborative study of modified AOAC method of analysis for nitrite and meat and meat products. *J. Assoc. Off. Anal. Chem.* 60:594-599.

- Follett, M.J., and P. W. Ratcliff. 1963. Determination of nitrite and nitrate in meat products. *J. Sci. Food. Agric.* 14:138-144.
- Food and Drug Administration. 1979. Report: Nitrite Task Force. Unpublished report of the Nitrite Task Force, Bureau of Foods, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C. 43 pp + tables and figures.
- Food and Drug Administration. 1980. Compliance Program Report of Findings: FY 77 Total Diet Studies--Adult (7320.73). Industry Programs Branch (HFF-326), Bureau of Foods, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C. [32] pp.
- Fornal, L., J. Fornal, M. Soral-Smietana, and E. Strzalkowska. 1975. [In Polish; English summary.] [Nitrate and nitrite content in wheat dried in internal-combustion dryers.] *Rocz. Nauk Roln. Ser. C* 71:37-44.
- Fox, J. B., Jr., and R. A. Nicholas. 1974. Nitrite in meat. Effect of various compounds on loss of nitrite. *J. Agric. Food Chem.* 22:302-306.
- Fudge, R., and R. W. Truman. 1973. The nitrate and nitrite contents of meat products--A survey by Public Analysts' laboratories in South Wales and the South West of England. *J. Assoc. Public Anal.* 11:19-27.
- Fujimaki, M., M. Emi, and A. Okitani. 1975. Fate of nitrite in meat-curing model systems composed of myoglobin, nitrite and ascorbate. *Agr. Biol. Chem.* 39:371-377.
- Fuqua, B. D., J. E. Leggett, and J. L. Sims. 1974. Accumulation of nitrate and chloride by burley tobacco. *Can. J. Plant Sci.* 54:167-174.
- Fuqua, B. D., J. L. Sims, J. E. Leggett, J. F. Benner, and W. O. Atkinson. 1976. Nitrate and chloride fertilization effects on yield and chemical composition of burley tobacco leaves and smoke. *Can. J. Plant Sci.* 56:893-899.
- Garman, W. H. 1969. Nitrogen facts and fallacies. *Agric. Chem.* 24(4):10-14.
- Gerritse, R. G. 1979. Rapid simultaneous determination of nitrate and nitrite by high-performance liquid chromatography using ultraviolet detection. *J. Chromatogr.* 171:527-529.

- Gori, G. B. 1976. Low-risk cigarettes: A prescription. *Science* 194:1243-1246.
- Greenberg, R. A. 1977. Nitrosopyrrolidine in United States cured meat products. Pp. 203-210 in B. J. Tinbergen and B. Krol, eds. *Proceedings of the Second International Symposium on Nitrite in Meat Products*, September 7-10, 1976, Zeist, the Netherlands. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Hall, C. B., J. R. Hicks, and R. E. Stall. 1977. Nitrites in inoculated carrot juice as a function of nitrate content and temperature. *J. Food Sci.* 42:549-550.
- Hamilton, J. E. 1976. Collaborative study of the colorimetric determination of nitrate and nitrite in cheese. *J. Assoc. Off. Anal. Chem.* 59:284-288.
- Hänni, H. 1954. [In German; English summary.] Über den Nitratnachweis in Milch. *Mitt. Geb. Lebensmittelunters. Hyg.* 45:502-508.
- Hanway, J. J., J. B. Herrick, T. L. Willrich, P. C. Bennett, and J. T. McCall. 1963. The nitrate problem. Special Report No. 34, Cooperative Extension Service in Agriculture and Home Economics. Iowa State University of Science and Technology, Ames, Iowa. 20 pp.
- Harada, M., Y. Nakamura, and A. Tanimura. 1972. [In Japanese; English summary.] [Studies on nitrosamines in foods. IX. Distribution of nitrite in various foods.] *J. Food Hyg. Soc. Jpn.* 13:36-40.
- Harmeson, R. H., F. W. Sollo, Jr., and T. E. Larson. 1971. The nitrate situation in Illinois [streams]. *J. Am. Water Assoc.* 63:303-310.
- Hartman, P. E. 1981. Nitrates and nitrites: Ingestion, pharmacodynamics and toxicology. Pp. 211-294 in F. J. de Serres and A. Hollaender, eds. *Chemical Mutagens*, Vol. 7. Plenum Press, New York.
- Haynes, R. J., and K. M. Goh. 1978. Ammonium and nitrate nutrition of plants. *Biol. Rev.* 53:465-510.
- Health and Welfare Canada. 1975. Food and Drug Regulations, Amendment. Privy Council Order No. 1975-774. *Canada Gazette*, Part II, 109:757-762 (April 23, 1975).
- Heimer, Y. M., and P. Filner. 1971. Regulation of the nitrate assimilation pathway in cultured tobacco cells. III. The nitrate uptake system. *Biochim. Biophys. Acta* 230:362-372.

- Heisler, E. G., J. Siciliano, S. Krulick, W. L. Porter, and J. W. White, Jr. 1973. Nitrate and nitrite content of market potatoes. *J. Agric. Food Chem.* 21:970-973.
- Helms, G. T., J. H. Southerland, K. R. Woodard, I. J. Hindawi, and D. H. Coventry. 1970. Chattanooga, Tennessee--Rossville, Georgia. Interstate Air Quality Study, 1967-1968. National Air Pollution Control Administration. U. S. Government Printing Office, Washington, D. C. 120 pp. Available from the National Technical Information Service, Springfield, Virginia as PB-195 145.
- Herring, H. K. 1973. Effect of nitrite and other factors of the physico-chemical characteristics and nitrosamine formation in bacon. Pp. 47-60 in *Proceedings of the Meat Industry Research Conference, March 22-23, 1973. Meeting Sponsored by the American Meat Science Association in cooperation with the American Meat Institute Foundation, both of Chicago, Illinois.*
- Herrmann, K. 1972. [In German.] Über den Nitrat- und Nitritgehalt des Gemüses, Obstes und Wassers und deren Bedeutung für die Ernährung. *Ernaehr. Umsch.* 11:398-402.
- Hochheiser, S. 1965. Field comparison of methods of determining atmospheric nitric oxide and nitrogen dioxide. P. 124 in *Preprints of Papers Presented at the 150th National Meeting of the American Chemical Society, September 12-17, 1965, Atlantic City, N. J. American Chemical Society, Washington, D. C.*
- Hoffmann, D., G. Rathkamp, and Y. Y. Liu. 1975. Chemical studies on tobacco smoke. XXVI. On the isolation and identification of volatile and non-volatile N-nitrosamines and hydrazines in cigarette smoke. Pp. 159-165 in P. Bogovski and E. A. Walker, eds. *N-Nitroso Compounds in the Environment, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.*
- Hoffmann, D., T. C. Tso, and G. B. Gori. 1980. The less harmful cigarette. *Prev. Med.* 9:287-296.
- Hoffmann, D., J. D. Adams, K. D. Brunnemann, and S. S. Hecht. In press. Formation, occurrence and carcinogenicity of N-nitrosamines in tobacco products. In R. A. Scanlan and S. R. Tannenbaum, eds. *N-Nitroso Compounds, ACS Symposium Series. American Chemical Society, Washington, D. C.*
- Horwitz, W., ed. 1975. *Official Methods of Analysis of the Association of Official Analytical Chemists, 12th ed. Association of Official Analytical Chemists, Arlington, Virginia. 1094 pp.*

- Høyem, T. 1974. Nitrate and nitrite contents in Norwegian food. Pp. 466-470 in Proceedings of the IV International Congress of Food Science and Technology. Available from Instituto de Aroquímica y Tecnología de Alimentos, c/ Jaime Roig, 11, Valentia - 10, Spain.
- Huguet, C., M. Bonafous, and G. Ducaillar. 1976. [In French; English summary.] Étude sur la présence des nitrates dans les fruits a pépins et a noyau. *Ann. Nutr. Aliment.* 30:673-682.
- Intersociety Committee. 1972. *Methods of Air Sampling and Analysis.* American Public Health Association, Washington, D. C. 480 pp.
- Jackson, W. A., J. S. Steel, and V. R. Boswell. 1967. Nitrates in edible vegetables and vegetable products. *Proc. Am. Soc. Hortic. Sci.* 90:349-352.
- Jacobs, M. B., and S. Hochheiser. 1958. Continuous sampling and ultramicrodetermination of nitrogen dioxide in air. *Anal. Chem.* 30:426-428.
- Jägerstad, M., and R. Nilsson. 1976. Intake of nitrate and nitrite of some Swedish consumers as measured by the duplicate portion technique. Pp. 283-287 in B. J. Tinbergen and B. Krol, eds. *Second International Symposium on Nitrite in Meat Products, September 7-10, 1976, Zeist, the Netherlands.* Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Jägerstad, M., Å. Nordén, and R. Nilsson. 1976. Dietary intake of nitrate and nitrite using the duplicate-sampling portion technique. *Ambio* 6:276-277.
- Jenkins, R. A., and B. E. Gill. 1980. Determination of oxides of nitrogen (NO_x) in cigarette smoke by chemiluminescent analysis. *Anal. Chem.* 52:925-928.
- Kačmár, P., and M. Bartík. 1965. Certain quantitative relations of nitrate and nitrite metabolism in farm animals. IV. Normal nitrate levels in the organs and body liquids of swine. *Folia Vet.* 9:59-63.
- Kamm, L., G. G. McKeown, and D. M. Smith. 1965. New colorimetric method for the determination of the nitrate and nitrite content of baby foods. *J. Assoc. Off. Agric. Chem.* 48:892-897.
- Keating, J. P., M. E. Lell, A. W. Strauss, H. Zarkowsky, and G. E. Smith. 1973. Infantile methemoglobinemia caused by carrot juice. *N. Engl. J. Med.* 288:824-826.

- Kelly, G. J., and E. Latzko. 1980. Prospect of a specific enzymatic assay for ascorbic acid (vitamin C). *J. Agric. Food Chem.* 28:1320-1321.
- Kemp, J. D., J. D. Fox, and W. G. Moody. 1974. Cured ham properties as affected by nitrate and nitrite and fresh pork quality. *J. Food Sci.* 39:972-976.
- Kemp, J. D., B. E. Langlois, J. D. Fox, and W. Y. Varney. 1975. Effects of curing ingredients and holding times and temperatures on organoleptic and microbiological properties of dry-cured sliced ham. *J. Food Sci.* 40:634-636.
- Kenny, T. A., and P. E. Walshe. 1975. Nitrate and nitrite contents of vegetables and fruit in Ireland. *Ir. J. Agric. Res.* 14:349-355.
- Kerr, R. H., C. T. N. Marsh, W. F. Schroeder, and E. A. Boyer. 1926. The use of sodium nitrite in the curing of meat. *J. Agric. Res.* 33:541-551.
- Kilgore, L., A. R. Stasch, and B. F. Barrentine. 1963. Nitrate content of beets, collards, turnip greens. *J. Am. Diet. Assoc.* 43:39-42.
- Kilgore, L., A. R. Stasch, and B. F. Barrentine. 1964. Relation of ascorbic acid to nitrate content of turnip greens and to methemoglobin formation. *Am. J. Clin. Nutr.* 14:52-55.
- Klepper, R. 1978. Nitrogen fertilizer and nitrate concentrations in tributaries of the upper Sangamon River in Illinois. *J. Environ. Qual.* 7:13-22.
- Klepper, L. A. 1979. An improved method for nitrite extraction from plants. *J. Agric. Food Chem.* 27:438-441.
- Kohl, D. H., G. B. Shearer, and B. Commoner. 1971. Fertilizer nitrogen: Contribution to nitrate in surface water in a corn belt watershed. *Science* 174:1331-1334.
- Kolari, O. E., and W. J. Aunan. 1972. The residual levels of nitrite in cured meat products. P. 422-434 in Proceedings of the 18th Meeting of Meat Research Workers, University of Guelph, Ontario, Canada.
- Krehl, W. A., and R. W. Winters. 1950. Effect of cooking methods on retention of vitamins and minerals in vegetables. *J. Am. Diet. Assoc.* 26:966-972.

- Larson, T. E. 1963. Mineral Content of Public Ground Water Supplies in Illinois, Circular No. 90. Illinois State Water Survey, Urbana, Illinois. 28 pp.
- Larson, T. E., and L. Henley. 1966. Occurrence of nitrate in well water. Final Report. Project 65-OSG. University of Illinois Water Resources Center, Urbana, Illinois. 8 pp. + tables and figures.
- Lee, D. H. K. 1970. Nitrates, nitrites, and methemoglobinemia. *Environ. Res.* 3:484-511.
- Lee, C. Y., R. S. Shallenberger, D. L. Downing, G. S. Stoewsand, and N. M. Peck. 1971. Nitrate- and nitrite-nitrogen in fresh, stored and processed table beets and spinach from different levels of field nitrogen fertilisation. *J. Sci. Food Agric.* 22:90-92.
- Lee, C. Y., G. S. Stoewsand, and D. L. Downing. 1972. Nitrate problems in foods. *N. Y. Food Life Sci. Q.* 5:8-9.
- Lemoigne, M., P. Monguillon, and R. Desveaux. 1937. [In French.] Réduction de l'acide nitreux par les végétaux supérieurs. Première phase de la réaction. Rôle de l'acide ascorbique. *Bull. Soc. Chim. Biol.* 19:1350-1360.
- Leuenberger, U., R. Gouch, K. Rieder, and E. Baumgartner. 1980. Determination of nitrate and bromide in foodstuffs by high-performance liquid chromatography. *J. Chromatogr.* 202:461-468.
- Lewis, T. R. 1980. Criteria relevant to an occupational health standard for nitrogen dioxide. Pp. 361-375 in S. D. Lee, ed. *Nitrogen Oxides and Their Effects on Health.* Ann Arbor Science, Ann Arbor, Michigan.
- Li, M., P. Li, and B. Li. 1980. Recent progress in research on esophageal cancer in China. *Adv. Cancer Res.* 33:173-249.
- Liedtke, M. A., and C. E. Meloan. 1976. Rapid screening determination of nitrate in baby food using the nitrate-selective electrode. *J. Agric. Food Chem.* 24:410-412.
- Lin, J.-K., and J.-Y. Yen. 1980. Changes in the nitrate and nitrite contents of fresh vegetables during cultivation and post-harvest storage. *Food Cosmet. Toxicol.* 18:597-603.
- Lorenz, O. A. 1978. Potential nitrate levels in edible plant parts. Pp. 201-219 in D. R. Nielsen and J. G. MacDonald, eds. *Nitrogen in the Environment.* Academic Press, New York, San Francisco, and London.

- Luh, B. S., N. Ukai, and J. I. Chung. 1973. Effects of nitrogen nutrition and day temperature on composition, color and nitrate in tomato fruit. *J. Food Sci.* 38:29-33.
- Lyng, R. 1978. Testimony. Pp. 103 in Food Safety and Quality: Nitrites, Part III. Hearings before the Subcommittee on Agricultural Research and General Legislation, September 13, 14, 15, and 25, 1978, Committee on Agriculture, Nutrition, and Forestry, United States Senate, 95th Congress, 2nd session. U. S. Government Printing Office, Washington, D. C.
- Manning, P. B., S. T. Coulter, and R. Jenness. 1968. Determination of nitrate and nitrite in milk and dry milk products. *J. Dairy Sci.* 51:1725-1730.
- Martinoia, E., U. Heck, and A. Wiemken. 1981. Vacuoles as storage compartments for nitrate in barley leaves. *Nature* 289:292-294.
- Massey, R. C., M. J. Dennis, C. Crews, D. J. McWeeny, and R. Davies. 1980. Model system studies on N-nitrosamine formation in relation to cured meat: The non-polar phase and S-nitroso peptides. Pp. 291-303 in E. A. Walker, M. Castegnaro, L. Gričiute, and M. Börzsönyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Matsui, H. 1944. [In Japanese.] [On the oxidizing substance which appears in Shiozuke extracts of various vegetables.] *J. Agric. Chem. Soc. Jpn.* 16:1167-1168.
- Maynard, D. N. 1978. Potential nitrate levels in edible plant parts. Pp. 221-233 in D. R. Nielsen and J. G. MacDonald, eds. Nitrogen in the Environment, Vol. 2, Soil-Plant-Nitrogen Relationship. Academic Press, New York.
- Maynard, D. N., and A. V. Barker. 1972. Nitrate content of vegetable crops. *HortScience* 7:224-226.
- Maynard, D. N., and A. V. Barker. 1974. Nitrate accumulation in spinach as influenced by leaf-type. *J. Am. Soc. Hortic. Sci.* 99:135-138.
- Maynard, D. N., A. V. Barker, P. L. Minotti, and N. H. Peck. 1976. Nitrate accumulation in vegetables. *Adv. Agronomy* 28:71-118.
- McCabe, L. J., J. M. Symons, R. D. Lee, and G. G. Robeck. 1970. Survey of community water supply systems. *J. Am. Water Works Assoc.* 62:670-687.

- McCaslin, B. D., W. T. Franklin, and M. A. Dillon. 1970. Rapid determination of nitrate nitrogen in sugarbeets with the specific ion electrode. *J. Am. Soc. Sugar Beet Technol.* 16:64-70.
- McNamara, A. S., L. A. Klepper, and R. H. Hageman. 1971. Nitrate content of seeds of certain crop plants, vegetables, and weeds. *J. Agric. Food Chem.* 19:540-542.
- Melia, R. J. W., C. du V. Florey, S. C. Darby, E. D. Palmes, and B. D. Goldstein. 1978. Differences in NO₂ levels in kitchens with gas or electric cookers. *Atmos. Environ.* 12:1379-1381.
- Mills, H. A., A. V. Barker, and D. N. Maynard. 1976. Effects of nitrapyrin [the nitrification suppressor] on nitrate accumulation in spinach. *J. Am. Soc. Hortic. Sci.* 101:202-204.
- Minotti, P. L., and D. L. Stankey. 1973. Diurnal variation in the nitrate concentration of beets. *HortScience* 8:33-34.
- Mirvish, S. S., L. Wallcave, M. Eagen, and P. Schubik. 1972. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. *Science* 177:65-68.
- Mizusaki, S., H. Okamoto, A. Akiyama, and Y. Fukuhara. 1977a. Relation between chemical constituents of tobacco and mutagenic activity of cigarette smoke condensate. *Mutat. Res.* 48:319-325.
- Mizusaki, S., T. Takashima, and K. Tomaru. 1977b. Factors affecting mutagenic activity of cigarette smoke condensate in Salmonella typhimurium TA 1538. *Mutat. Res.* 48:29-36.
- Moschandreas, D. J., J. W. C. Stark, J. E. McFadden, and S. S. Morse. 1978. *Indoor Air Pollution in the Residential Environment, Vol. 1: Data Collection, Analysis and Interpretation.* U. S. Environmental Protection Agency. Report No. EPA 600/7-78-229a. Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Research Triangle Park, North Carolina. 201 pp. Available from National Technical Information Service, Springfield, Virginia as PB-290 999/ZW.
- National Academy of Sciences. 1977a. *Drinking Water and Health, Vol. 1.* A report prepared for the U. S. Environmental Protection Agency by the Safe Drinking Water Committee, Advisory Center on Toxicology, Assembly of Life Sciences, National Research Council. National Academy of Sciences, Washington, D. C. 939 pp.

- National Academy of Sciences. 1977b. Nitrogen Oxides. A report prepared for the U. S. Environmental Protection Agency by the Subcommittee on Nitrogen Oxides, Committee on Medical and Biological Effects of Environmental Pollutants, National Research Council. National Academy of Sciences, Washington, D. C. 333 pp.
- National Academy of Sciences. 1978. Nitrates: An Environmental Assessment. A report prepared for the U. S. Environmental Protection Agency by the Panel on Nitrates of the Coordinating Committee for Scientific and Technical Assessments of Environmental Pollutants, Environmental Studies Board, Commission on Natural Resources, National Research Council. National Academy of Sciences, Washington, D. C. 723 pp.
- National Institute for Occupational Safety and Health. 1976. Criteria for a Recommended Standard: Occupational Exposure to Oxides of Nitrogen. DHEW Publ. No. NIOSH 76-149. National Institute for Occupational Safety and Health, U. S. Department of Health, Education, and Welfare, Washington, D. C. 196 pp.
- Newmark, H. L., and W. J. Mergens. 1981. Alpha-tocopherol (vitamin E) and its relationship to tumor induction and development. Pp. 127-168 in M. Zedeck and M. Lipkin, eds. Inhibition of Tumor Induction and Development. Plenum Press, New York.
- Nicholas, R. A., and J. B. Fox. 1973. Critical evaluation of the AOAC method of analysis for nitrite in meat. J. Assoc. Off. Anal. Chem. 56:922-925.
- Nordin, H. R. 1969. The depletion of added sodium nitrite in ham. Can. Inst. Food Technol. J. 2:79-85.
- Norman, V., and C. H. Keith. 1965. Nitrogen oxides in tobacco smoke. Nature 205:915-916.
- Oda, H., H. Tsubone, H. Suzuki, T. Ichinose, and K. Kubota. 1981. Alterations of nitrite and nitrate concentrations in the blood of mice exposed to nitrogen dioxide. Environ. Res. 25:294-301.
- Olday, F. C., A. V. Barker, and D. N. Maynard. 1976. A physiological basis for different patterns of nitrate accumulation in two spinach cultivars. J. Am. Soc. Hortic. Sci. 101:217-219.
- Palmes, E. D., C. Tomczyk, and J. Dimattio. 1977. Average NO₂ concentration in dwellings with gas or electric stoves. Atmos. Environ. 11:869-872.
- Panalaks, T., J. R. Iyengar, and N. P. Sen. 1973. Nitrate, nitrite, and dimethylnitrosamine in cured meat products. J. Assoc. Off. Anal. Chem. 56:621-625.

- Panalaks, T., J. R. Iyengar, B. A. Donaldson, W. F. Miles, and N. P. Sen. 1974. Further survey of cured meat products for volatile N-nitrosamines. *J. Assoc. Off. Anal. Chem.* 57:806-812.
- Parks, N. J., K. A. Krohn, C. A. Mathis, J. H. Chasko, K. R. Geiger, M. E. Gregor, and N. F. Peek. 1981. Nitrogen-13-labeled nitrite and nitrate: Distribution and metabolism after intratracheal administration. *Science* 212:58-61.
- Paul, J. L., and R. M. Carlson. 1968. Nitrate determination in plant extracts by the nitrate electrode. *J. Agric. Food Chem.* 16:766-768.
- Pedersen, E., J. Thomsen, and H. Werner. 1980. Investigations on formation and occurrence of volatile nitrosamines in Danish cheese. Pp. 493-501 in E. A. Walker, M. Castegnaro, L. Gričiute, and M. Bőrzsönyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Pennington, J. A. T., and H. N. Church. 1980. Pp. 136-148 in Bowes and Church's Food Values of Portions Commonly Used. Harper & Row, New York, Cambridge, Hagerstown, Philadelphia, San Francisco, London, Mexico City, São Paulo, and Sydney.
- Pfeiffer, S. L., and J. Smith. 1975. Nitrate determination in baby food, using the nitrate ion selective electrode. *J. Assoc. Off. Anal. Chem.* 58:915-919.
- Phillips, W. E. J. 1966. Effect of dietary nitrite on the liver storage of vitamin A in the rat. *Can. J. Biochem.* 44:1-7.
- Phillips, W. E. J. 1968a. Changes in the nitrate and nitrite contents of fresh and processed spinach during storage. *J. Agric. Food Chem.* 16:88-91.
- Phillips, W. E. J. 1968b. Nitrate content of foods--Public health implications. *Can. Inst. Food Technol. J.* 1:98-103.
- Phillips, W. E. J. 1971. Naturally occurring nitrate and nitrite in foods in relation to infant methaemoglobinaemia. *Food Cosmet. Toxicol.* 9:219-228.
- Pivnick, H., L. J. Rubin, H. W. Barnett, H. R. Nordin, P. A. Ferguson, and C. H. Perrin. 1967. Effect of sodium-nitrite and temperature on toxinogenesis by Clostridium botulinum in perishable cooked meats vacuum-packed in air-impermeable plastic pouches. *Food Technol.* 21:204-206.

- Rammell, C. G., and M. M. Joerin. 1972. Determination of nitrite in cheese and other dairy products. *J. Dairy Res.* 39:89-94.
- Richardson, W. D. 1907. The occurrence of nitrates in vegetable foods, in cured meats and elsewhere. *J. Am. Chem. Soc.* 29:1757-1767.
- Ridder, W. E., and F. W. Oehme. 1974. Nitrates as an environmental animal, and human hazard. *Clin. Toxicol.* 7:145-159.
- Rooma, M. Ya. 1971. [In Russian; English summary.] [The content of nitrates, nitrites and hydroxylamines in food products.] *Gig. Sanit.* 36(8):46-50.
- Saffigna, P. G., and D. R. Kenney. 1977. Nitrate and chloride in ground water under irrigated agriculture in central Wisconsin. *Ground Water* 15:170-177.
- Saltzman, B. E. 1954. Colorimetric microdetermination of nitrogen dioxide in the atmosphere. *Anal. Chem.* 26:1949-1955.
- Sander, J. 1967. [In German; English summary.] Kann Nitrit in der menschlichen Nahrung Ursache einer Krebsentstehung durch Nitrosaminbildung sein? *Arch. Hyg. Bakteriol.* 15:22-28.
- Schuphan, W. 1974. The significance of nitrates in food and potable waters. *Qualitas Plantarum-Plant Foods for Human Nutrition* 24:19-36.
- Sebranek, J. G. 1974. Studies on the Ultimate Fate and Distribution of Nitrite in a Cured Meat Product. Ph.D. thesis, University of Wisconsin, Madison, Wisconsin. 203 pp.
- Selenka, F., and D. Brand-Grimm. 1976. [In German; English summary.] [Nitrate and nitrite in human food calculation of the daily intake and estimation of its range.] *Zbl. Bakt. Hyg., I. Abt. Orig. B.* 162:449-466.
- Sen, N. P., and M. McPherson. 1978. Interference by ascorbic acid in the determination of nitrite by the separate color reagent addition technique. *J. Food Saf.* 1:247-255.
- Sen, N. P., J. R. Iyengar, B. A. Donaldson, and T. Panalaks. 1974. Effect of sodium nitrite concentration on the formation of nitrosopyrrolidine and dimethylnitrosamine in fried bacon. *J. Agric. Food Chem.* 22:540-541.

- Sen, N. P., B. A. Donaldson, and C. Charbonneau. 1975. Formation of nitrosodimethylamine from the interaction of certain pesticides and nitrite. Pp. 75-79 in P. Bogovski and E. A. Walker, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Sen, N. P., B. Donaldson, S. Seaman, B. Collins, and J. Y. Iyengar. 1977. Recent nitrosamine analyses in cooked bacon. *J. Inst. Can. Sci. Technol. Aliment.* 10:A13-A15.
- Shaner, D. L., and J. S. Boyer. 1976. Nitrate reductase activity in maize (*Zea mays* L.) leaves. I. Regulation by nitrate flux. *Plant Physiol.* 58:499-504.
- Siciliano, J., S. Krulick, E. G. Heisler, J. H. Schwartz, and J. W. White, Jr. 1975. Nitrate and nitrite content of some fresh and processed market vegetables. *J. Agric. Food Chem.* 23:461-464.
- Sims, J. L., L. P. Bush, and W. O. Atkinson. 1970. Alkaloid and nitrate nitrogen concentrations of two isogenic strains of burley tobacco. *J. Agric. Food Chem.* 18:381-384.
- Sims, J. L., W. O. Atkinson, and F. Benner. 1979. Nitrogen fertilization and genotype effects on selected constituents of smoke from all-burley cigarettes. *Tob. Sci.* 23:11-13.
- Skovgaard, N. 1980. [In Danish; English summary.] Nitrat, nitrit og nitrosaminer i levnedsmidler. Forbedret tilvirkningshygiejne som alternativ til nitrit. Nitrate, nitrite, and nitrosamines in foods. [Improved production hygiene as an alternative to nitrite]. *Nord. Veterinaermed.* 32:387-399.
- Smith, G. E. 1966. Causes of nitrate accumulation in plants and water supplies. Paper presented at the 18th Annual Midwest Fertilizer Conference, Chicago, Ill.
- Sohier, Y., A.-M. Poumarat, and P. Berges. 1976. [In French.] Les nitrates dans les épinards en conserve: Influence des traitements technologiques. *Ann. Nutr. Alim.* 30:689-694.
- Spalding, R. F., G. A. Junk, and J. J. Richard. 1980. Pesticides in ground water beneath irrigated farmland in Nebraska, August 1978. *Pestic. Monit. J.* 14:70-73.
- Statens Levnedsmiddelinstitut [State Food Institute]. 1981. [In Danish.] Nitrat og Nitrit i Kødvarer. Rapport fra in Intern Arbejdsgrube [Report from the Internal Working Group]. Søborg, Denmark. 63 pp.

- Stephany, R. W., and P. L. Schuller. 1978. The intake of nitrate, nitrite and volatile N-nitrosamines and the occurrence of volatile N-nitrosamines in human urine and veal calves. Pp. 443-460 in E. A. Walker, M. Castegnaro, L. Gričute, and R. E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Thayer, J. R., and R. C. Huffaker. 1980. Determination of nitrate and nitrite by high-pressure liquid chromatography: Comparison with other methods for nitrate determination. Anal. Biochem. 102:110-119.
- Tremp, E. 1980. [In German; English summary.] Die Belastung der schweizerischen Bevölkerung mit Nitraten in der Nahrung. Mitt. Geb. Lebensmittelunters. Hyg. 71:182-194.
- Turek, B., D. Hlavsová, J. Tuček, J. Waldman, and J. Černá. 1980a. [In Czechoslovakian; English summary.] [Hygienic importance of nitrates in living environment.] Cesk. Hyg. 25:301-305.
- Turek, B., D. Hlavsová, J. Tuček, J. Waldman, and J. Černá. 1980b. The fate of nitrates and nitrites in the organism. Pp. 625-632 in E. A. Walker, M. Castegnaro, L. Gričute, and M. Bőrzsönyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- U. S. Department of Agriculture. 1926. Regulations Governing the Meat Inspection of the United States Department of Agriculture. BAI Order 211, Amendment 4, Revision. Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D.C. 1 p.
- U. S. Department of Agriculture. 1976. Methodology for Large-Scale Surveys of Household and Individual Diets. Home Economics Research Report No. 40, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C. 88 pp.
- U. S. Department of Agriculture. 1978a. Final Report on Nitrites and Nitrosamines. Report to the Secretary of Agriculture by the Expert Panel on Nitrites and Nitrosamines. Food Safety and Quality Service, U. S. Department of Agriculture, Washington, D. C. 127 pp.
- U. S. Department of Agriculture. 1978b. Nitrates, nitrites, and ascorbates (or isoascorbates) in bacon. Fed. Regist. 43(95):20992-20995.

- U. S. Department of Agriculture. 1980. Food and Nutrient Intakes of Individuals in 1 Day in the United States, Spring, 1977. Nationwide Food Consumption Survey 1977-78 Preliminary Report No. 2. Science and Education Administration, U. S. Department of Agriculture, Washington, D. C. 121 pp.
- U. S. Environmental Protection Agency. 1971a. Air Quality Criteria for Nitrogen Oxides. Air Pollution Control Office Publication No. AP-84. U. S. Government Printing Office, Washington, D. C. 154 pp. + appendix.
- U. S. Environmental Protection Agency. 1971b. National primary and secondary ambient air quality standards. Fed. Regist. 36(84):8186-8201.
- U. S. Environmental Protection Agency. 1972. National ambient air quality standards: Approval and promulgation of state implementation plans. Fed. Regist. 37:11826.
- U. S. Environmental Protection Agency. 1973. Compilation of Air Pollutant Emission Factors, 2nd Ed.; Publ. No. AP-42. Office of Air Quality Planning and Standards, Office of Air and Water Programs, U. S. Environmental Protection Agency. U. S. Government Printing Office, Washington, D. C. 290 pp.
- U. S. Environmental Protection Agency. 1976a. Nitrogen dioxide in the atmosphere: Measurement principle and calibration procedure. Fed. Regist. 41(53):11258-11266.
- U. S. Environmental Protection Agency. 1976b. National primary and secondary ambient air quality standards: Nitrogen dioxide measurement principle and calibration procedure. Fed. Regist. 41(232):52686-52692.
- Usher, C. D., and G. M. Telling. 1975. Analysis of nitrate and nitrite in foodstuffs: Critical review. J. Sci. Food Agric. 26:1793-1805.
- Voogt, V. P. 1969. [In German; English summary.] Die Bestimmung von Nitrat im Spinat mittels einer nitratselektiven Elektrode. Dtsch. Lebensm. Rundsch. 65:196-198.
- Wade, H. A., H. B. Elkins, and B. P. W. Ruotolo. 1950. Composition of nitrous fumes from industrial processes. Arch. Ind. Hyg. Occup. Med. 1:81-89.
- Wade, W. A., III, W. A. Cote, and J. E. Yocom. 1975. Indoor air quality. J. Air Pollut. Control Assoc. 25:933-939.

- Walker, R. 1976. Naturally occurring nitrate/nitrite in foods. *J. Sci. Food Agric.* 26:1735-1742.
- Walters, C. L., M. J. Downes, R. J. Hart, S. Perse, and P. L. R. Smith. 1978. Determination of nitrite at low level without prior extraction. *Z. Lebensm. Unters. Forsch.* 167:229-232.
- Walton, G. 1951. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. *Am. J. Public Health* 41:986-995.
- Whelan, M. 1935a. Determination of nitrate in animal tissues. *J. Lab. Clin. Med.* 20:755-757.
- Whelan, M. 1935b. XC. The nitrate content of animal tissues, and the fate of ingested nitrate. *Biochem. J.* 29:782-787.
- White, J. W., Jr. 1975. Relative significance of dietary sources of nitrate and nitrite. *J. Agric. Food Chem.* 23:886-891.
- White, J. W., Jr. 1976. Correction: Relative significance of dietary sources of nitrate and nitrite. *J. Agric. Food Chem.* 24:202.
- Whitehead, D. C., L. H. P. Jones, and R. J. Barnes. 1978. The influence of fertiliser N plus K on N, S and other mineral elements in perennial ryegrass at a range of sites. *J. Sci. Food Agric.* 29:1-11.
- Wilson, J. K. 1949. Nitrate in foods and its relation to health. *Agron. J.* 41:20-22.
- Woerner, F., and A. Fricker. 1960. [In German.] Die Nitrat- und Nitritbestimmung in Käse verschiedenen Alters. *Dtsch. Molk. Ztg.* 81:1345-1348.
- Woll, R. S. 1978. Maryland Ground-Water Information: Chemical Quality Data. Water Resources Basic-Data Report No. 10, Department of Natural Resources, Maryland Geological Survey, Merryman Hall, The Johns Hopkins University, Baltimore, Maryland. 125 pp. + maps and tables.
- World Health Organization. 1978. Nitrates, Nitrites, and N-Nitroso Compounds: Environmental Health Criteria No. 5. World Health Organization, Geneva, Switzerland. Available from WHO Publications Centre, Albany, New York. 107 pp.
- Wright, M. J., and K. L. Davison. 1964. Nitrate accumulation in crops and nitrate poisoning in animals. *Adv. Agron.* 16:197-247.

Wu, K.-Y., and C. E. McDonald. 1976. Effect of nitrogen fertilizer on nitrogen fractions of wheat and flour. *Cereal Chem.* 53:242-249.

Wynder, E. L., and D. Hoffmann. 1968. Experimental tobacco carcinogenesis. *Science* 162:862-871.

Yanagihara, T., H. Komoda, H. Yoneyama, and M. Yamada. 1963. Nitrate content in juice of various vegetables. *J. Food Hyg. Soc. Jpn.* 4:343-347.

CHAPTER 6

ENVIRONMENTAL DISTRIBUTION AND EXPOSURE OF HUMANS TO NITROSATABLE SUBSTRATES AND MODIFIERS OF NITROSATION REACTIONS

| | <u>Page</u> |
|---|-------------|
| NITROSATABLE SUBSTRATES: AMINO COMPOUNDS AND RELATED SUBSTANCES..... | 6-3 |
| Environmental Distribution..... | 6-4 |
| Occupational Exposure..... | 6-13 |
| Endogenous Production..... | 6-13 |
| Possible Exposure of Humans to Amino Compounds..... | 6-14 |
| Summary and Discussion..... | 6-14 |
| AGENTS ENHANCING OR INHIBITING <u>IN VIVO</u> NITROSATION..... | 6-14 |
| Catalysts..... | 6-16 |
| Inhibitors..... | 6-19 |
| Summary..... | 6-21 |
| OVERALL SUMMARY AND CONCLUSIONS..... | 6-22 |
| RECOMMENDATIONS..... | 6-23 |
| REFERENCES..... | 6-24 |

CHAPTER 6

ENVIRONMENTAL DISTRIBUTION AND EXPOSURE OF HUMANS TO NITROSATABLE SUBSTRATES AND MODIFIERS OF NITROSATION REACTIONS

Exposure of humans to nitrosating agents (nitrate, nitrite, and nitrogen oxides) may contribute to the formation of N-nitroso compounds in vivo if nitrosatable substrates such as amines and amides are also present. Thus, data on the environmental distribution and exposure of humans to these nitrosatable substances will provide further indication of the potential for in vivo nitrosation reactions. Chemical agents that may inhibit or enhance the in vivo formation of N-nitroso compounds must also be considered when determining the overall potential for in vivo nitrosation. Thus, a review of data concerning the environmental distribution and exposure of humans to the various chemicals that may participate in, enhance, or block the formation of N-nitroso compounds is also included in this chapter.

NITROSATABLE SUBSTRATES: AMINO COMPOUNDS AND RELATED SUBSTANCES

Amino compounds are a diverse group of chemicals whose reactivity with nitrosating agents varies considerably among the different compounds (see Chapter 4) and also varies according to the environmental medium containing the compounds (e.g., air, food, or drugs). Most secondary amines, N-alkylureas, and N-alkylcarbamates react readily with nitrite to produce N-nitroso compounds, whereas primary, tertiary, and quaternary amines, simple N-alkylamides, and N-alkylguanidines usually react much more slowly to form N-nitroso compounds. The environmental distribution of these compounds is reviewed in this section as are data suggesting that amino compounds in the environment can react with nitrosating agents to form N-nitroso compounds.

The ubiquity and large numbers of amino compounds in the environment precludes a review of all such chemicals in this report. Thus, for discussion purposes, this chapter provides examples of the major amino compounds present in each environmental medium. For the same reasons, the committee has made no attempt to estimate the exposure of humans to these compounds. Also, since most of the reports in the literature pertain to volatile amines, the discussion will focus on exposure to this class of amines. However, certain non-volatile amines are also nitrosatable and may be important in understanding total exposure of humans to nitrosatable substances.

Environmental Distribution

Food. Food is a major source of a variety of nitrosatable amines (Table 6-1). Keay and Hardy (1972) described a gas-liquid chromatographic method for the separation of dimethylamine and trimethylamine in fish as a means for monitoring spoilage since these were the major amines present. In addition to these two compounds, volatile amines including methylamine, ethylamine, n-propylamine, and isopropylamine were found in uncured pork by Patterson and Mottram (1974), who reported that the concentrations of dimethylamine, trimethylamine, n-propylamine, and isopropylamine increased during the manufacture of bacon. Patterson and Edwards (1975) reported that dimethylamine and trimethylamine reached sufficiently high concentrations in spoiled pork meat to lead to the formation of nitrosodimethylamine (NDMA).

Among the amines that have been measured in pork bellies are the free amino acids -- proline, alanine, isoleucine, leucine, methionine, phenylalanine, tyrosine, valine, glutamic acid, cysteine, and aspartic acid -- as well as hydroxyproline and N-methylglycine (sarcosine). The concentration of most of the amino acids (especially proline) increases during storage (Lakritz *et al.*, 1976). Proline, hydroxyproline, and N-methylglycine can be nitrosated to form nitrosamines. For example, Janzowski *et al.* (1978) suggested that nitrosohydroxyproline is probably formed in foods from hydroxyproline during processing with nitrate and/or nitrite. However, there is no evidence that N-nitroso compounds formed from amino acids are carcinogenic (see Chapter 9).

Fujinaka *et al.* (1976) found detectable levels of methylguanidine in fish and meat products consumed in the Japanese diet. They estimated that the daily intake was approximately 125 μ g. The authors speculated that the precursor of methylguanidine might be creatinine or creatine, both of which are abundant in meats. High concentrations of a related guanidine derivative, agmatine, were found in the muscles of fresh abalone and top-shell and in dried squid (Kawabata *et al.*, 1978).

Recently, Mirvish and Cairnes (1981) reported that creatinine was the precursor to methylurea formed in a Japanese dried bonito fish. In this study, the nitrosation of creatinine followed by a denitrosation reaction resulted in the formation of methylurea, possibly via nitrosomethylurea. High concentrations of creatine (3 to 6 g/kg) and its dehydration product creatinine (150 to 200 mg/kg) are found in fresh pork and beef (Velišek *et al.*, 1975). Nitrosation products of the reaction of creatinine with sodium nitrite under acidic conditions were identified as creatinine-5-oxime and 1-methylhydantoin-5-oxime, and nitrososarcosine was formed from creatine (Archer *et al.*, 1971). A dried fish product and fried bacon were found to contain creatinine at 2 to 3 g/kg (Mirvish and Cairnes, 1981). Since these nitrosation reactions occurred with a very large molar excess of sodium nitrite, it is doubtful whether these compounds could be formed in nitrite-containing meat, unless there were high local concentrations of nitrite.

Sen et al. (1973) found that nitrosopiperidine (NPiP) and nitrosopyrrolidine (NPYR), both cyclic nitrosamines, were present in nitrite-treated black pepper and paprika, respectively. The NPiP in pepper was probably derived by the nitrosation of the tertiary amine piperine. However, the obvious precursors, pyrrolidine or piperidine, have not been found in these spices. Singer and Lijinsky (1976a) have reported that piperidine and pyrrolidine are present in milk and meat products.

Polyamines such as cadaverine, putrescine, spermidine, and spermine were found in the germs of cereals such as barley, rice, oats, corn, wheat, and sorghum (Moruzzi and Calderera, 1964). Analysis of amines in pork and hams indicated that the concentrations of these polyamines were higher than those of monoamines, including histamine, typtamine, tryamine, and ethanolamine (Lakritz et al., 1975).

Maga (1978) has prepared a comprehensive review of the various amines in food.

Drugs. During the past decade, considerable attention has been directed toward the nitrosation of drugs that are secondary and tertiary amines. Examples of secondary amine drugs include phemmetrazine (an anorexic), which was nitrosated to nitrosophemmetrazine in vivo in rabbits and rats (Lijinsky and Taylor, 1976), morpholine (an anesthetic), which can be converted to nitrosomorpholine (NMOR), and piperazine (an antihelmintic that yields mono- and dinitroso piperazine). Nitrosamines produced by nitrosation of some drugs are listed in Table 6-2.

Lijinsky and Greenblatt (1972) found that aminopyrine, which is a tertiary amine used as an analgesic, reacts with sodium nitrite under mildly acidic conditions both in vivo and in vitro to produce the potent carcinogen NDMA. Lijinsky et al. (1972) reported that a number of tertiary amine drugs reacted with nitrite under acidic conditions. Among these drugs are oxytetracycline (an antibiotic), aminopyrine (an analgesic), disulfiram (an antialcoholic), nikethamide (a respiratory stimulant), and tolazamide (an oral hypoglycemic). Two tertiary amines (oxytetracycline and aminopyrine) produced high yields of NDMA, whereas the dialkylamides gave rather lower yields. Tolazamide, which is a dialkylhydrazine and a substituted urea, formed nitrosohexamethyleneimine -- a potent liver carcinogen in rats. Nitrosation of tertiary amine drugs may not proceed through the amines (Lijinsky and Singer, 1975). These same authors did report, however, that such nitrosation reactions could occur at body temperature. Furthermore, long-chain aliphatic tertiary amines give higher yields than do the short-chain compounds (Lijinsky and Singer, 1975).

TABLE 6-1

Some Amino Compounds in Food

| <u>Compound</u> | <u>Source</u> | <u>Reference</u> |
|--|---|---|
| <u>Alkyl amines:</u> | | |
| Dimethylamine | Fish meal, fish products, cereals, teas Cod Pork, bacon Ham, frankfurters, evaporated milk, coffee, tea, beer, wine | Lijinsky and Epstein, 1970 Keay and Hardy, 1972 Patterson and Mottram, 1974; Patterson and Edwards, 1975 Singer and Lijinsky, 1976a |
| Diethylamine | Fish meal, fish products, cereals, teas Pork, bacon manufacture | Lijinsky and Epstein, 1970 Patterson and Mottram, 1974 |
| Trimethylamine | Cod Pork, bacon | Keay and Hardy, 1972 Patterson and Mottram, 1974; Patterson and Edwards, 1975 |
| Methylamine) Ethylamine) n-Propylamine) Isopropylamine) | Uncured and cured pork, bacon manufacture | Patterson and Mottram, 1974 |
| Dipropylamine | Fish | Singer and Lijinsky, 1976a |
| Methylethylamine | Coffee | Singer and Lijinsky, 1976a |
| Methyl-n-butyl- amine | Evaporated milk | Singer and Lijinsky, 1976a |
| <u>Other monoamines:</u> | | |
| Histamine) Tryptamine) Tyramine) Ethanolamine) | Pork, cured and smoked ham | Lakritz <u>et al.</u> , 1975 |
| <u>Amino acids:</u> | | |
| Proline | Pork bellies Protein | Lakritz <u>et al.</u> , 1976 Mirvish <u>et al.</u> , 1973 |
| Hydroxyproline | Collagen | Mirvish <u>et al.</u> , 1973 |
| Sarcosine (N-methylglycine) | Muscles of cartilaginous and ganoid fishes | Mirvish <u>et al.</u> , 1973 |

| | | |
|--|--|--|
| Alanine) | | |
| Glycine) | | |
| Valine) | | |
| Leucine) | | |
| Isoleucine) | | |
| Aspartic acid) | Pork bellies | Lakritz <u>et al.</u> , 1976 |
| Glutamic acid) | | |
| Phenylalanine) | | |
| Tyrosine) | | |
| Methionine) | | |
| Cystine) | | |
| <u>Cyclic amines:</u> | | |
| Pyrrolidine (also formed from heating putrescine) | Spices (including paprika) Cooked meat and fish, wine Evaporated milk, coffee, wine | Sen <u>et al.</u> , 1973 Lijinsky and Epstein, 1970 Singer and Lijinsky, 1976a |
| Piperidine (also formed from heating) cadaverine | Spices (including black pepper) Cooked meat and fish Ham, evaporated and whole milk, coffee | Sen <u>et al.</u> , 1973 Lijinsky and Epstein, 1970 Singer and Lijinsky, 1976a |
| 3-Hydroxy- pyrrolidine | Cured meats | Janzowski <u>et al.</u> , 1978 |
| Morpholine | Unintentional additive to foods such as canned hams Fish, ham, frankfurters, coffee, beer, wine | Shank and Newberne, 1972 Singer and Lijinsky, 1976a |
| <u>Polyamines:</u> | | |
| Spermine) Spermidine) Putrescine) | Pork, cured ham, soybeans, cereals | Lakritz <u>et al.</u> , 1975; Moruzzi and Caldarera, 1964 |
| Cadaverine | Pork, cured ham, fish, cereals | Lakritz <u>et al.</u> , 1975; Moruzzi and Caldarera, 1964 |
| <u>Miscellaneous:</u> | | |
| Methylguanidine | Fresh, dried, or canned fish and meat Fish, beef | Fujinaka <u>et al.</u> , 1976 Kawabata <u>et al.</u> , 1978 |
| Agmatine (decarboxy- lated arginine) | Muscles of fresh albalone and top-shell | Kawabata <u>et al.</u> , 1978 |
| Creatine) Creatinine) | Fresh pork and beef, constituent of blood plasma, fried bacon | Velišek <u>et al.</u> , 1975; Mirvish and Cairnes, 1981 |

TABLE 6-2

Reaction of Drugs with Nitrite^a

| <u>Drug</u> | <u>Use</u> | <u>Nitrosamine Formed</u> |
|---|---------------------|----------------------------|
| Aminopyrine | Analgesic | NDMA |
| Chlorpheniramine | Antihistamine | NDMA |
| Chlorpromazine | Tranquilizer | NDMA |
| Cyclizine | Motion sickness | NDMA |
| Dextropropoxyphene | Tranquilizer | NDMA |
| Disulfiram | Antialcoholic | NDMA |
| Hexahydroazepinyl- nitropropiofenone | Antihashish agent | Nitrosohexamethyleneimine |
| Lucanthone | Antischistosomiasis | Nitrosodiethylamine (NDEA) |
| Methadone | Narcotic | NDMA |
| Methapyrilene | Antihistamine | NDMA |
| Nikethamide | Stimulant | NDMA |
| Oxytetracycline | Antibiotic | NDMA |
| Quinacrine | Antimalarial | NDEA |
| Tolazamide | Hypoglycemic | Nitrosohexamethyleneimine |

^a From Lijinsky, 1974.

Scheunig and Ziebarth (1976) studied the nitrosation of more than 30 drugs. In addition to aminopyrine, they found that analgine (whose structure is similar to that of aminopyrine) and piperazine produced nitrosamines when incubated with sodium nitrite in the gastric juice of humans.

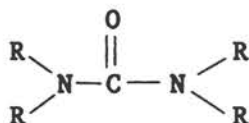
Eisenbrand *et al.* (1978) found concentrations of NDMA ranging from 10 to 371 $\mu\text{g}/\text{kg}$ in 68 drugs containing aminopyrine. They speculated that the nitrosation of aminopyrine, which contains a dimethyl-amino group, might be caused by the high reactivity of the solid drug with nitrogen oxides, even in the presence of ascorbic acid. Since nitric oxide and nitrogen dioxide can reach levels of approximately 1.9 mg/m^3 (1 ppm) in urban atmospheres (Chapter 5), NDMA might be easily formed and accumulate on the surface of the drug.

Cimetidine has been used effectively for 5 years to treat peptic ulcers and disorders of the esophagus, stomach, and duodenum (Jensen and Magee, 1981). The drug can be nitrosated to nitrosocimetidine which can methylate DNA *in vitro* in a manner similar to the reaction of the related compound 1-methyl-3-nitro-1-nitrosoguanidine, which is known to be carcinogenic in animals.

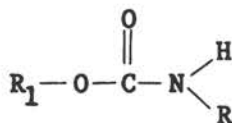
Cosmetics. Although many amines may be present in cosmetic products and are listed in the CTFA Cosmetic Ingredient Dictionary (Cosmetic, Toiletry and Fragrance Association, 1977), triethanolamine (an antiallergenic) is of special interest to the Food and Drug Administration (Wenninger, 1980) because 2-bromo-2-nitro-1,3-propanediol (bronopol), which is used as a preservative, may nitrosate this compound, resulting in the formation of nitrosodiethanolamine (NDELA), which does occur in cosmetics (see Chapter 7) (Ong and Rutherford, 1980; Schmeltz and Wenger, 1979).

In an ongoing investigation, Hecht (1981) has conducted model nitrosation studies with some typical cosmetic ingredients and sodium nitrite over a range of pH values at 37°C or 90°C. The compounds investigated included stearalkonium chloride, lauramine oxide, dimethylstearamine, and triisopropanolamine. Nitrosation of stearalkonium chloride produced nitrosobenzylmethylamine (NBMA), nitrosomethylstearylamine (NMSA), and NDMA. Nitrosation of lauramine oxide produced nitrosododecylmethylamine (NDOMA), nitrosation of dimethylstearamine gave NMSA, and nitrosation of triisopropanolamine gave nitrosodiisopropanolamine (NDILA). The yields of these reactions varied from 0.3% to 31.9%, depending on the precursor and conditions of the experiment.

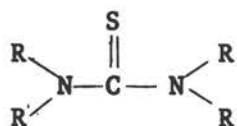
Agricultural Chemicals. Many agricultural chemicals are derivatives of alkylureas and alkylcarbamic esters, and such compounds can react with nitrite under mild acidic conditions to form N-nitroso derivatives (Elespuru and Lijinsky, 1973). Examples of these derivatives are:



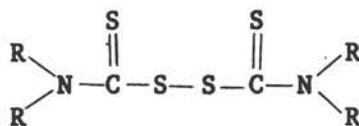
N-Alkylurea



N-Alkylcarbamate



N-Alkylthiourea

Bis(dialkylthiocarbamyl)
disulfide

Nitrosatable insecticides, fungicides, and pesticides include such compounds as aldicarb [2-methyl-2-(methylthio)propanol O-[(methylamino)carbonyl]oxime], Baygon [2-(1-methylethoxy)phenol methylcarbamate], Bux-Ten [3-(1-ethylpropyl)phenyl methylcarbamate and 3-(1-methylbutyl)phenyl methylcarbamate], carbaryl (1-naphthalenol methylcarbamate), carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate), Landrin (mixture of 2,3,5- and 3,4,5-trimethylphenyl methylcarbamate), and methomyl (N-[(methylamino)carbonyl]oxy)ethanimidothioic acid methyl ester). The last compound produced forestomach tumors in rats after nitrosation (Lijinsky and Schmähl, 1978).

Eisenbrand et al. (1975a) have studied the nitrosation of atrazine (2-chloro-4-ethylamino-6-isopropylamine-S-triazine), simazine (6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine), and thiram (tetramethylthioperoxydicarbonic diamide); and Sen et al. (1975) reported the formation of NDMA from the reaction of nitrite with ferbam [tris(dimethylcarbamodithioato-S,S')iron] and succinic acid 2,2-dimethylhydrazide. Nitrosation of carbaryl with nitrite occurs at pH 1, which can be reached in the stomach. Subcutaneous administration of a single high dose (1,000 mg/kg) of nitrosocarbaryl to Wistar rats induced local sarcomas at the site of injection (Eisenbrand et al., 1975b).

More recently, Oliver (in press) observed 0.09% to 1.54% in vivo nitrosation of carbaryl by nitrite in the stomachs of guinea pigs at pH 1.3 to 1.6, which is similar to the normal range of pH in the human stomach. Extrapolation of these data to a 70 kg human consuming 100 g of produce containing 5 μ mol of insecticide would result in the formation of 11.2 μ g of nitrosocarbaryl. Oliver postulated that nitrite is the most probable nitrosating agent in soil and that it accumulates in alkaline soils during nitrification. However, the author pointed out that the formation of nitrosamines in natural media (e.g, soil, air, and water) requires artificially high concentrations of added nitrite.

In organic solvents, lipid-soluble pesticides such as carbaryl react very readily with nitrogen oxides (Mirvish et al., 1978). Therefore, nitrosation by nitrogen oxides may be more likely than nitrosation by nitrite (Mirvish et al., 1978).

Air. The amount of information regarding nitrosatable amines in the ambient air is limited. In addition to the many unidentified amines that could be produced during combustion processes, in sewage, in lubricating oils, etc., Iqbal et al. (1980) have reported the presence of morpholine in the ambient air. Two nitrosation products -- NDMA and NMOR -- have been found in crankcase emissions from diesel engines (Goff et al., 1980), and Fine et al. (1976a,b,c) and Fan et al. (1976) have identified NDMA as an air pollutant, mainly outside factories that use amines.

Water. Although there are no specific data concerning the presence of nitrosatable substrates in drinking water, it is likely that such amines as pesticides and herbicides are carried into water supplies by run-off from soil and, possibly, chemical dumps.

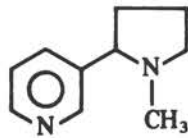
Tobacco. The occurrence of nitrosatable amines in tobacco smoke has been studied and summarized by Hoffmann et al. (1980). Precursor amines for volatile, nonvolatile, and tobacco-specific nitrosamines are derived from protein, agricultural chemicals, and alkaloids in tobacco products (Hoffmann et al., in press). Tobacco smoke also contains nitric oxide and trace amounts of nitrogen dioxide and nitrous oxide (Chapter 5). Schmeltz and Hoffmann (1977) have identified more than 600 nitrogen-containing compounds in tobacco smoke.

Singer and Lijinsky (1976b) have observed that pyrrolidine and dimethylamine were predominant among the naturally occurring secondary amines in unburned tobacco and cigarette smoke condensate. Among the volatile amines in tobacco, methylamine and aniline occur in the highest concentrations. Although Spincer and Westcott (1976) measured levels of dimethylamine and nitrogen oxides in smoke from different tobaccos, they believe that it would be difficult to make quantitative predictions of nitrosamine formation from measurements of nitrosamine precursors. Although NDEA and NPYR have been found in tobacco, their amine precursors have not been identified (Hoffmann et al., in press).

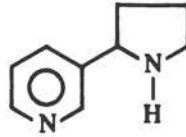
The chemical structures of some common tobacco alkaloids, including nicotine, and their concentration in cigarettes, are presented in Figure 6-1. Hoffmann et al. (in press) have shown that three of these alkaloids -- nicotine, nor nicotine, and anabasine -- are nitrosated during the processing and smoking of tobacco.

Trace amounts of some aromatic amines are found in trace amounts in cigarette smoke. Among these compounds are β -naphthylamine, o-toluidine, and 4-aminobiphenyl (U.S. Public Health Service, 1979). Much higher concentrations of aromatic amines are found in sidestream smoke (smoke from the burning cigarette tip) than in mainstream smoke (smoke that passes through the cigarette) (Patrianakos and Hoffmann, 1979).

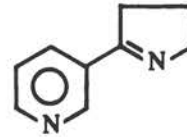
Hoffmann et al. (in press) have listed several amines, amides, and carbonates in agricultural chemicals used for the cultivation of tobacco crops. Among these compounds are dimethyldodecylamine acetate, maleic hydrazide diethanolamine, and carbaryl, which have been found in small quantities in harvested tobacco. Thus far, only diethanolamine has been studied as a possible precursor of nitrosamines in tobacco and in tobacco smoke.



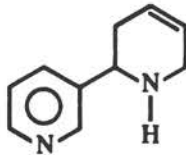
Nicotine
1,000-25,000



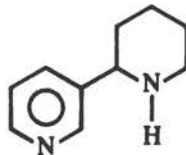
Nor nicotine
100-1,000



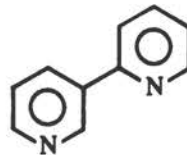
Myosmine
50-150



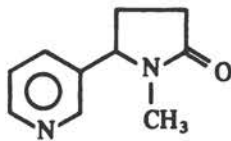
Anatabine
100-1,000



Anabasine
80-200



3,2'-Bipyridyl
10-150



Cotinine
40-200

FIGURE 6-1. Chemical structures of major tobacco alkaloids in U.S. cigarette tobacco and the concentrations ($\mu\text{g/g}$) in which they have been found in cigarettes. From Hoffmann et al., in press.

Occupational Exposure

The industrial production of nitrosamines and their precursors has been reviewed by the U.S. Environmental Protection Agency (1977). Included in this document are sources of nitrosatable amines such as methyl-, ethyl-, n-propyl-, n-butyl-, n-hexyl-, dimethyl-, and trimethyl-amine, which have been identified in air as pollutants resulting from the decomposition of manure from livestock, including poultry. These amines may react with nitrite in wastes or on dust or with atmospheric nitrogen oxides. Exhausts from rendering plants in which animal parts are cooked also produce such amines as ethyl-, diethyl-, and triethylamine as well as putrescine and cadaverine. Amines produced by industry include antioxidants (e.g., aryl and alkyl amines used to inhibit oxidation of lubricants), vulcanization accelerators (e.g., thiram sulfides and dialkyldithiocarbamates), pharmaceuticals, self-polishing waxes and corrosion inhibitors (e.g., morpholine), pesticides, synthetic detergents (e.g., dimethylamine), solvents (e.g., dimethylamine used in synthesis of dimethylformamide), animal glues, and photographic and leather products. The EPA report also contains production figures for amines, but the list is probably incomplete.

Analytical monitoring of occupational environments will probably lead to the identification of additional nitrosamines and their precursors. Fine (1978) has prepared a review of the exposure of humans to nitrosamines from industrial emissions and commercial products.

Endogenous Production

In addition to the amines from exogenous sources, humans are exposed to amines formed in the body. For example, bacteria in the gut can produce secondary amines from amino acid precursors (Asatoor et al., 1967; Johnson, 1977):

| | | |
|-----------|---|---------------|
| lysine | → | piperidine |
| ornithine | → | pyrrolidine |
| arginine | → | pyrrolidine |
| choline | → | dimethylamine |

Other amino products may be formed by the metabolism of tryptophan in the liver and by gut flora. The amino compounds are excreted in the urine (Hill, 1980).

Table 6-3 lists some endogenous amines and their sources.

Endogenous nitrosatable amides include waste products such as urea, creatinine, and uric acid. These substrates might be metabolized by gut bacteria to unknown compounds. Wrong (1978) has provided evidence suggesting that creatinine is converted to a number of metabolites including methylguanidine, 1-methylhydantoin, and sarcosine.

Possible Exposure of Humans to Amino Compounds

Quantitative data on exogenous exposure and endogenous production of amines have not been adequately developed. Thus, no estimates of human exposure have been developed by the committee.

Summary and Discussion

Amino compounds that may interact with nitrite to form N-nitroso compounds are present in the environment. Foods, drugs, cosmetics, agricultural chemicals (e.g., pesticides), and tobacco products are all significant sources of nitrosatable amino compounds. Amines may also be present in drinking water, although this subject has not been specifically studied. Amines are probably not present to a significant extent in the air, except in certain environments such as animal feedlots. In addition to exogenous sources of amines, nitrosatable amino compounds are synthesized in vivo.

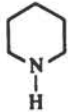

Although there are no quantitative data on exogenous exposure and only few data on the endogenous production of amines, it is likely that sufficient quantities of amines are present in humans to participate in nitrosation reactions under appropriate conditions. It is also probable that certain environments, as well as exposure to tobacco and certain drugs, will result in increased exposure to these compounds. A sufficient body of evidence demonstrates that amines derived from both exogenous and endogenous sources could participate in in vivo nitrosation reactions to produce nitrosamines, many of which are carcinogenic.

AGENTS ENHANCING OR INHIBITING IN VIVO NITROSATION

The formation of N-nitroso compounds can be increased, decreased, or even completely blocked by the presence of certain agents in the reaction mixture (Douglass et al., 1978) (see Chapter 4). The ability of many chemicals to catalyze and inhibit nitrosation has been demonstrated in simple chemical systems (Boyland et al., 1971; Mirvish et al., 1972), in model food systems (Gray and Dugan, 1975; Massey et al., 1978), and in simulated gastric or salivary contents (Tannenbaum et al., 1977; Ziebarth and Scheunig, 1976). Nitrosation reactions have also been demonstrated in vivo in assays for acute hepatotoxicity

TABLE 6-3

Endogenous Amines

| <u>Amine</u> | <u>Structure</u> | <u>Source</u> | <u>Reference</u> |
|---------------------|--|---|--|
| Betaine | $(\text{CH}_3)_3\text{N}^+-\text{CH}_2-\text{COO}^-$ | Widely distributed in plants and animals | Fiddler <i>et al.</i> , 1972 |
| Cadaverine | $\text{H}_2\text{N}-(\text{CH}_2)_5-\text{NH}_2$ | Bacterial decarboxylation of lysine | Johnson, 1977 |
| Carnitine | $(\text{CH}_3)_3\text{N}^+-\text{CH}_2-\underset{\text{OH}}{\text{CH}}-\text{CH}_2-\text{COO}^-$ | Constituent of striated muscle and liver; meat | Fiddler <i>et al.</i> , 1972 |
| Choline | $(\text{CH}_3)_3\text{NCH}_2\underset{\text{OH}}{\text{CH}_2}$ | Metabolism of lecithin by bacterial phospholipases; can also be dealkylated to form dimethylamine | Johnson, 1977 |
| N,N-Dimethylglycine | $\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{N}-\text{CH}_2-\text{COOH} \\ \diagup \\ \text{CH}_3 \end{array}$ | Unknown | Friedman and McClanahan, 1973; Mirvish, 1975 |
| Neurine chloride | $\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_2=\text{CH}-\text{N}^+\text{CH}_3 \\ \\ \text{CH}_3 \end{array} \text{Cl}^-$ | Egg yolk, bile in cadavers | Fiddler <i>et al.</i> , 1972 |
| Piperidine |  | Metabolism of lysine by gut flora | Hawksworth and Hill, 1971; Hill, 1979; Johnson, 1977 |
| Putrescine | $\text{H}_2\text{N}-(\text{CH}_2)_4-\text{NH}_2$ | Bacterial decarboxylation of ornithine | Johnson, 1977 |
| Pyrrolidine |  | Metabolism of ornithine and arginine by gut flora | Johnson, 1977 |

(Cardesa et al., 1974; Greenblatt, 1973; Kamm et al., 1973; Lijinsky and Greenblatt, 1972), in carcinogenicity assays (Ivankovic et al., 1975; Mirvish et al., 1975) and in biological materials that were analyzed for the presence of N-nitroso compounds (Kamm et al., 1975).

The assessment of the significance of catalysts and inhibitors for the in vivo formation of N-nitroso compounds requires a discussion of the environmental distribution and possible exposure of humans to both catalysts and inhibitors. The catalysts that are considered in this section are thiocyanate, halide ions, and certain phenols. The inhibitors reviewed herein include ascorbic acid, bisulfite ion, and several phenols and thiols. Not all modifying agents of possible significance in vivo are included; rather, the discussions concern agents that illustrate some of the complexities involved in predicting both qualitative and quantitative outcome of nitrosation reactions. Although information on possible exposures of humans may indicate which chemicals could be important in vivo, the absolute intake of any one substance is less meaningful in predicting catalysis or inhibition than is the intake of these modifying agents relative to the intake of nitrate, nitrite, and nitrosatable substrates.

Other important factors that determine the effect of catalysts and inhibitors are the timing of intake (relative to the intake of nitrate, nitrite, and amino substrates), the persistence of the various substances in vivo, and the reactivity of the modifying agents. For example, approximately 25% of ingested nitrate is recycled in the bloodstream to the mouth where some of it is reduced to nitrite. This contribution of salivary nitrite to the total nitrite content of the stomach will continue over time. Thus, in order to be effective, inhibitors of nitrosation should either be persistent or administered frequently.

In addition, both the pH and the nature of the medium (i.e., hydrophilic or lipophilic) influence the outcome of nitrosation reactions in the presence of modifying agents. Much of the information about the mediatory effects of various compounds has been obtained from investigations with model systems that have used relatively high concentrations of single compounds in homogeneous media. Thus, the extrapolation of these findings to the heterogeneous conditions that may exist in vivo may be an oversimplification and may lead to the possibility of synergistic or interfering effects being overlooked.

Catalysts

Several nucleophilic anions, especially thiocyanate and iodide, may catalyze the nitrosation reactions of nitrite under acidic conditions in the stomach, but not at the neutral pH of normal saliva.

Under the same conditions, certain phenols that are capable of undergoing C-nitrosation may also enhance the formation of N-nitroso compounds. On the other hand, aldehydes and polyhydric alcohols act as catalysts at pH above 7.5 and might be important to nitrosation reactions in the lung, mouth, hypoaacidic stomach, or lower gastrointestinal tract.

Of the anionic catalysts studied thus far, thiocyanate has been found to have the greatest effect (Boyland, 1972; Boyland *et al.*, 1971; Fan and Tannenbaum, 1973; Mirvish, 1975). In one study of factors influencing the rate at which morpholine is nitrosated, Fan and Tannenbaum (1973) found that the optimal pH for catalysis by thiocyanate was 2.3 and that catalysis did not occur as readily above pH 3 and below pH 2.

Both the dependency on pH and the intensity of thiocyanate catalysis are strongly influenced by the structure of the amino substrate. Thus, no pH maximum is observed for the nitrosation of weakly basic amines such as N-methylaniline, where catalysis by thiocyanate is strong (Boyland *et al.*, 1971). By contrast, thiocyanate does not catalyze the nitrosation of ureas, amides, guanidines, and urethanes (see Chapter 4).

Halide ions are also catalytic. Iodide is more reactive than bromide, which is more reactive than chloride (Boyland, 1972; Fan and Tannenbaum, 1973; Schweinsberg, 1975). The effect of pH on these compounds is very similar to that described for thiocyanate (Fan and Tannenbaum, 1973).

Nitrosation by the thiocyanate-NO and halide-NO reagents should be more important for low rather than high nitrite concentrations because the concentration of N_2O_3 (which regulates noncatalyzed nitrosation of secondary amines) depends on the square of the concentration of nitrite, whereas the concentration of the nitrosating agents in the presence of thiocyanate and halide ions (SCN-NO and X-NO) depends simply on the level of nitrite. As a consequence, the levels of nitrosamines formed *in vivo*, when the concentration of nitrite is low, may be underestimated if the reaction is assumed to occur via N_2O_3 and if the presence of catalytic anions is not taken into consideration.

All phenolic compounds can combine irreversibly with nitrosating agents to form nitroso products, which can catalyze the formation of nitrosamines from nitrous acid under acidic conditions (see Chapter 4). Phenols that can act as catalysts following their nitrosation are monohydroxy compounds without 2-, 4-, or 6-substituents and 1,3-dihydroxy compounds. Thus, certain phenols, including a group of naturally occurring compounds (e.g., catechin, quercetin, and kaempferol) tested by Pignatelli *et al.* (1980), can catalyze the formation of nitrosamines by nitrous acid, when the concentration of nitrous acid exceeds that

of the phenol. However, a large excess of the phenol can inhibit the nitrosations.

Environmental Distribution and Exposure of Humans to Catalysts

Thiocyanate is present in the saliva of humans at concentrations ranging from 11.7 to 33 mg/100 ml (Diem, 1962); in the saliva of smokers, concentrations of this compound are nearly 3 to 4 times higher (Boyland and Walker, 1975; Druckrey et al., 1967; Schievelbein et al., 1969). The anion is also present in gastric secretions at 1 mg/100 ml (Lane and Bailey, 1973).

Varying amounts of iodide occur in food, water, medications, and air (Underwood, 1977). The most important sources include iodized salt, bread, milk, marine fish, and other seafood (Kidd et al., 1974). Average dietary intake ranges from 0.24 to 0.74 mg/day (Oddie et al., 1970).

Bromide is found in certain medications and brominated vegetable oils. The average daily intake has not been estimated.

The chloride ion has been shown to catalyze the diazotization of aromatic amines, which proceeds via formation of the primary nitrosamine (Ridd, 1961), but it has never been shown to catalyze the formation of N-nitroso compounds. The occurrence of chloride is briefly reviewed below in case future studies demonstrate that it does have an effect on nitrosation.

Chloride is found primarily in food (mainly as sodium chloride) and water. Rich dietary sources include table salt, breakfast cereals, breads, dried skim milk, teas, eggs, margarine, salted butter, bacon, ham, salted beef, canned meats, canned fish, canned vegetables, salted snack foods, and olives (Harper et al., 1977). Dietary levels of chloride depend largely on the intake of table salt. Sodium chloride alone is estimated to provide from 2,400 to 14,400 mg/day (Dahl, 1960). The EPA has estimated that the average chloride content of drinking water is 21 mg/liter (U.S. Environmental Protection Agency, 1975). Assuming that 2 liters of water are consumed daily, this source may contribute 42 mg/day or just less than 2% of the lower estimate for the daily intake from sodium chloride.

Many of the phenols that are capable of catalyzing nitrosation reactions are found in plants and, hence, in food prepared from these plants. The average daily intake of phenols in the diet has not been determined; however, the total phenolic content of certain fruits and vegetables may be quite high. For example, the phenolic content of unripe persimmons is 3 g/kg (Nakayama and Chichester, 1963). Coffee and tea may contribute 1 g to the total intake of phenols per day (Anonymous, 1969). However, when considering the intake of phenols from fruits and vegetables, one should remember

that most of these compounds are likely to act as inhibitors rather than as catalysts of nitrosation reactions. The inhibitory action of these compounds is discussed in the following section.

Inhibitors

In general, the most effective inhibitors of nitrosation reactions act by rapidly reducing the nitrosating agent to either nitric oxide or nitrogen. Ascorbic acid (vitamin C) blocks the formation of nitrosamines in a number of systems (Archer *et al.*, 1975; Bruce *et al.*, 1979; Ivankovic *et al.*, 1975; Kamm *et al.*, 1975; Mirvish *et al.*, 1972). This agent is most effective at pH 1 to 4 in the absence of oxygen when its concentration is at least equal to that of nitrite (Archer *et al.*, 1975; Mirvish, 1981a). As expected, the amines that are rapidly nitrosated are less susceptible to inhibition than are the ones that are nitrosated more slowly (Mirvish, 1981b). Because vitamin C is water soluble, it is less effective in lipophilic media (Pensabene *et al.*, 1976).

α -Tocopherol (vitamin E) is also capable of blocking nitrosation reactions. It can be as effective as ascorbic acid, but only in lipophilic media or in emulsions (Kamm *et al.*, 1977; Mergens *et al.*, 1978; Tannenbaum and Mergens, 1980; Walters *et al.*, 1976). Again, the relative concentrations of the inhibitor and nitrite are important in determining the outcome. The optimum pH range for the reaction is 2 to 3. As the pH is increased, the reaction between α -tocopherol and nitrite slows down until the pH reaches 5, when less than 5% of the nitrite reacts with this agent (Mergens *et al.*, 1978); however, the formation of nitrosamines also decreases with increasing pH.

Certain phenols can also inhibit the formation of nitroso compounds by competing with the amino substrates and combining irreversibly with the nitrosating species. In the presence of excess nitrosating agent, however, subsequent interactions between the nitrosated phenol and the nitrosating agent may then catalyze the formation of nitrosamines (see above). Other phenols, which contain two hydroxyl groups in the 1,2 or 1,4 positions, inhibit the formation of nitroso compounds by reducing the nitrosating agent to nitric oxide. In principle, these phenols should be as effective as ascorbic acid as blocking agents when used at pH 1 to 4 under anaerobic conditions and when the concentration of phenol is at least equal to that of nitrite.

Sodium bisulfite has also been extensively tested. At a strongly acidic pH, the effectiveness of this compound has been found to equal that of ascorbic acid and α -tocopherol (Mirvish, 1975). At pH 1 to 4, bisulfite reduces nitrite in two steps: first to nitric oxide and then to nitrous oxide (Hisatsune, 1961).

Thiols that are present in meat as cysteine and glutathione also reduce acidified nitrite to nitric oxide through the formation of nitrite thioesters. These interactions may inhibit the formation of nitroso compounds. However, thionitrite esters are stable above pH 7 and in lipophilic media (Davies *et al.*, 1978), and they may promote rather than inhibit the formation of nitrosamines under such conditions.

Environmental Distribution and Exposure of Humans to Inhibitors

Ascorbic acid is present in a variety of fresh fruits and vegetables in a wide range of concentrations. For example, oranges contain 50 mg/100 g, whereas apples contain 4 mg/100 g (U.S. Department of Agriculture, 1963). The concentration in fresh foods may be reduced considerably during storage or cooking. Ascorbic acid or ascorbate is also added to many food items, including baked goods, cereals, milk, frozen dairy products, meat (including cured meats), poultry, fish, processed vegetables, jam, soups, nonalcoholic beverages, beer, candy, and infant formulas.

The U.S. Department of Agriculture (1980) has estimated that the average daily dietary intake of vitamin C is 87 mg/person. However, a committee of the National Academy of Sciences (1973) estimated that the daily intake of ascorbic acid added to food could be as high as 550 mg. Not included in either of these figures is the contribution of vitamin supplements. According to unpublished data from the same USDA survey (personal communication), approximately 24% of all Americans surveyed were taking some form of vitamin supplementation. An additional 7% were specifically taking vitamin C supplements; however, the quantities were not reported.

α -Tocopherol is a natural constituent of certain foods. Fresh pork bellies may contain up to 20 mg/kg, which declines to less than 5 mg/kg upon processing into bacon. Other dietary sources include milk products, poultry, gelatin, soups, breakfast cereals, and infant formulas. Current estimates of daily intake of α -tocopherol derived from foods range from 3.8 to 11.8 mg/day (U.S. Department of Agriculture, personal communication). However, if concentrations of α -tocopherol added to foods are also considered, an estimated 54.9 mg may be consumed per person per day (National Academy of Sciences, 1973). α -Tocopherol may also be present in multiple vitamin formulations taken by 24% of the U.S. population, and it is taken by itself as a supplement by 4% those surveyed by the USDA (personal communication). However, the unesterified form of α -tocopherol is required to block nitrosamine formation. That form of the vitamin occurs naturally in foods. Most vitamin supplements contain the acetate ester form of the vitamin, which does not act as a blocking agent (Tannenbaum and Mergens, 1980).

Tannins (tannic acid), propyl gallate, vanillin, chlorogenic acid, and thymol are phenols that may have an inhibitory effect on the formation of N-nitroso compounds. Tannic acid is a naturally occurring component of foods prepared from plants. The total intake of tannins may be as high as 1 g per day for persons who drink coffee and tea (Singleton and Kratzer, 1973). Chlorogenic acid is found in coffee and in many plant materials. The possible exposure of humans to phenols that are added to foods may be 3.9 mg/day for propyl gallate; up to 500 mg/day for vanillin (if all forms of vanilla are added together); and 3.5 mg for thymol (National Academy of Sciences, 1973). Concentrations of phenols as high as 300 mg/kg have also been found in smoked meats (Knowles et al., 1975).

Sodium bisulfite is a food additive. It is used in baked goods, processed fruits and vegetables, beverages, and relishes. Daily intake has been estimated to be 187.2 mg/day (National Academy of Sciences, 1973).

Free thiol groups, equivalent to 21-25 mM, have been found in meat (Hamm and Hofmann, 1966).

The amounts of inhibitors ingested by humans are not as important as the ratio of inhibitors to nitrite in the stomach. Thus, determining the level of nitrite present in human gastric juice is an important first step in determining the concentration of the inhibitor that is required (Ruddell et al., 1977) (see Chapter 8). In an investigation conducted in humans, Ohshima and Bartsch (1981) studied the amount of inhibitors required to block N-nitroso compound formation. These investigators showed that the formation of nitrosoproline following ingestion of proline and red beet juice was completely inhibited by a large excess of ascorbic acid and partially blocked by an excess of α -tocopherol. The implication of this finding for predicting the amount of inhibitors generally needed to block nitrosation in the human stomach, where most in vivo nitrosation is likely to occur, remains unknown.

In addition, the persistence of the inhibitor in the stomach is also an important factor since nitrite may be continually introduced into the stomach from swallowed saliva. Investigators have found that tissue levels of α -tocopherol can be raised in many organs of rats by supplementing their diet with the vitamin (Mergens et al., 1978). The persistence of α -tocopherol in certain organs, such as the stomach and lung, may make this inhibitor of nitrosation reactions of greater importance than inhibitors that do not persist as long.

Summary

A complex mixture of the various substrates, catalysts, and inhibitors of nitrosation reactions may occur within the human body. The in vivo concentrations and reactivity of these compounds vary

with the amounts ingested and the conditions prevailing in the saliva and the stomach. Because of the many variables involved, it is impossible to predict the outcome of reactions within such mixtures solely on the basis of the estimated intakes of catalysts and inhibitors, even when such data are compared to intakes of the reactants. Hence, although it seems likely that modifying agents play a role in in vivo nitrosation reactions, the eventual outcome cannot be predicted with any degree of precision.

Although one study in humans has demonstrated that the nitrosation of proline was inhibited by ascorbic acid and α -tocopherol, the amount of inhibitors required for this blocking effect remains unknown. Determining the nitrite content of gastric juice at various times would be an important first step in ascertaining the level of inhibitors needed.

Decreasing the intake of catalysts or increasing the intake of inhibitors (if it could be easily accomplished) may not necessarily reduce the in vivo formation of N-nitroso compounds. First, the adjustment must be timed to coincide with the consumption of nitrite. Moreover, some compounds, such as certain phenols and thiols, can act either as catalysts or inhibitors, depending on the reaction conditions. Another complication is that many dietary sources of ascorbic acid also contain nitrate (see Chapter 5). Thus, the effect on in vivo nitrosation from such dietary sources will depend on the ratio of ascorbate to nitrate. Moreover, an increased intake of inhibitors may be unwise if adequate toxicological information is not available or if the agent is clearly toxic. The elimination of catalysts must also be viewed with caution because they may be essential constituents of the diet.

More information on the in vivo effects of the various agents is needed because some modifiers may be of greater significance than others, depending on their reactivity and/or the extent to which humans are exposed to them. Persistence is another important consideration. For example, α -tocopherol has been shown to persist in various organs such as the lung and stomach of laboratory animals.

OVERALL SUMMARY AND CONCLUSIONS

The formation of N-nitroso compounds in vivo is dependent on the relative concentrations of nitrate, nitrite, nitrosatable substances, and agents that can either enhance or inhibit nitrosation reactions.

Nitrosatable substances, especially amines, are present throughout the environment and may be produced endogenously as well. Although there are no quantitative estimates of average exposure of humans to

amines (both exogenous and endogenous), exposure to these compounds is likely to be significant and may be enhanced in certain occupational settings, in smokers, and in individuals taking certain drugs.

In addition to the amounts and types of nitrosating species and substrates, there are many other factors that influence the nitrosation reaction. Thus, it is difficult to predict the extent to which N-nitroso compounds will be formed endogenously from a given mixture of reactants. One important consideration is the presence of modifiers (catalysts and inhibitors) of the nitrosation reaction. Compounds such as thiocyanate, iodide, and bromide, can catalyze nitrosation, whereas ascorbic acid, α -tocopherol, and thiols can inhibit the reaction. Some compounds, such as phenols, can either inhibit or catalyze, depending on the conditions. The presence of many other catalysts and inhibitors not discussed in this chapter may also influence the amount of N-nitroso compounds formed endogenously.

RECOMMENDATIONS

In certain circumstances, some subgroups of the population may be exposed to excessive levels of nitrosatable amines or amides. The committee recommends that such exposures be reduced, when feasible. For example, pesticides produced as secondary and tertiary amine salts could be substituted by other formulations, and certain readily nitrosatable drugs could be replaced by drugs that have the same therapeutic effect but are not nitrosatable. In addition, further research should be performed to identify amino compounds that could be nitrosated in vivo--especially those that are readily nitrosated or to which there is a large exposure.

The committee recommends that further research be conducted to study inhibition and catalysis of nitrosation reactions in vivo, specifically to determine the amount of nitrite that is destroyed in the human stomach and the extent to which nitrosation reactions are modified by the various inhibitors. Attention should also be directed toward interactions among inhibitors, catalysts, and other food-derived substances.

REFERENCES

- Anonymous. 1969. Tannic acid again! *Food Cosmet. Toxicol.* 7:364-365.
- Archer, M. C., S. D. Clark, J. E. Thilly, and S. R. Tannenbaum. 1971. Environmental nitroso compounds: Reaction of nitrite with creatine and creatinine. *Science* 174:1341-1343.
- Archer, M. C., S. R. Tannenbaum, T.-Y. Fan, and M. Weisman. 1975. Reaction of nitrite with ascorbate and its relation to nitrosamine formation. *J. Natl. Cancer Inst.* 54:1203-1205.
- Asatoor, A. M., M. J. Chamberlain, B. T. Emmerson, J. R. Johnson, A. J. Levi, and M. D. Milne. 1967. Metabolic effects of oral neomycin. *Clin. Sci.* 33:111-124.
- Boyland, E. 1972. The effect of some ions of physiological interest on nitrosamine synthesis. Pp. 124-126 in P. Bogovski, R. Preussmann, and E. A. Walker, eds. *N-Nitroso Compounds: Analysis and Formation*, IARC Scientific Publication No. 3. International Agency for Research on Cancer, Lyon, France.
- Boyland, E., and S. A. Walker. 1975. Thiocyanate catalysis of nitrosamine formation and some dietary implications. Pp. 132-136 in P. Bogovski and E. A. Walker, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Boyland, E., E. Nice, and K. Williams. 1971. The catalysis of nitrosation by thiocyanate from saliva. *Food Cosmet. Toxicol.* 9:639-643.
- Bruce, W. R., A. J. Varghese, S. Wang, and P. Dion. 1979. The endogenous production of nitroso compounds in the colon and cancer at that site. Pp. 221-227 in E. C. Miller, J. A. Miller, I. Hirono, T. Sugimura, and S. Takayama, eds. *Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis*. University Park Press, Baltimore, Maryland.
- Cardesa, A., S. S. Mirvish, G. T. Haven, and P. Shubik. 1974. Inhibitory effect of ascorbic acid on the acute toxicity of dimethylamine plus nitrite in the rat (37761). *Proc. Soc. Exp. Biol. Med.* 145:124-128.

- Cosmetic, Toiletry and Fragrance Association. 1977. CTFA Cosmetic Ingredient Dictionary, 2nd ed. Cosmetic, Toiletry and Fragrance Association, Washington, D.C. 513 pp.
- Dahl, L. K. 1960. Possible role of salt intake in the development of essential hypertension. Pp. 53-65 in K. D. Bock and P. T. Cottier, eds. Essential Hypertension: An International Symposium. Springer-Verlag, Berlin, Göttingen, and Heidelberg, Federal Republic of Germany.
- Davies, R., M. J. Dennis, R. C. Massey, and D. J. McWeeny. 1978. Some effects of phenol- and thiol-nitrosation reactions on N-nitrosamine formation. Pp. 183-197 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Diem, K. 1962. Synopsis of saliva. Pp. 517-519 in Scientific Tables, 6th Ed. Geigy Pharmaceuticals, Ardsley, New York.
- Douglass, M. L., B. L. Kabacoff, G. A. Anderson, and M. C. Cheng. 1978. The chemistry of nitrosamine formation, inhibition and destruction. J. Soc. Cosmet. Chem. 29:581-606.
- Druckrey, H., R. Preussmann, S. Ivankovic, D. Schmähl, J. Afkham, G. Blum, H. D. Mennel, and M. Müller, P. Petropoulos, and H. Schneider. 1967. [In German; English summary.] [Organotropic carcinogenic effects of 65 different N-nitroso-compounds on BD-rats.] Z. Krebsforsch. 69:103-201.
- Eisenbrand, G., O. Ungerer, and R. Preussmann. 1975a. Formation of N-nitroso compounds from agricultural chemicals and nitrite. Pp. 71-74 in P. Bogovski and E. A. Walker, eds. N-Nitroso Compounds in the Environment, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Eisenbrand, G., O. Ungerer, and R. Preussmann. 1975b. The reaction of nitrite with pesticides. II. Formation, chemical properties and carcinogenic activity of the N-nitroso derivative of N-methyl-1-naphthyl carbamate (carbaryl). Food Cosmet. Toxicol. 13:365-367.
- Eisenbrand, G., B. Spiegelhalter, C. Janzowski, J. Kann, and R. Preussmann. 1978. Volatile and non-volatile N-nitroso compounds in foods and other environmental media. Pp. 311-324 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.

- Elespuru, R. K., and W. Lijinsky. 1973. The formation of carcinogenic nitroso compounds from nitrite and some types of agricultural chemicals. *Food Cosmet. Toxicol.* 11:807-817.
- Fan, T.-Y., and S. R. Tannenbaum. 1973. Factors influencing the rate of formation of nitrosomorpholine from morpholine and nitrite: Acceleration by thiocyanate and other anions. *J. Agric. Food Chem.* 21:237-240.
- Fan, S., D. Fine, R. Ross, P. Rounbehler, A. Silvergleid, and L. Song. 1976. Determination of N-nitroso pesticides in air, water, and soil. Paper presented at the 172nd American Chemical Society National Meeting, August 29 - September 3, 1976, San Francisco, California. Abstract 99 (Pesticides).
- Fiddler, W., J. W. Pensabene, R. C. Doerr, and A. E. Wasserman. 1972. Formation of N-nitrosodimethylamine from naturally occurring quaternary ammonium compounds and tertiary amines. *Nature* 236:307.
- Fine, D. H. 1978. An assessment of human exposure to N-nitroso compounds. Pp. 267-278 in E. A. Walker, M. Castegnaro, L. Gričič, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19, International Agency for Research on Center, Lyon, France.
- Fine, D. H., D. P. Rounbehler, N. M. Belcher, and S. S. Epstein. 1976a. N-Nitroso compounds: Detection in ambient air. *Science* 192:1328-1330.
- Fine, D. H., D. P. Rounbehler, E. D. Pellizzari, J. E. Bunch, R. W. Berkley, J. McCrae, J. T. Bursey, E. Sawicki, K. Krost, and G. A. DeMarrais. 1976b. N-Nitrosodimethylamine in air. *Bull. Environ. Contam. Toxicol.* 15:739-746.
- Fine, D. H., D. P. Rounbehler, E. Sawicki, K. Krost, and G. A. DeMarrais. 1976c. N-Nitroso compounds in the ambient community air of Baltimore, Maryland. *Anal. Lett.* 9:595-604.
- Friedman, M. A., and H. McClanahan. 1973. Biosynthesis of nitrosamines: Reaction of sodium nitrite with dimethylglycine produces nitrososarcosine. *Proc. Am. Assoc. Cancer Res.* 14:127. Abstract 508.
- Fujinaka, N., Y. Masuda, and M. Kuratsune. 1976. Methylguanidine content in food. *Gann* 67:679-683.
- Goff, E. U., J. R. Coombs, D. H. Fine, and T. M. Baines. 1980. Determination of N-nitrosamines from diesel engine crankcase emissions. *Anal. Chem.* 52:1833-1836.

- Gray, J. I., and L. R. Dugan, Jr. 1975. Inhibition of N-nitrosamine formation in model food systems. *J. Food Sci.* 40:981-984.
- Greenblatt, M. 1973. Ascorbic acid blocking of aminopyrine nitrosation in NZO/BI mice. *J. Natl. Cancer Inst.* 50:1055-1056.
- Hamm, R., and K. Hofmann. 1966. [In German.] Bestimmung von Sulfhydryl- und Disulfid-Gruppen in Myofibrillen und Muskelgeweben mit Hilfe der amperometrischen Titration. *Z. Lebensm. Unters. Forsch.* 130(3):133-145.
- Harper, H. A., V. W. Rodwell, and P. A. Mayes. 1977. Water and mineral metabolism. Pp. 516-541 in *Review of Physiological Chemistry*, 16th Ed. Lange Medical Publications, Los Altos, California.
- Hawksworth, G. M., and M. J. Hill. 1971. Bacteria and the N-nitrosation of secondary amines. *Br. J. Cancer* 25:520-526.
- Hecht, S. S. 1981. Investigation of new nitrosamine contaminants of cosmetics--Report on FDA contract studies. Paper presented at the SCC-FDA Scientific Seminar, Society of Cosmetic Chemists, Mid-Atlantic Chapter, April 15, 1981, held at the Food and Drug Administration, Washington, D.C.
- Hill, M. J. 1979. Bacterial metabolism and colon cancer. *Nutr. Cancer* 1:46-50.
- Hill, M. J. 1980. Bacterial metabolism and human carcinogenesis. *Br. Med. Bull.* 36:89-94.
- Hisatsune, I. C. 1961. Thermodynamic properties of some oxides of nitrogen. *J. Phys. Chem.* 65:2249-2253.
- Hoffmann, D., J. D. Adams, J. J. Plade, and S. S. Hecht. 1980. Chemical studies on tobacco smoke LXVIII. Analysis of volatile and tobacco-specific nitrosamines in tobacco products. Pp. 507-516 in E. A. Walker, M. Castegnaro, L. Gričiute, and M. Börzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Hoffmann, D., J. D. Adams, K. D. Brunnemann, and S. S. Hecht. In press. Formation, occurrence and carcinogenicity of N-nitrosamines in tobacco products. In R. A. Scanlan and S. R. Tannenbaum, eds. *N-Nitroso Compounds*, ACS Symposium Series. American Chemical Society, Washington, D.C.

- Iqbal, Z. M., K. Dahl, and S. S. Epstein. 1980. Role of nitrogen dioxide in the biosynthesis of nitrosamines in mice. *Science* 207:1475-1477.
- Ivankovic, S., R. Preussmann, D. Schmähel, and J. W. Zeller. 1975. Prevention by ascorbic acid of in vivo formation of N-nitroso compounds. Pp. 101-102 in P. Bogovski and E. A. Walker, eds. N-Nitroso Compounds in the Environment, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Janzowski, C., G. Eisenbrand, and R. Preussmann. 1978. Occurrence and determination of N-nitroso-3-hydroxypyrrolidine in cured meat products. *J. Chromatogr.* 150:216-220.
- Jensen, D. E., and P. N. Magee. 1981. Methylation of DNA by nitrosocimetidine in vitro. *Cancer Res.* 41:230-236.
- Johnson, K. A. 1977. The production of secondary amines by the human gut bacteria and its possible relevance to carcinogenesis. *Med. Lab. Sci.* 34:131-143.
- Kamm, J. J., T. Dashman, A. H. Conney, and J. J. Burns. 1973. Protective effect of ascorbic acid on hepatotoxicity caused by sodium nitrite plus aminopyrine. *Proc. Natl. Acad. Sci. USA* 70:747-749.
- Kamm, J. J., T. Dashman, A. H. Conney, and J. J. Burns. 1975. Effect of ascorbic acid on amine-nitrite toxicity. *Ann. N.Y. Acad. Sci.* 258:169-174.
- Kamm, J. J., T. Dashman, H. Newmark, and W. J. Mergens. 1977. Inhibition of amine-nitrite hepatotoxicity by α -tocopherol. *Toxicol. Appl. Pharmacol.* 41:575-583.
- Kawabata, T., H. Ohshima, and M. Ino. 1978. Occurrence of methylguanidine and agmatine, nitrosatable guanidino compounds, in foods. *J. Agric. Food Chem.* 26:334-338.
- Keay, J. N., and R. Hardy. 1972. The separation of aliphatic amines in dilute aqueous solution by gas chromatography and application of this technique to the quantitative analysis of tri- and dimethylamine in fish. *J. Sci. Food Agric.* 23:9-19.
- Kidd, P. S., F. L. Trowbridge, J. B. Goldsby, and M. Z. Nichaman. 1974. Sources of dietary iodine. *J. Am. Diet. Assoc.* 65:420-422.

- Knowles, M. E., J. Gilbert, and D. J. McWeeny. 1975. Phenols in smoked, cured meats: Nitrosation of phenols in liquid smokes and in smoked bacon. *J. Sci. Food Agric.* 26:267-276.
- Lakritz, L., A. M. Spinelli, and A. E. Wasserman. 1975. Determination of amines in fresh and processed pork. *J. Agric. Food Chem.* 23:344-346.
- Lakritz, L., A. M. Spinelli, and A. E. Wasserman. 1976. Effect of storage on the concentration of proline and other free amino acids in pork bellies. *J. Food Sci.* 41:879-881.
- Lane, R. P., and M. E. Bailey. 1973. The effect of pH on dimethyl-nitrosamine formation in human gastric juice. *Food Cosmet. Toxicol.* 11:851-854.
- Lijinsky, W. 1974. Reaction of drugs with nitrous acid as a source of carcinogenic nitrosamines. *Cancer Res.* 34:255-258.
- Lijinsky, W., and S. S. Epstein. 1970. Nitrosamines as environmental carcinogens. *Nature* 225:21-23.
- Lijinsky, W., and M. Greenblatt. 1972. Carcinogen dimethylnitrosamine produced in vivo from nitrite and aminopyrine. *Nature New Biol.* 236:177-178.
- Lijinsky, W., and D. Schmähl. 1978. Carcinogenesis by nitroso derivatives of methylcarbamate insecticides and other nitrosamides in rats and mice. Pp. 495-501 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Lijinsky, W., and G. M. Singer. 1975. Formation of nitrosamines from tertiary amines and nitrous acid. Pp. 111-114 in P. Bogovski and E. A. Walker, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Lijinsky, W., and H. W. Taylor. 1976. Carcinogenicity tests of N-nitroso derivatives of two drugs, phemetrazine and methylphenidate. *Cancer Lett.* 1:359-363.
- Lijinsky, W., E. Conrad, and R. Van de Bogart. 1972. Carcinogenic nitrosamines formed by drug/nitrite interactions. *Nature* 239:165-167.
- Maga, J. A. 1978. Amines in foods. *Crit. Rev. Food Sci. Nutr.* 10:373-403.

- Massey, R. C., C. Crews, R. Davies, and D. J. McWeeny. 1978. A study of the competitive nitrosations of pyrrolidine, ascorbic acid, cysteine and p-cresol in a protein-based model system. *J. Sci. Food Agric.* 29:815-821.
- Mergens, W. J., J. J. Kamm, H. L. Newmark, W. Fiddler, and J. Pensabene. 1978. Alpha-tocopherol: Uses in preventing nitrosamine formation. Pp. 199-212 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Mirvish, S. S. 1975. Formation of N-nitroso compounds: Chemistry, kinetics, and in vivo occurrence. *Toxicol. Appl. Pharmacol.* 31:325-351.
- Mirvish, S. S. 1981a. Ascorbic acid inhibition of N-nitroso compound formation in chemical, food, and biological systems. Pp. 101-126 in M. S. Zedeck and M. Lipkin, eds. *Inhibition of Tumor Induction and Development*. Plenum Press, New York and London.
- Mirvish, S. S. 1981b. Inhibition of the formation of carcinogenic N-nitroso compounds by ascorbic acid and other compounds. Pp. 557-587 in J. H. Burchenal and H. F. Oettgen, eds. *Achievements, Challenges, and Prospects for the 1980's*, Vol. 1. Grune and Stratten, Inc., New York.
- Mirvish, S. S., and D. A. Cairnes. 1981. Identification of the compound in a fish product yielding methylurea (MU) on nitrosation-denitrosation as creatinine (CRN). *Proc. Am. Assoc. Cancer Res.* 22:140. Abstract 555.
- Mirvish, S. S., L. Wallcave, M. Eagen, and P. Shubik. 1972. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. *Science* 177:65-68.
- Mirvish, S. S., J. Sams, T. Y. Fan, and S. R. Tannenbaum. 1973. Kinetics of nitrosation of the amino acids proline, hydroxyproline, and sarcosine. *J. Natl. Cancer Inst.* 51:1833-1839.
- Mirvish, S. S., A. Cardesa, L. Wallcave, and P. Shubik. 1975. Induction of mouse lung adenomas by amines or ureas plus nitrite and by N-nitroso compounds: Effect of ascorbate, gallic acid, thiocyanate, and caffeine. *J. Natl. Cancer Inst.* 55:633-636.

- Mirvish, S. S., K. Karlowski, J. P. Sams, and S. D. Arnold. 1978. Studies related to nitrosamide formation: Nitrosation in solvent: Water and solvent systems, nitrosomethylurea formation in the rat stomach and analysis of a fish product for ureas. Pp. 161-174 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Moruzzi, G., and C. M. Caldarera. 1964. Letter to the Editor: Occurrence of polyamines in the germs of cereals. Arch. Biochem. Biophys. 105:209-210.
- Nakayama, T. O. M., and C. O. Chichester. 1963. Astringency of persimmons (Diospyros kaki, L.). Nature 199:72-73.
- National Academy of Sciences. 1973. A Comprehensive Survey of Industry on the Use of Food Chemicals Generally Recognized as Safe (GRAS): Table 16; Part D: All NAS Appendix A and FEMA Questionnaire Substances (Groups I, II, III) Ranked by Possible Average Daily Intake. A report prepared for the Food and Drug Administration, Report No. FDABF-GRAS-124, by the Subcommittee on Review of the GRAS List--Phase II, Committee on Food Protection, Food and Nutrition Board, Division of Biology and Agriculture, National Research Council, National Academy of Sciences, Washington, D.C. Available from the National Technical Information Service, Springfield, Virginia, as PB-221 947.
- Oddie, T. H., D. A. Fisher, W. M. McConahey, and C. S. Thompson. 1970. Iodine intake in the United States: A reassessment. J. Clin. Endocrinol. Metab. 30:659-665.
- Ohshima, H., and H. Bartsch. 1981. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. Cancer Res. 41:3658-3662.
- Oliver, J. E. In press. Pesticide-derived nitrosamines: Occurrence and environmental fate. In R. A. Scanlan and S. R. Tannenbaum, eds. N-Nitroso Compounds, ACS Symposium Series. American Chemical Society, Washington, D.C.
- Ong, J. T. H., and B. S. Rutherford. 1980. Some factors affecting the rate of N-nitrosodiethanolamine formation from 2-bromo-2-nitropropane-1,3-diol and ethanolamines. J. Soc. Cosmet. Chem. 31:153-159.

- Patrianakos, C., and D. Hoffmann. 1979. Chemical studies on tobacco smoke LXIV. On the analysis of aromatic amines in cigarette smoke. *J. Anal. Toxicol.* 3:150-154.
- Patterson, R. L. S., and R. A. Edwards. 1975. Volatile amine production in uncured pork during storage. *J. Sci. Food Agric.* 26:1371-1373.
- Patterson, R. L. S., and D. S. Mottram. 1974. The occurrence of volatile amines in uncured and cured pork meat and their possible role in nitrosamine formation in bacon. *J. Sci. Food Agric.* 25:1419-1425.
- Pensabene, J. W., W. Fiddler, J. Feinberg, and A. E. Wasserman. 1976. Evaluation of ascorbyl monoesters for the inhibition of nitrosopyrrolidine formation in a model system. *J. Food Sci.* 41:199-200.
- Pignatelli, B., M. Friesen, and E. A. Walker. 1980. The role of phenols in catalysis of nitrosamine formation. Pp. 95-109 in E. A. Walker, M. Castegnaro, L. Gričute, and M. Bőrzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Ridd, J. H. 1961. Nitrosation, diazotisation, and deamination. *Q. Rev. Chem. Soc.* 15:418-441.
- Ruddell, W. S. J., L. M. Blendis, and C. L. Walters. 1977. Nitrite and thiocyanate in the fasting and secreting stomach and in saliva. *Gut* 18:73-77.
- Scheunig, G., and D. Ziebarth. 1976. Formation of nitrosamines by interaction of some drugs with nitrite in human gastric juice. Pp. 269-277 in E. A. Walker, P. Bogovski, and L. Gričute, eds. *Environmental N-Nitroso Compounds: Analysis and Formation*, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.
- Schievelbein, H., E. Werle, E. K. Schulz, and R. Baumeister. 1969. The influence of tobacco smoke and nicotine on thiocyanate metabolism. *Naunyn Schmiedebergs Arch. Pharmakol. Exp. Pathol.* 262:358-365.

- Schmeltz, I., and D. Hoffmann. 1977. Nitrogen-containing compounds in tobacco and tobacco smoke. *Chem. Rev.* 77:295-311.
- Schmeltz, I., and A. Wenger. 1979. 2-Bromo-2-nitropropane-1,3-diol as a nitrosating agent for diethanolamine: A model study. *Food Cosmet. Toxicol.* 17:105-109.
- Schweinsberg, F. 1975. Catalysis of nitrosamine synthesis. Pp. 80-85 in P. Bogovski and E. A. Walker, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Sen, N. P., W. F. Miles, B. Donaldson, T. Panalaks, and J. R. Iyengar. 1973. Formation of nitrosamines in a meat curing mixture. *Nature* 245:104-105.
- Sen, N. P., B. A. Donaldson, and C. Charbonneau. 1975. Formation of nitrosodimethylamine from the interaction of certain pesticides and nitrite. Pp. 75-79 in P. Bogovski and E. A. Walker, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Shank, R. C., and P. M. Newberne. 1972. Letter to the Editor: Nitrite-morpholine-induced hepatomas. *Food Cosmet. Toxicol.* 10:887-888.
- Singer, G. M., and W. Lijinsky. 1976a. Naturally occurring nitrosatable compounds. I. Secondary amines in foodstuffs. *J. Agric. Food Chem.* 24:550-553.
- Singer, G. M., and W. Lijinsky. 1976b. Naturally occurring nitrosatable amines. II. Secondary amines in tobacco and cigarette smoke condensate. *J. Agric. Food Chem.* 24:553-555.
- Singleton, V. L., and F. H. Kratzer. 1973. Plant phenolics. Pp. 309-345 in *Toxicants Occurring Naturally in Foods*, 2nd Ed. National Academy of Sciences, Washington, D.C.
- Spincer, D., and D. T. Westcott. 1976. Formation of nitrosodimethylamine in smoke from cigarettes manufactured from different tobacco types. Pp. 133-139 in E. A. Walker, P. Bogovski, and L. Gričiute, eds. *Environmental N-Nitroso Compounds: Analysis and Formation*, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.

- Tannenbaum, S. R., and W. Mergens. 1980. Reaction of nitrite with vitamins C and E. *Ann. N.Y. Acad. Sci.* 355:267-281.
- Tannenbaum, S. R., M. C. Archer, J. S. Wishnok, P. Correa, C. Cuello, and W. Haenszel. 1977. Nitrate and the etiology of gastric cancer. Pp. 1609-1625 in H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds. *Origins of Human Cancer, Book C: Human Risk Assessment*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Underwood, E. J. 1977. *Trace Elements in Human and Animal Nutrition*, 4th Ed. Academic Press, New York, San Francisco, and London. 545 pp.
- U. S. Department of Agriculture. 1963. *Composition of Foods: Raw, Processed, Prepared; Agriculture Handbook No. 8. Consumer and Food Economics Institute, Agricultural Research Service, U. S. Department of Agriculture, Washington, D.C.* 189 pp.
- U. S. Department of Agriculture. 1980. Milk and milk products; eggs; legumes, nuts and seeds. Pp. 6-8, 48-50 in *Food and Nutrient Intakes of Individuals in 1 Day in the United States, Spring 1977. Nationwide Food Consumption Survey 1977-78, Preliminary Report No. 2.* Science and Education Administration, U. S. Department of Agriculture, Washington, D.C.
- U. S. Environmental Protection Agency. 1975. *Region V Joint Federal/State Survey of Organics and Inorganics in Selected Drinking Water Supplies.* U. S. Environmental Protection Agency, Chicago, Illinois. 82 pp. + appendices.
- U. S. Environmental Protection Agency. 1977. *Scientific and Technical Assessment Report on Nitrosamines.* EPA-600/6-77-001. Office of Research and Development, U. S. Environmental Protection Agency, Washington, D.C.
- U. S. Public Health Service. 1979. *Smoking and Health: A Report of the Surgeon General.* DHEW Publication No. (PHS) 79-50066. Office on Smoking and Health, Office of the Assistant Secretary for Health, U. S. Public Health Service, U. S. Department of Health, Education, and Welfare, Washington, D.C. 1197 pp.
- Vélišek, J., J. Davídek, S. Klein, M. Karásková, and I. Vykouková. 1975. The nitrosation products of creatine and creatinine in model systems. *Z. Lebensm. Unters. Forsch.* 159:97-102.
- Walters, C. L., M. W. Edwards, T. S. Elsey, and M. Martin. 1976. The effect of antioxidants on the production of volatile nitrosamines during the frying of bacon. *Z. Lebensm. Unters. Forsch.* 162:377-385.

Wenninger, J. A. 1980. FDA focus on cosmetics. Drug Cosmet. Ind. 127:62-69, 117-118.

Wrong, O. 1978. Nitrogen metabolism in the gut. Am. J. Clin. Nutr. 31:1587-1593.

Ziebarth, D., and G. Scheunig. 1976. Effects of some inhibitors on the nitrosation of drugs in human gastric juice. Pp. 279-290 in E. A. Walker, P. Bogovski, and L. Gričiute, eds. Environmental N-Nitroso Compounds Analysis and Formation, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.

CHAPTER 7

N-NITROSO COMPOUNDS: ENVIRONMENTAL DISTRIBUTION AND
EXPOSURE OF HUMANS

| | <u>Page</u> |
|---|-------------|
| ANALYTICAL METHODS..... | 7-3 |
| CONCENTRATIONS OF NITROSAMINES IN VARIOUS ENVIRONMENTAL SOURCES..... | 7-6 |
| Occupational Settings..... | 7-6 |
| Tobacco and Tobacco Smoke..... | 7-11 |
| Food..... | 7-15 |
| Cosmetics..... | 7-22 |
| Pharmaceuticals..... | 7-23 |
| Pesticides..... | 7-23 |
| Water..... | 7-27 |
| Air..... | 7-28 |
| EXPOSURE OF HUMANS TO N-NITROSO COMPOUNDS..... | 7-28 |
| Individual Exposures, By Source..... | 7-28 |
| Estimated Exposure of the U.S. Population to Nitrosamines..... | 7-34 |
| SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS..... | 7-35 |
| REFERENCES..... | 7-39 |

CHAPTER 7

N-NITROSO COMPOUNDS: ENVIRONMENTAL DISTRIBUTION AND EXPOSURE OF HUMANS

Several analytical methods have been used to determine the levels of volatile nitrosamines in a variety of environmental media. In this chapter, the committee has described these methods and their limitations, and has reviewed the resultant data on nitrosamine concentrations in tobacco, food, alcoholic beverages, cosmetics, pharmaceuticals, pesticides, water, the atmosphere, and occupational settings. Estimates of individual exogenous exposures to nitrosamines for workers in certain occupations, for tobacco users, and for the general population of several countries, based on concentrations of nitrosamines in food and alcoholic beverages are also presented. In addition, the committee has used the concentrations of nitrosamines in various environmental sources to develop estimates of average exposures to nitrosamines for the U.S. population.

ANALYTICAL METHODS

Methods that have withstood the test of extensive interlaboratory collaborative studies exist only for N-nitroso compounds that are amenable to analysis by gas chromatography (GC). Procedures for N-nitroso compounds that decompose upon heating or are not sufficiently volatile to be analyzed by GC are more complex. Only a few compounds with such properties have been analyzed by high pressure liquid chromatography (HPLC) with a Thermal Energy Analyzer (TEA®) or an ultra-violet detector. Recently, a method for the detection of nitrosamides has been reported (Saul *et al.*, in press).

Techniques used for the analysis of volatile and nonvolatile N-nitroso compounds include thin-layer chromatography, polarography, spectrophotometry, and chemical denitrosation reactions. Recently, the TEA and the high-resolution mass spectrometer, when used as detectors for gas chromatographs, have become the instruments of choice for volatile N-nitroso compounds (International Agency for Research on Cancer, 1978a,b, 1980).

Gough *et al.* (1977c) compared the GC-TEA procedure for food and urine samples with three GC-mass spectrometric (GC-MS) techniques. Low-resolution MS was performed by monitoring two key ions for each compound, one of the ions being the parent ion. High-resolution MS with precision ion monitoring was attained by focusing precisely on each parent ion, and peak matching was accomplished by continuously scanning the mass range close to the parent ion and

comparing the peak to that of a suitable standard. The investigators demonstrated that only the high-resolution MS with peak matching provided data that were in agreement with those obtained with the TEA. For example, a urine sample was found to contain more than 100 $\mu\text{g/liter}$ (ppb) nitrosodimethylamine (NDMA) by low-resolution MS in which two peaks were monitored, 15 $\mu\text{g/liter}$ by high-resolution MS with precise ion monitoring, but only 2 $\mu\text{g/liter}$ by high-resolution MS with peak matching. The TEA result was 1.2 $\mu\text{g/liter}$. In a sample of Chinese food, low-resolution MS detected nitrosopyrrolidine (NPYR) at a concentration of 70 $\mu\text{g/kg}$, whereas high-resolution MS with precise ion monitoring detected NPYR at 3 $\mu\text{g/kg}$. Yet, NPYR was not detectable either by high-resolution MS with peak matching or by TEA. In this case, not only was the quantitation in error, but an inappropriate use of MS led to a positive result when no nitrosamine was present. The work of Gough and his colleagues clearly demonstrates the need to ensure that the MS is carried out with appropriate safeguards.

Havery et al. (1978) found no discrepancies when comparing 106 cured meat samples by GC-TEA and GC-MS. Webb et al. (1979) demonstrated good agreement, even at levels less than 1 pg, when the extracts of 98 substrates were compared on GC-TEA and GC-MS. These papers demonstrate that GC-TEA and GC-MS (high resolution with peak matching) can be used for the reliable identification of trace levels of volatile nitrosamines extracted from complex matrices.

Although both the TEA analyzer and the high-resolution spectrometer have been shown to be reliable tools for identifying nitrosamines in extracts, even at the picogram level, it is still difficult to ensure that the nitrosamines detected were present in the sample before extraction. Artifacts arise because precursors of nitrosamines are generally present in much larger amounts than the nitrosamines themselves. Furthermore, depending upon the matrix and precursors, the formation of artifacts can be enhanced, or the nitrosamine destroyed, by the action of acid, alkali, heat, light, radiation, and other factors. Artifactual formation presents especially acute problems when attempting to detect nitrosamines in biological fluids where levels are likely to be less than 1 $\mu\text{g/liter}$. The control of artifacts during analysis for nitrosamines has been discussed in detail by Krull et al. (1978, 1979a). In general, many investigators now agree that whenever an N-nitroso compound is first reported to be present in a new sample matrix, the data should be presumed to indicate that artifacts have been formed during analysis, unless all or most of the following specific steps have been taken:

- Nitrosation inhibitors, such as α -tocopherol (fat soluble) and ascorbic acid (water soluble), have been added in excess of the amount required for inhibition.

- A suitable nitrosamine has been added to the sample as an internal control for recovery efficiency.

- A readily nitrosatable amine, such as 2,6-dimethylmorpholine, has been added to the sample. (The presence of the N-nitroso derivative of this marker amine in the final extract can be used to estimate the extent to which artifacts have formed.)

- Glassware, solvents, and other testing equipment and materials have been checked daily for contamination.

- The minimum possible number of analytical steps have been used. If possible, at least one data point is checked by introducing the crude sample directly into a GC-TEA (Fan *et al.*, 1977b). Even then, care must be exercised to prevent formation of artifacts in the GC injector itself (Fan and Fine, 1978).

- Consideration has been given to the fact that many solvents (Eisenbrand *et al.*, 1978), amines, deionized water (Kimoto *et al.*, 1980), and all rubber products are contaminated with nitrosamines.

- Undue exposure to ambient air is avoided since nitrogen oxides, even at ambient levels, can nitrosate amines upon contact (Eisenbrand *et al.*, 1978). (See Chapter 4.)

Specific analytical procedures for nonvolatile nitroso compounds, such as the N-nitroso ureas, amides, and carbamates have only recently been developed. For example, a colorimetric method for urea can be used to determine nitrosoureas and nitrosocyanamides (Mirvish *et al.*, 1979), and Saul *et al.* (in press) have recently reported a method for detecting nitrosamides. There are also some broad screening techniques that can be used for some nonvolatile N-nitroso compounds (Fan *et al.*, 1978a; Fine, 1980b). For example, screening techniques have been used successfully to identify a variety of nonvolatile nitrosamines in pesticide products (Wolf *et al.*, 1980; Zweig and Garner, in press). Final detection has generally been made by HPLC-UV (ultraviolet) or HPLC-TEA techniques. In tobacco and tobacco smoke, the presence of tobacco-specific nonvolatile nitrosamines has been detected by HPLC-TEA and other HPLC procedures (Hecht *et al.*, 1978; Hoffmann *et al.*, 1979).

Procedures have been developed to analyze foodstuffs for such compounds as the nitrosamino acids. Nitroso-3-hydroxypyrrolidine, which was found in cured meat products (Janzowski *et al.*, 1978), was determined by trifluoroacetylation, followed by GC-TEA and GC-high-resolution MS. Sen *et al.* (1977a) have also described a mass spectrometric method to detect this compound in cooked bacon. More recently, Roussin's red methyl ester $[(NO)_2Fe(CH_3S)]_2$ was isolated and identified by GC-MS from the ether extracts of Chinese vegetables (Lu *et al.*, 1981). The total N-nitroso compound content of food samples has been assayed by Walters *et al.* (1978), who used a chemical denitrosation procedure, followed by the detection of the nitrosyl radical by its chemiluminescence reaction with ozone.

The ability to detect C-, O-, and S-nitroso compounds is also important because of the potential of these agents to form N-nitroso compounds via transnitrosation reactions (see Chapter 4). These nitroso compounds are generally thermolabile and readily decompose to release nitric oxide (Krull *et al.*, 1979a). Specific analytical procedures have not yet been identified, although some authors have reported that these compounds can be detected with the TEA. Krull *et al.* (1979a) have described procedures for distinguishing these compounds from N-nitroso compounds.

CONCENTRATIONS OF NITROSAMINES IN VARIOUS ENVIRONMENTAL SOURCES

Occupational Settings

The highest known concentrations of exogenous nitrosamines occur in the workplace, especially in the rubber and leather-tanning industries.

Rubber Industry. The rubber industry uses nitrosodiphenylamine (NDPhA) as a vulcanization retarder. This nitrosamine has been shown to be carcinogenic in rats (Cardy *et al.*, 1979). It is very labile, can participate in transnitrosation reactions (see Chapters 4 and 6), and may contribute to the formation of other carcinogenic N-nitroso compounds.

Fajen *et al.* (1979) measured levels of nitrosamines in three rubber factories. As expected, NDPhA was found at the 0.2 to 47 $\mu\text{g}/\text{m}^3$ level in the air of a factory where the compound was being manufactured. The entire area was contaminated with NDPhA; its concentration in a mud scraping from the floor contained 15,000 mg/kg. In addition to the presence of NDPhA in chemical manufacturing areas, curing and extrusion sections of the rubber factories were found to contain nitrosomorpholine (NMOR) in concentrations ranging from 0.5 to 27 $\mu\text{g}/\text{m}^3$. (The NMOR presumably arises from the use of bismorpholinecarbonylsulfenamide, which is used as an accelerator.) NDMA was detected at lower levels (0.05 to 0.5 $\mu\text{g}/\text{m}^3$) as an air pollutant in several of the factories.

More recently, McGlothlin *et al.* (1981) conducted an in-depth study of nitrosamine levels in a rubber factory. Initial measurements indicated that one area air sample contained NMOR at 250 $\mu\text{g}/\text{m}^3$. Within 7 months, airborne nitrosamine levels were reduced dramatically (McGlothlin *et al.*, 1981). As shown in Figure 7-1, 250 $\mu\text{g}/\text{m}^3$ was the highest atmospheric concentration of NMOR found at the beginning of the study in August 1979. Within 2 months, the concentration had been reduced to 120 $\mu\text{g}/\text{m}^3$ by venting the building. Two months later, a further reduction to 63 $\mu\text{g}/\text{m}^3$ was achieved, mainly by the use of exhaust canopies. By February 1980, 7 months after the study was initiated, the highest detectable NMOR concentration was only 14 $\mu\text{g}/\text{m}^3$. The final reduction was achieved by substituting a phthalimide derivative for NDPhA.

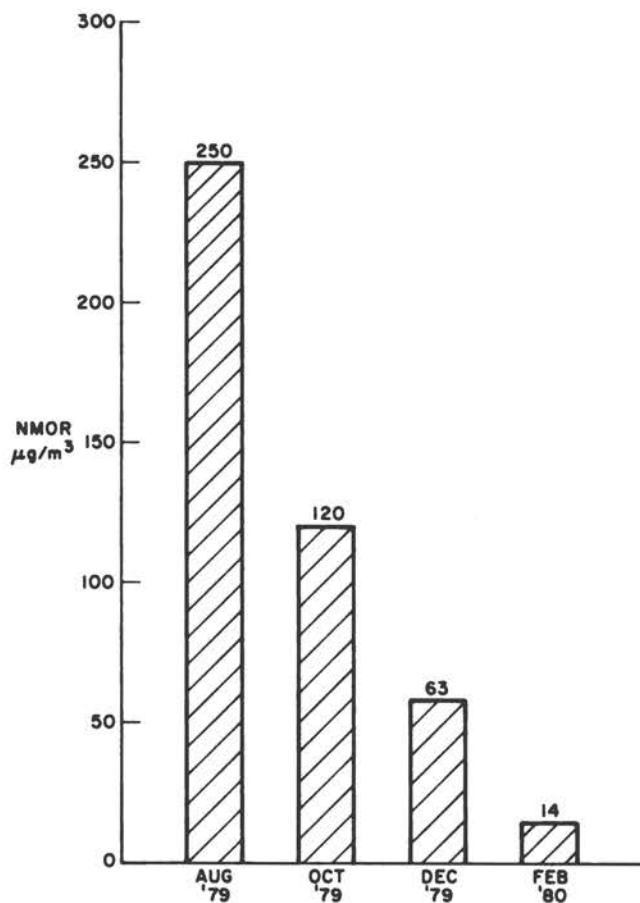


FIGURE 7-1. Highest NMOR concentrations in area air samples obtained from a rubber factory between August 1979 and February 1980, showing reductions achieved. The October 1979 reduction was attributed to venting, the December 1979 reduction to the installation of exhaust canopies, and the February 1980 reduction to the use of a phthalimide derivative instead of NDPhA. From Fine and Rounbehler, in press.

Preussmann et al. (1980; in press) measured nitrosamine levels in 19 rubber factories in the Federal Republic of Germany. Initial concentrations of NDMA and NMOR ranged from 1 to 20 $\mu\text{g}/\text{m}^3$. In one working area (tire tube curing), an extremely high concentration of NDMA (140 $\mu\text{g}/\text{m}^3$) was detected. Subsequently, levels in that area were reduced 1 to 2 $\mu\text{g}/\text{m}^3$ by substituting the NDPhA with cyclohexylthiophthalimide. More recently, Spiegelhalder and Preussmann (in press) have reported that air in certain areas of rubber factories in Germany may contain extremely high concentrations of nitrosamines--1,060 $\mu\text{g}/\text{m}^3$ NDMA and 4,700 $\mu\text{g}/\text{m}^3$ NMOR.

Leather Tanning. Because of Acheson's (1976) report of a possible increase in nasal cancer incidence among leather workers, an investigation of airborne nitrosamine levels in a typical leather tannery was undertaken. In their initial study, Rounbehler et al. (1979) reported that NDMA was detected as an airborne pollutant at all sites in the tannery on three separate visits. The highest level, 47 $\mu\text{g}/\text{m}^3$, was found in the retanning, coloring, and fat-liquoring areas. The average NDMA level on the first and second visits was 19 $\mu\text{g}/\text{m}^3$. Small amounts of NDMA were also found in the process water and wastewater. By the time of the third visit, the tannery had been thoroughly cleaned and NDMA levels had been reduced to a range of 0.1 to 3.4 $\mu\text{g}/\text{m}^3$.

This group has now surveyed eight leather-tanning facilities throughout the United States (Fajen et al., in press; New England Institute for Life Sciences, in press). Four of the eight plants were found to have airborne concentrations of NDMA greater than 0.5 $\mu\text{g}/\text{m}^3$. Table 7-1 lists the type of operations of each plant and the highest levels of NDMA found at that plant. The data in the table indicate that the use of dimethylamine sulfate (DMAS), a precursor of NDMA, as a depilatory agent, is associated with the presence of airborne NDMA. Even a facility that had recently discontinued the use of DMAS, and another that used DMAS only on an experimental basis, contained airborne NDMA.

The agents nitrosating the DMAS are probably oxides of nitrogen formed by the combustion of fossil fuels in gas-powered forklift trucks or in open gas heaters; however, because all the tanneries that used DMAS had a possible source of nitrogen oxides, this could not be determined conclusively.

In further studies aimed at determining the source of NDMA, airborne nitrosation potential and levels of amines and nitrosamines were measured simultaneously throughout a complete tanning operation

(Fine and Rounbehler, in press). None of the bulk samples that were collected contained NDMA (sensitivity limit 0.5 µg/ml), including the fresh DMAS, the hide depilatory solution, and even water on the floor near the depilatory operation. This negative finding demonstrates conclusively that the source of NDMA is not an impurity in the DMAS, nor is NDMA being formed in the depilatory solutions. However, NDMA was present in the air at all test sites in the tannery at the time the bulk samples were collected. The data on airborne concentrations also show that dimethylamine (DMA) was always present (Table 7-2). In all cases, the amount of airborne NDMA was approximately equal to 1% of the amount of airborne DMA. In contrast, NDMA levels did not vary with measured nitrosation capacity presumably because nitrosation capacity (i.e., nitrogen oxide levels) did not vary sufficiently among the different areas in the tannery.

As shown in Table 7-2, DMA, presumably formed from the DMAS, is clearly needed to produce detectable levels of NDMA. Yet, as was shown in a previous study, the DMAS itself and its aqueous solutions do not contain NDMA. A much higher level of DMA, as well as sufficient airborne nitrosation capacity, is required for the production of airborne NDMA. Thus, the NDMA must be formed from DMA outside the solution -- either in the gas phase or on surfaces.

TABLE 7-1

Summary of NDMA Concentrations Measured at Eight
Leather-Tanning Facilities^a

| Description of Tannery ^b | DMAS Used | Source of Nitrogen Oxides | Highest NDMA Concentration Measured, µg/m ³ |
|--|--------------------------|------------------------------|---|
| All operations | Yes | Fork-lift trucks | 47 |
| All operations | Yes | Fork-lift trucks | 11 |
| All operations | No | Fork-lift trucks | 0 |
| All operations | No | Fork-lift trucks | 0 |
| Partial-wet | Recently discontinued | Fork-lift trucks | 8 |
| Partial-wet | Used experimentally | Open gas heaters | 3 |
| Partial-dry | No | Fork-lift trucks | 0.05 |
| Partial-dry | No | None | 0 |

^aFrom Fine and Rounbehler, in press.

^bDMAS is used primarily in "wet" areas of the tanning operation.

TABLE 7-2

Analyses of Air Samples Collected at a Tannery
in Milwaukee, August 1980^a

NOTE: Some concentrations have been rounded off
to two significant figures.

| Sample ^b | Nitrogen Oxides, ppb ^c | DMA, $\mu\text{g}/\text{m}^3$ | NDMA, $\mu\text{g}/\text{m}^3$ | NDMA/DMA, % |
|---------------------|---|----------------------------------|-----------------------------------|----------------|
| 1 | 58 | 490 | 4.6 | 0.9 |
| 2 | 37 | 280 | 5 | 1.8 |
| 3 | 67 | 180 | 1.2 | 0.6 |
| 4 | 88 | 260 | 3.3 | 1.3 |
| 5 | 80 | 280 | 3.6 | 1.3 |

^aFrom Fine and Rounbehler, in press; each concentration given is the mean of three samples taken on three different days.

^bSamples were taken at various locations within the plant.

^cNitrogen oxides were measured indirectly as airborne nitrosation potential (see text).

Amine Factories. Bretschneider and Matz (1973, 1976) reported the presence of trace levels of NDMA in the air of a factory producing "fat" chemicals and one manufacturing pharmaceuticals. They also reported levels between 1 and 43 $\mu\text{g}/\text{m}^3$ on the site of a plant manufacturing DMA. Fine et al. (1976b) reported NDMA concentrations ranging from 0.01 to 1 $\mu\text{g}/\text{m}^3$ in the ambient air outside a factory in Belle, W. Va., in which DMA was manufactured and used. Subsequent studies (Fine et al., 1977a) showed that the source of the NDMA was a vent from a pilot chemical manufacturing operation (levels up to 130 $\mu\text{g}/\text{m}^3$). The NDMA was apparently produced as an unwanted by-product. Atmospheric concentrations of NDMA in the neighboring towns of Belle and Charleston ranged from 0.001 to 0.04 $\mu\text{g}/\text{m}^3$. Apparently, most of the NDMA detected had been produced in the chemical plant and not by the reaction of DMA with oxides of nitrogen in the atmosphere (Fine et al., 1977a).

Rocket Fuel Factory. Fine et al. (1976a,c) reported that NDMA was present as an air pollutant in Baltimore, Md. The prime source was subsequently found to be a chemical plant that used NDMA in the manufacture of a rocket fuel -- unsymmetrical-dimethylhydrazine (UDMH).

Typical NDMA levels ranged from 6 to 36 $\mu\text{g}/\text{m}^3$ at the factory site. Approximately 1 $\mu\text{g}/\text{m}^3$ was measured in the residential neighborhood adjacent to the factory, and approximately 0.1 $\mu\text{g}/\text{m}^3$ was found approximately 3.2 km away in downtown Baltimore (Fine et al., 1976a,b, 1977a,b,c).

A leak of NDMA as small as 130 g (4.7 oz) per hour could have been responsible for all of the airborne concentrations of NDMA found in Baltimore. The UDMH factory had been in operation for 17 years (from 1956 to 1973) before it was rebuilt in 1973 as a sealed system to comply with safety guidelines issued at that time by the Occupational Safety and Health Administration (OSHA) for handling carcinogens such as NDMA. Unfortunately, no data on airborne NDMA from that source were available prior to August 1975, and this factory ceased manufacturing rocket fuel in 1976.

Machine Shops. The finding of nitrosodiethanolamine (NDELA) in some industrial fluids at the 3% level (Fan et al., 1977b) triggered an effort to determine the extent of worker exposure to airborne NDELA. Fortunately, NDELA was not detected as an airborne pollutant in factories making the cutting fluids or in large and small machine shops using the fluids (New England Institute for Life Sciences, in press).

Despite the fact that NDELA is not sufficiently volatile to pose a problem as an air pollutant, significant exposure of workers can still occur by dermal contact, either through splashing or by handling metal parts that have been soaked in the fluids. Concern for these workers has increased as a result of recent reports that NDELA penetrates the skin of both rats (Lijinsky et al., 1981) and humans (Bronaugh et al., 1981; Edwards et al., 1979) and that it is a more potent carcinogen than was previously surmised (Lijinsky et al., 1980; Preussmann et al., in press a). NDELA is also present in cosmetics. This is discussed in a later section of the chapter.

Tobacco and Tobacco Smoke

At the time of harvesting, fresh tobacco leaves do not contain measurable amounts of nitrosamines ($< 5 \mu\text{g}/\text{kg}$). However, these compounds are formed during curing, aging, and fermentation. Their concentrations depend primarily on the amount of nitrosatable precursors -- proteins, alkaloids, agricultural chemicals -- and the amount of nitrate present in the fresh tobacco as well as on the processing conditions, which lead to the reduction of the nitrate to nitrite. The volatile nitrosamines NDMA and nitrosodiethylamine (NDEA) have been detected in various tobacco products (Table 7-3); however, the amounts detected are much less than those for pesticide-derived and tobacco-specific nitrosamines (Table 7-4). For example, NDELA has been detected in levels as high as 6,800 $\mu\text{g}/\text{kg}$ in snuff. It is

TABLE 7-3

Volatile Nitrosamines in Tobacco^a

NOTE: Some concentrations have been rounded off to two significant figures.

| Tobacco | Concentrations, $\mu\text{g}/\text{kg}$ | |
|--------------------------------------|---|-----------------|
| | NDMA | NDEA |
| Cigar (Pennsylvania) ^b | 6.9 | NR ^c |
| Robinson, high nitrate ^d | 9.5 | 15 |
| Catterton, high nitrate ^d | 16 | 12 |
| French cigarette | 188 | 12 |
| Fine-cut chewing tobacco | 56 | 8.6 |

^aAdapted from Brunnemann *et al.*, 1977.

^bCommercial product bought in open market.

^cNo data reported.

^dTobacco from experimental cigarettes with high nitrate levels provided by the U.S. Department of Agriculture.

TABLE 7-4

Nitrosamines in Snuff and Chewing Tobacco^a

NOTE: Some concentrations have been rounded off to two significant figures.

| Tobacco Product | NDELA, $\mu\text{g}/\text{kg}$ | Tobacco-Specific Nitrosamines, mg/kg | | | |
|-----------------|-----------------------------------|---|--------|---------|--------|
| | | NAT | NNN | NNK | Total |
| Snuff, fresh | 6,800 | - | - | - | - |
| Snuff, aged | 3,200 | - | - | - | - |
| U.S. snuff | - | 2-44 | 3.5-39 | 1.3-4.6 | 6.8-88 |
| Bavarian snuff | - | 4 | 6 | 1.5 | 12 |
| Chewing tobacco | 220-280 | - | - | - | - |

^aData adapted from Hoffmann and Adams, in press.

also present in tobacco (Schmeltz and Hoffmann, 1977) and tobacco smoke (Brunnemann and Hoffmann, in press) of cigarettes. NDELA is believed to be derived from diethanolamine (DELA), which is present in maleic hydrazide-diethanolamine (MH-30), a pesticide used in the cultivation of tobacco. Pesticide formations also contain numerous other nitrosatable amines (Chapter 6), which could give rise to nitrosamines not only in the pesticides themselves, but also in the tobacco to which they are applied. However, despite the fact that residues of such amine-containing pesticides as methylcarbamate and carbaryl (Sevin®) have been detected in harvested tobacco (Sheets and Leidy, 1979), there have been no studies of the amines in these pesticides as precursors to nitrosamines in tobacco.

Alkaloids present in tobacco may serve as precursors for another class of nitrosamines -- the tobacco-specific nitrosamines. Tobaccos used for commercial products in the United States contain between 0.5% and 2.7% alkaloids. Nicotine constitutes between 85% and 95% of the total alkaloid content (Hecht et al., 1974; Piade and Hoffmann, 1980). Important minor alkaloids are nornicotine, anatabine, anabasine, cotinine, and N-formylnornicotine. Several of these alkaloids are secondary and tertiary amines and can be nitrosated. Tobacco-specific nitrosamines identified in tobacco and tobacco smoke include nitroso-nornicotine (NNN), 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and nitrosoanatabine (NAT). In model experiments, nitrosation of nicotine also yielded 4-(N-methyl-N-nitrosamino)-4-(3-pyridyl) butanol (NNA) (Hoffman et al., in press). These nitrosamines are found in tobacco at levels ranging from 1.3 mg/kg to as high as 88 mg/kg (Table 7-4). This wide variation was found to stem from differences in the levels of nitrate and alkaloids in fresh tobacco leaves and the methods used to cure and ferment the tobacco (Hoffmann et al., 1979).

As constituents of chewing tobacco and snuff, carcinogenic tobacco-specific nitrosamines come into direct and prolonged contact with tissues of the oral cavity when the user places and retains the tobacco product between the gum and cheek. Snuff users have a significantly increased risk for oral cancer compared to individuals who do not use tobacco (Pindborg, 1980; Winn et al., 1981a,b). In other studies, a correlation between the increased use of snuff and an increased risk for cancer of the oral cavity has been reported (Christen et al., 1979; Modeer et al., 1980).

Volatile nitrosamines are also formed during smoking, and Hoffmann et al. (in press) have measured a maximum of 70 ng of volatile nitrosamines per cigarette in the mainstream smoke (smoke that passes through the cigarette) in unfiltered U.S. cigarettes (Table 7-5).

TABLE 7-5

Nitrosamines in Mainstream Tobacco Smoke^a

| Tobacco Product | Volatile Nitrosamines, ng/Cigarette or Cigar | | | | Pesticide- Derived Nitrosamines, ng/Cigarette or Cigar | Tobacco-Specific Nitrosamines, ng/Cigarette or Cigar | | |
|--|---|-------------------|------|-------|--|--|-------|-------|
| | NDMA | NEMA ^b | NDEA | NPYR | NDELA | NAT | NNN | NNK |
| U.S. cigarettes: | | | | | | | | |
| Cellulose-acetate filter tip | 6-7 | 0.5 | 1 | 5-9 | 24 | 370 | 310 | 150 |
| Nonfilter | 13-27 | 1-2 | 1-8 | 11-33 | 36 | 330 | 240 | 110 |
| Charcoal-cellulose-acetate filter tip | 14 | 0.6 | 8 | 8 | - | - | - | - |
| Small cigar | - | - | - | - | 68 | 1,700 | 5,500 | 4,200 |
| Large cigar | - | - | - | - | 10 | 1,900 | 3,200 | 1,900 |
| French cigarettes: | | | | | | | | |
| Cellulose-acetate filter tip | 4 | 0.5 | 0.1 | 11 | 0 | 190 | 1,000 | 190 |
| Nonfilter | 29 | 2.7 | 0.6 | 25 | 0 | 640 | 3,200 | 430 |

^aData adapted from Hoffmann *et al.*, in press.

^bNEMA = N-nitrosoethylmethylamine.

The amount of volatile nitrosamines in mainstream smoke from cigarettes with cellulose acetate filters is considerably less (Table 7-5). Apparently, as much as 90% of the volatile nitrosamines generated during smoking is retained by cellulose-acetate filters (Brunnemann et al., 1977; Hoffmann et al., 1980). Another important consideration in determining the exposures of humans to volatile nitrosamines from tobacco smoke is that the concentration of volatile nitrosamines, especially NDMA, in sidestream smoke (smoke from the burning tip of the cigarette) can be as much as 50 times higher than in mainstream smoke (Brunnemann et al., 1980; Hoffmann et al., 1980; Rühl et al., 1980).

In addition to the volatile nitrosamines formed during smoking, each cigarette smoked produces tobacco-specific nitrosamines (Table 7-5). Similar quantities are found in both mainstream and sidestream smoke. The quantities of tobacco-specific nitrosamines in the smoke are dependent on the concentrations of nitrate, nitrite, tobacco alkaloids, and tobacco-specific nitrosamines present in the tobacco itself.

Food

Extensive compilations of the volatile nitrosamine content of foodstuffs in various diets have been published by Gray (in press), Havery et al. (1978), the International Agency for Research on Cancer (1978b), Kawabata et al. (1979), Preussmann et al. (1979), Scanlan (1975), and Schmähl (1980).

Meats. Over the past 9 years, the meat industry and various government and research laboratories have developed techniques to reduce volatile nitrosamines in cooked bacon. Although nitrosamines have not been eliminated, their concentrations have been reduced considerably (Sen et al., 1977b). Data on NPYR, accumulated by the Food and Drug Administration (FDA) from 1971 to 1977, show this reduction clearly (Havery et al., 1978; Table 7-6). The trend toward lower NPYR levels in cooked bacon is partially explained by the use of reduced levels of nitrite and increased levels of the nitrosation inhibitor, ascorbate, in the bacon-curing mixture (Havery et al., 1978). The amount of NPYR formed in cooked bacon is also influenced by the method of cooking, frying temperature, and cooking time (Gray, in press).

In England, Gough et al. (1978) analyzed a variety of foodstuffs typical of the diet in that country. All 50 samples of fried bacon examined contained concentrations of NPYR ranging from 1 to 20 $\mu\text{g}/\text{kg}$, and occasionally up to 200 $\mu\text{g}/\text{kg}$. In addition, all samples contained nitrosopiperidine (NPiP) (in concentrations up to 0.25 $\mu\text{g}/\text{kg}$) and NDMA (in concentrations as high as 5 $\mu\text{g}/\text{kg}$). These data are summarized in Figure 7-2.

TABLE 7-6

Concentrations of NPYR in Commercial U.S. Cooked Bacon^a

NOTE: Some concentrations have been rounded off to two significant figures.

| Year | Concentration ($\mu\text{g}/\text{kg}$), by Brand ^b | | | | | | | | |
|------|--|-------|----------|-----|-----|----|-------|-------|----|
| | A | B | C | D | E | F | G | H | I |
| 1971 | - | - | - | - | 100 | 77 | 73 | - | - |
| 1972 | 110 | - | 20,13,24 | 100 | - | - | 95 | - | 25 |
| 1973 | 34 | 58,36 | - | 25 | 39 | 29 | 86,79 | - | 25 |
| 1974 | 17 | - | 14,8 | 7 | 10 | 29 | 96 | 45,13 | 38 |
| 1975 | - | 18 | 5 | 12 | 19 | 17 | 65 | 13,10 | 12 |
| 1976 | 18 | 25 | - | 3 | 23 | 7 | 33 | 6 | 21 |
| 1977 | 29 | 14 | 5 | 5 | 12 | - | 75 | 10 | 9 |

^aData adapted from Havery *et al.*, 1978.

^bNine brands of bacon purchased at various intervals from 1971 to 1977 in a Washington, D.C. retail market.

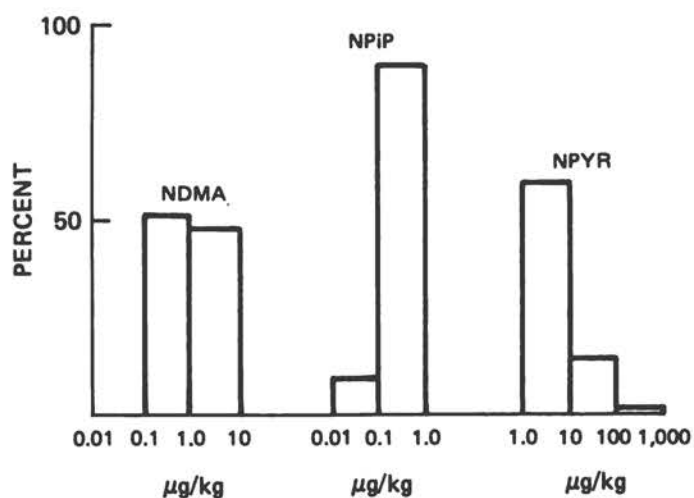


FIGURE 7-2. Measurement of volatile nitrosamines in fried bacon (50 samples). From Webb and Gough, 1980.

In recent surveys conducted in the United States to determine the nitrosamine content of cured meats other than bacon (Nitrite Safety Council, 1980; Sen et al., 1979), the majority of the positive samples contained extremely low levels of nitrosamines -- usually less than 1 $\mu\text{g}/\text{kg}$. These low levels could be attributed in part to the discontinuation of the use of nitrite-spice premixes in the mid-1970's. Some of those premixes contained NPYR, NPiP, and NDMA (Sen et al., 1973, 1974). In England, Gough et al. (1978) found that cured meats other than bacon also contained NPYR, NPiP, and NDMA; however, NPYR did not exceed 1 $\mu\text{g}/\text{kg}$. Some cured meats simultaneously contained several nitrosamines in concentrations ranging from 0.1 to 1 $\mu\text{g}/\text{kg}$.

Dairy Products. Cheeses of the Gouda and Edam types as produced in certain European countries could contain nitrosamines because of the addition of nitrate to prevent the growth of clostridia (Gray et al., 1979). Gough et al. (1977a) examined 21 different varieties of cheese commonly available in England, including cheeses to which nitrate had been added during manufacture. NDMA was not found more frequently in these samples than in cheese made without added nitrate. This corresponds to the finding that the nitrate content is not higher in cheeses to which nitrate has been added (see Chapter 5). Levels of NDMA were similar for all cheeses (1 to 5 $\mu\text{g}/\text{kg}$), except for one sample of Stilton, which contained 13 $\mu\text{g}/\text{kg}$. A similar range of concentrations was measured by Sen et al. (1978) in 31 samples of cheese imported into Canada, many of which were known to have been prepared with the addition of nitrate. Havery et al. (1976) failed to detect any of 14 nitrosamines in 17 samples of cheese, 10 of which had been processed with nitrate as an additive.

Fish. Because of its relatively high amine content, fish has been regarded as a likely source of nitrosamines. However, fish products in the United States rarely contain nitrosamines in excess of 1 $\mu\text{g}/\text{kg}$.

In a study conducted in England (Gough et al., 1978; Webb and Gough, 1980), approximately 80% of the uncooked and fried fish sampled were found to contain NDMA (Figure 7-3), but among 70 samples tested, the only other nitrosamine detected was NPYR, which was present at a concentration of 0.01 $\mu\text{g}/\text{kg}$ in one sample. Five of 24 samples of salted, pickled, smoked, and canned fish also contained NDMA.

The Japanese diet contains a relative large amount of fish, some of which contains high levels of nitrosamines. Table 7-7, taken from Kawabata et al. (1979), shows the NDMA and NPYR levels in various fresh and cooked fish in Japan. These investigators also reported that fish prepared in a gas oven contained higher levels of nitrosamines than fish cooked in an electric oven. Presumably, nitrogen oxides in the combustion products were the source of the nitrosating agents (see Chapter 5). Although

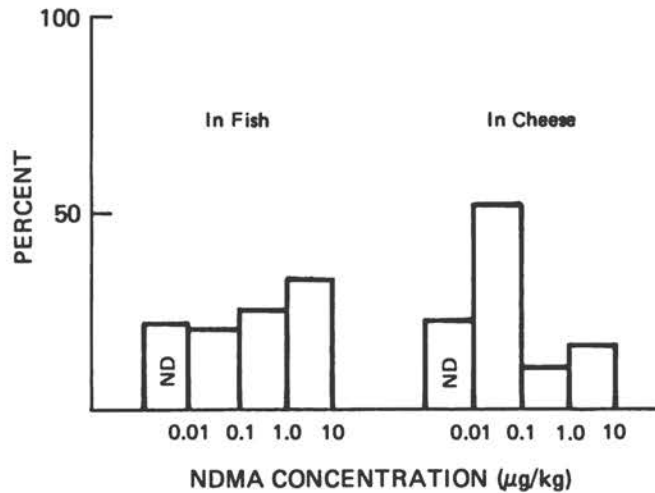


FIGURE 7-3. Measurement of the volatile nitrosamine NDMA in fish (94 samples) and in cheese (76 samples). ND = Not detected. Limit of detection 0.01 µg/kg. From Webb and Gough, 1980.

parallel studies have not been conducted on U.S. foods, it is reasonable to expect that U.S. gas ovens would also lead to elevated nitrosamine levels.

Fruits and Vegetables. Nitrosamine levels lower than 1 µg/kg have occasionally been found in a variety of fruits and vegetables. However, data at these very low levels (<0.2 µg/kg) are unreliable because of the possibility of artifact formation. In one study, 15 baby foods commonly used in the United Kingdom were all free of volatile nitrosamines in concentrations above the detection limit in this assay of 0.01 µg/kg (Webb and Gough, 1980). They also reported that NDMA was found in 5 of 12 samples of canned fruit (< 0.1 µg/kg). Sixteen samples of different varieties of vegetables contained no volatile nitrosamines, and three of 30 soups tested contained NDMA (< 0.09 µg/kg).

Edible Oils. Early reports of the presence of volatile nitrosamines in edible oils have not proven reliable (Hedler and Marquart, 1975; Hedler *et al.*, 1972; White *et al.*, 1974). In a recent paper, Hedler *et al.* (1979) again reported finding NDMA and NDEA at concen-

TABLE 7-7

Changes in Nitrosamine Content of Japanese Salt-Dried Fish and Shellfish after Broiling^a

NOTE: Some of the concentrations have been rounded to two significant figures.

| Sample | Method of Broiling | | Nitrosamine Content, $\mu\text{g}/\text{kg}$ | | Sample | Method of Broiling | | Nitrosamine Content, $\mu\text{g}/\text{kg}$ | |
|---|----------------------|------------------|--|-----------------|---|-----------------------|-----|--|------|
| | | | NDMA | NPYR | | | | NDMA | NPYR |
| Salt-dried horse mackerel (aji-hiraki) | (1) ^b | Raw ^c | 1.0 | NR ^d | Salt-dried chub mackerel (saba-hiraki) | Raw | 0.5 | NR | |
| | | Gas ^e | 6.0 | NR | | Al. foil ^f | 5.8 | NR | |
| | (2) | Raw | 2.4 | NR | Electric ^g | 7.3 | NR | | |
| | | Gas | 7.8 | NR | Gas | 12 | NR | | |
| | (3) | Raw | 4.9 | NR | Salt-dried round herring (urume-iwashi) | Raw | 5.0 | NR | |
| | | Al. foil | 3.4 | NR | | Al. foil | 2.5 | NR | |
| | | Electric | 5.9 | NR | | Electric | 4.5 | NR | |
| | | Gas | 9.4 | NR | | Gas | 26 | NR | |
| | Salt-dried shishyamo | (1) | Raw | 0.5 | NR | Dried squid | Raw | 15 | NR |
| Gas | | | 1.0 | NR | Gas | | 24 | 7.2 | |
| (2) | | Raw | 4.0 | NR | (2) | Raw | 58 | NR | |
| | | Gas | 13 | NR | Gas | 140 | 2.4 | | |
| Salt-dried Pacific saury (sauma-hiraki) | Raw | Tr ^h | NR | (3) | Raw | 84 | NR | | |
| | Al. foil | 3.7 | NR | Gas | 310 | 9.7 | | | |
| | Electric | 1.1 | NR | (4) | Raw | 56 | NR | | |
| | Gas | 6.8 | NR | Gas | 110 | 13 | | | |
| Salt-dried flounder (karei-himono) | Raw | ND | NR | (5) | Raw | 18 | NR | | |
| | Gas | Tr | NR | Al. foil | 19 | NR | | | |
| | | | | Electric | 49 | 3.7 | | | |
| | | | | Gas | 69 | 3.3 | | | |

^aAdapted from Kawabata et al., 1979.^bNumbers presumably refer to different analyses of the same species of fish.^cRaw = uncooked fish.^dNR = no data reported.^eGas = broiled in a city gas range.^fAl. foil = samples covered with aluminum foil broiled in a city gas range.^gElectric = broiled in an electric range.^hTr = trace.

trations as high as 23 and 28 $\mu\text{g}/\text{kg}$, respectively, in one-third of 61 samples. This finding has not been confirmed and is presumed to be due to artifact formation (Preussmann, 1980). More recently, Fiddler et al. (1981) studied 21 edible oils and reported NDMA at levels of only 0.22 to 1.0 $\mu\text{g}/\text{kg}$.

Alcoholic Beverages. Considerable attention has been focused over the past few years on the presence of nitrosamines in beer and other alcoholic beverages. Spiegelhalder et al. (1979) analyzed 158 samples of different types of beer in the Federal Republic of Germany and reported that 70% of them contained NDMA (mean concentration, 2.7 $\mu\text{g}/\text{kg}$). Goff and Fine (1979) reported NDMA levels ranging from 0.4 to 7.0 $\mu\text{g}/\text{kg}$ in 18 brands of U.S. and imported beers. In addition, six of seven brands of Scotch whiskey, which is also made from malt, contained NDMA at levels between 0.3 and 2.3 $\mu\text{g}/\text{kg}$. Scanlan et al. (1980) reported NDMA in 23 of 25 beer samples, at levels ranging from 0 to 14 $\mu\text{g}/\text{kg}$ and averaging 5.9 $\mu\text{g}/\text{kg}$.

In a study conducted by the German Cancer Research Center, Spiegelhalder et al. (1980a,b) analyzed 215 beer samples in the Federal Republic of Germany, 141 of which contained NDMA. The mean NDMA concentration in beer was 2 to 5 $\mu\text{g}/\text{liter}$; one sample of a beer made with smoked malt (Rauchbier) contained 68 $\mu\text{g}/\text{liter}$. NDEA was detected in only 2 of the 215 beer samples, at levels of 3.0 and 0.5 $\mu\text{g}/\text{liter}$. The levels of NDMA found by these investigators in different types of beer in the Federal Republic of Germany and in other European countries are shown in Tables 7-8 and 7-9. Stephany and Schuller (1980), who also analyzed 57 samples of beer consumed in the Netherlands, found that 72% contained NDMA at levels ranging from 0 to 5.7 $\mu\text{g}/\text{liter}$.

Following the initial reports of nitrosamines in beer, efforts were made to determine the source(s) of these contaminants. The only significant source of NDMA was found to be the malt, which contains a variety of amine precursors including hordenine, gramine, and methyltyramine (Mangino et al., in press), that had been exposed to nitrogen oxides during the drying process (Scanlan et al., 1980; Spiegelhalder et al., 1980a,b). Consequently, changes in malting procedures were implemented, resulting in markedly reduced nitrosamine levels in both malts and beer (Havery et al., 1981; Preussmann et al., 1980, in press b). For example, since sulfur dioxide or products of sulfur combustion were found to reduce exposure of the malt to nitrogen oxides, sulfur was added to the open flame used to dry the malt, which led to reduced formation of nitrosamines (Preussmann et al., 1980). In addition, Preussmann et al. (1980) reported that the use of a burner that reduces nitrogen oxide synthesis resulted in the production of malt containing NDMA concentrations as low as 1 to 3 $\mu\text{g}/\text{kg}$ -- a 15- to 30-fold reduction.

TABLE 7-8

NDMA in Different Types of Beer
in the Federal Republic of Germany^a

| Type of Beer | No. of Samples | % positive (> 0.5 µg/liter) | Mean, µg/liter | Maximum, µg/liter |
|-----------------------------------|----------------|-----------------------------|----------------|-------------------|
| Top fermented pale ales | 22 | 23 | 0.2 | 1 |
| Alcohol-free and diet lager beers | 16 | 69 | 1.0 | 4 |
| Pale lager and export lager | 42 | 67 | 1.2 | 7 |
| Pilsen lager | 54 | 65 | 1.2 | 7 |
| Pale strong lager | 25 | 76 | 1.9 | 8 |
| Top fermented dark ales | 25 | 76 | 2.7 | 11 |
| Dark lager and dark strong lager | 22 | 68 | 6.0 | 47 |
| Rauchbier (from smoked malt) | 9 | 100 | 18 | 68 |
| All types | 215 | 66 | 2.5 | 68 |

^aFrom Spiegelhalder et al., 1980b.

TABLE 7-9

NDMA Levels in European Beers^a

| Country | No. of Samples | Mean, µg/liter | Maximum, µg/liter |
|----------------------------|----------------|----------------|-------------------|
| Austria | 12 | 3.0 | 10 |
| Belgium | 16 | 1.3 | 12 |
| Denmark | 5 | 0.5 | 0.5 |
| France | 21 | 1.7 | 7.0 |
| German Democratic Republic | 11 | 0.6 | 1.0 |
| Great Britain and Ireland | 14 | 2.7 | 8.0 |
| Soviet Union | 5 | 1.0 | 2.5 |
| Sweden | 5 | 0.3 | 0.8 |
| Switzerland | 10 | 1.6 | 9.0 |

^aFrom Spiegelhalder et al., 1980b.

The NDMA levels in beer have also dropped considerably. They are now generally well below the level (5 $\mu\text{g/liter}$) at which the FDA would consider regulatory action (Food and Drug Administration, 1980; Havery *et al.*, 1981). A recent survey of domestic and imported beers conducted by the FDA following implementation of this "action level" showed that NDMA in 180 samples of domestic beer ranged from undetectable levels (0.2 or 0.4 $\mu\text{g/liter}$) to 7 $\mu\text{g/liter}$, averaging less than 1 $\mu\text{g/liter}$. NDMA in 80 samples of imported beers ranged from undetectable levels (0.2 or 0.4 $\mu\text{g/liter}$) to 13 $\mu\text{g/liter}$, averaging 1 $\mu\text{g/liter}$ (Havery *et al.*, 1981).

Walker *et al.* (1979) and Goff and Fine (1979) screened other alcoholic beverages such as brandy, rum, wine, and cider. Goff and Fine were not able to detect volatile nitrosamines in U.S. beverages other than beer and Scotch whiskey. Walker and colleagues did find volatile nitrosamines in 74 of 145 French apple brandies and cognac at concentrations generally ranging from 0.18 to 0.6 $\mu\text{g/kg}$. The highest concentration found by these investigators was 10 $\mu\text{g/kg}$ in an apple brandy. They also found nitrosamines in 7 of 17 rums (average, 0.3 $\mu\text{g/kg}$), in 3 of 4 kirsch samples (average, 3.2 $\mu\text{g/kg}$), and in 2 of 2 mirabelle samples (average, 3.8 $\mu\text{g/kg}$).

Cosmetics

Many cosmetics, soaps, and shampoos are contaminated with NDELA (Fan *et al.*, 1977a). The source of the amine is triethanolamine, which is present in most cosmetic formulations (see Chapter 6). Bactericides such as 2-bromo-2-nitro-1,3-propanediol (bronopol) are generally considered to be the nitrosating agents (Fan *et al.*, 1978a; Ong and Rutherford, 1980). NDELA levels in cosmetics have been found to range from less than 1 $\mu\text{g/kg}$ to 48,000 $\mu\text{g/kg}$ (Fan *et al.*, 1977a).

The presence of nitrosamines in products containing lauramine oxide was analyzed by combined GC-TEA and HPLC-TEA (Hecht, 1981). Of seven products analyzed, six gave positive responses on both HPLC-TEA and GC-TEA for nitrosododecylmethylamine (NDOMA). The peak corresponding to NDOMA decreased upon photolysis, a reaction characteristic of nitrosamines. One sample was analyzed on a large scale, and the mass spectrum obtained was characteristic of NDOMA. The concentrations of NDOMA in these products ranged from 0.02 to 0.60 $\mu\text{g/kg}$. Apparently, some NDOMA is also present in the lauramine oxide ingredient itself. These results show that NDOMA, a bladder carcinogen, is present in cosmetics formulated with lauramine oxide.

Analyses for nitrosobenzylmethylamine (NBMA) and nitrosomethylstearylamine (NMSA) in products containing stearylalkonium chloride are currently in progress in Hecht's laboratories. Of eight samples screened by combined HPLC-TEA, three gave a positive response for NMSA and, tentatively, NBMA. Further work is needed to confirm the

presence of these nitrosamines in cosmetics. In addition, every cosmetic sample tested so far has given some positive TEA response, indicating the presence of other nitroso compounds, nitro compounds, or nitrite esters (Hecht, 1981).

Pharmaceuticals

Many drugs contain chemically bound nitrogen and have sites for possible nitrosation. For example, cimetidine, i.e., N-cyano-N'-methyl-N''-[2-[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]guanidine, which is a drug used for treatment of peptic ulcers, has received considerable attention recently, and its possible nitrosation products are being investigated (Henderson and Basilio, 1980; Jensen and Magee, 1981). In addition, vasodilators derived from nitrate or nitrite esters can behave as potential nitrosating agents. Nitrosation may occur exogenously (within the drug itself) or it may occur endogenously in humans after ingestion of the drug (see discussion in Chapter 8).

Eisenbrand *et al.* (1979) analyzed 68 commercial formulations of the drug aminopyrine for the presence of preformed nitrosamines. Although this drug was available in the Federal Republic of Germany, none of the formulations had been licensed for sale in the United States. All formulations contained NDMA in amounts varying from 1 to 370 $\mu\text{g}/\text{kg}$. These investigators also demonstrated that aminopyrine, a tertiary amine, reacts extremely rapidly with trace levels of nitrogen oxides in the air to form the NDMA.

Krull *et al.* (1979b) reported that nitrosamine impurities were absent from 68 of 73 pharmaceutical products, consisting of both prescription and over-the-counter drugs available in the United States. The methods used by these investigators were designed to detect both volatile and nonvolatile N-nitroso compounds at levels as low as 1 $\mu\text{g}/\text{kg}$.

Pesticides

Fan *et al.* (1976) reported that nitroaniline herbicides, and formulations prepared as the amine salt, contained substantial nitrosamine impurities. For example, a formulation of the herbicide trifluralin, i.e., α,α,α -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine, was shown to contain 154 mg of nitrosodipropylamine (NDPA) per liter (Ross *et al.*, 1977). Two experimental field studies have demonstrated that the nitrosamine impurity in trifluralin is not detectable as an air pollutant on farms, even during application, presumably because the pesticide is generally applied by incorporation into the soil (Ross *et al.*, 1978; West and Day, 1979). Furthermore, NDPA was not detected in crops such as tomatoes, soybeans,

cottonseed, peanuts, lettuce, carrots, cauliflower, or alfalfa (West and Day, 1979), which were harvested from fields that had been treated with trifluralin.

The NDPA in trifluralin was produced during synthesis when ring nitration with nitric and sulfuric acids was followed by the addition of dipropylamine (Fine et al., 1977b). If the residual nitrosating agent were still present during the addition of dipropylamine, then NDPA would be formed as an impurity. Within a few months after the discovery of the NDPA impurity, the manufacturer was able to reduce it 10-fold to 18 mg/liter (Fine, 1980b). The impurity has been further reduced to less than 1 mg/liter (Zweig and Garner, in press) by eliminating the nitrogen oxides formed during a nitration step before the synthesis step in which dipropylamine is introduced.

Similar success has been achieved in the reduction of nitrosamine contaminants in amine salt pesticides. Some dimethylamine salt formulations of 2,3,6-dichlorobenzoic acid had contained NDMA levels as high as 187, 195, and 640 mg/liter. The source of the nitrosating agent was traced to metal cans treated with sodium nitrite. A switch to plastic-lined cans reduced the NDMA concentration to less than 2 mg/liter.

Work by the EPA has extended the study of nitrosamine impurities in pesticides to cover more than 300 formulations (Bontoyan et al., 1979; Cohen et al., 1978; Zweig and Garner, in press). Analytical procedures that have been used to screen pesticide formulations for nitrosamines have been reviewed by Wolf et al. (1980). From these analyses, it was determined that only the following chemical classes of pesticides contained identifiable N-nitroso contaminants:

- substituted dinitroaniline derivatives
- dimethylamine salts of phenoxyalkanoic acid herbicides
- di- and triethanolamine salts of several pesticides
- some quaternary ammonium compounds
- dimethyl thiocarbamoyl disulfide (thiram)
- some morpholine derivatives

The EPA analyses of these pesticides showed that concentrations of N-nitroso compounds ranged from 1.2 to 430 µg/liter. The chemical identity of the contaminants was usually predictable on the basis of the chemical structure and route of synthesis of the pesticide compound. For example, pendimethalin [N(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine] yields nitrosopendimethalin, 2,4-D dimethylamine salt yields NDMA, and dinoseb triethanolamine salt yields NDELA. Many such nitrogen-containing pesticides were subsequently analyzed and found to contain less than 1 µg/liter of the corresponding N-nitroso compound contaminant (Zweig et al., 1980). Results of these analyses are tabulated in Table 7-10. The EPA has proposed a policy for

TABLE 7-10

Pesticides Contaminated with N-Nitroso Compounds in Concentrations
Lower than 1 µg/liter^a

| <u>Chemical Class</u> | <u>Pesticide</u> |
|----------------------------------|--|
| Amides | Alachlor Clonitralid Diphenamid |
| Carbamates and thiocarbamates | Carbaryl Ethiofencarb Methiocarb Propoxur Sulfallate Triallate |
| Triazines | Ametryn Anilazine Atrazine Cyanazine Metribuzin Propazine Simazine |
| Organophosphates | Acephate Azinphos-methyl Azodrin Dialifor Dicrotophos Fenamiphos Isufenphos Methamidophos Methyl parathion |
| Substituted ureas | Diuron Linuron |
| Miscellaneous classes | Chlorothalonil Fenaminosulf Maleic hydrazide Oryzalin Oxythioquinox Paraquat |

^aFrom Zweig et al., 1980.

pesticides contaminated with N-nitroso compounds (U.S. Environmental Protection Agency, 1980). This proposed policy is presented below to provide an example of how human exposure to N-nitroso compounds may be reduced.

The analysis of the N-nitroso compound content of the following classes of compounds would be required:

- dinitroanilines
- secondary and tertiary alkylamines, arylamines, and alkanolamines
- quaternary ammonium compounds, if a nitrosating agent such as nitrite has been added

Furthermore, the EPA would set the level of detection of nitrosamines at 1 mg/liter. This level is not based on the sensitivity of present instrumentation (e.g., the TEA can detect levels of N-nitroso compounds as low as 0.01 to 0.02 mg/liter) but, rather, on the practicality of directly and routinely analyzing formulations without extraction or clean-up. Moreover, because commercial formulations are diluted, 1 mg/liter concentrations of N-nitroso compounds in the technical grade material would be correspondingly lower in the final product.

According to the proposed EPA policy for products containing any of the above classes of compounds for use in the United States, registrants must submit to the EPA analytical data on the possible nitrosamine content of their products. Samples for analysis must be obtained from fresh production batches. If a nitrosating agent is present in or added to the final product, the same sample must be reanalyzed following storage at room temperature for 3 and 6 months. (This requirement may be slightly modified in the final regulations to reduce the number of samples.) If the analyses demonstrate that nitrosamine contamination does not exceed 1 mg/liter, the product is cleared for registration.

When nitrosamine concentrations exceed 1 mg/liter, the EPA proposals require that the registrant provide data on the carcinogenic potential of the contaminant and estimate potential exposure for persons coming into contact with the pesticide during normal application procedures and farm practices. On the basis of the exposure analysis and potential carcinogenic risk, the EPA staff will perform a risk estimation according to statistical methods such as linear extrapolation (one-hit model) or multistage analysis (Fishbein, 1980).

The EPA proposals suggest that the acceptable level of risk for N-nitroso compounds is approximately 10^{-6} . This level will be used by the agency to distinguish between high-risk (greater than 10^{-6}) and low-risk (less than 10^{-6}) products. However, before

the EPA proceeds on a regulatory action, registrants whose products fall into the high-risk category, i.e., exceed the 10^{-6} risk level, will be given the opportunity to lower potential exposure, thereby reducing the risk to users of their pesticides.

Suggested methods for reducing the nitrosamine content of the product include modification of the manufacturing process. This can be accomplished by using nitrosamine-free starting materials or intermediates or by eliminating or reducing all potential nitrosating agents formed or added during the manufacturing process (e.g., nitrous acid resulting from nitration). Nitrosating agents may also be eliminated by improving packaging technology. For example, the use of sodium nitrite as a corrosion inhibitor for metal containers could be discontinued or the metal containers could be lined with plastic to prevent exposure of pesticide formulations to metal treated with sodium nitrite. Exposure can also be reduced by modifying application techniques or by eliminating high-exposure uses (U.S. Environmental Protection Agency, 1980).

Water

Water is deionized with ion exchange resins for use in high-pressure steam production, chemical and food processing, mining, agriculture, laboratories, automobile batteries, steam irons, and, to a limited degree, in drinking water in areas with water of high salinity. Nitrosamines have been observed in deionized water by Fiddler *et al.* (1977), Cohen and Bachman (1978), and Gough *et al.* (1977b). Fiddler *et al.* (1977) reported that 13 of 19 samples of water exposed to regenerated deionizing resins contained NDMA in concentrations ranging from 0.03 to 0.34 $\mu\text{g}/\text{kg}$. NDMA concentrations of 0.25 $\mu\text{g}/\text{kg}$ and lower were also detected in deionized water by Cohen and Bachman (1978). The mechanism of nitrosamine formation in deionized water is not yet clearly understood (Angeles *et al.*, 1978; Gough *et al.*, 1977b; Kimoto *et al.*, 1980). Volatile nitrosamines such as NDMA have also been identified in industrial wastewater in concentrations ranging from 0.2 to 5 $\mu\text{g}/\text{liter}$ (Cohen and Bachman, 1978; Fine *et al.*, 1977b). In well water with a high nitrate content, NDEA and NDMA have been found at levels lower than 0.01 $\mu\text{g}/\text{liter}$ (Fine and Rounbehler, 1976).

Volatile nitrosamines have been shown to be absent in drinking water in Baltimore, Md. (Fine *et al.*, 1977c); Belle, W. Va.; Boston and Waltham, Mass. (Fine *et al.*, 1977a); New Orleans, La. (Fine and Rounbehler, 1976); Cincinnati, Ohio; Philadelphia, Penna.; Washington, D.C. (Fan *et al.*, 1978b); Kansas City, Ks.; Lexington, Mo.; and Memphis, Tenn. (Fine, 1980b). However, the water in most of these cities contained several nonvolatile low-polarity compounds, which gave a positive response on the TEA. For example, ethylene glycol dinitrate, a TEA-responsive compound that is not an N-nitroso

compound, has been shown to be present in drinking water in Cincinnati, Philadelphia, and Washington, D.C. (Fan *et al.*, 1978b). The majority of the TEA-responsive compounds in drinking water have not been identified.

Air

Amines and oxides of nitrogen can react to form nitrosamines (see Chapter 4). This reaction is partially responsible for the nitrosamine contamination of workroom air in tanneries and rubber factories (see earlier discussion on occupational settings).

NMOR and NDMA have been found in the interior air of new 1979 model automobiles by Rounbehler *et al.* (1980). In the 38 automobiles tested, the concentrations ranged from 0.07 to 0.83 $\mu\text{g}/\text{m}^3$ (average, 0.3 $\mu\text{g}/\text{m}^3$) for NDMA, from 0.07 to 2.5 $\mu\text{g}/\text{m}^3$ (average, 0.67 $\mu\text{g}/\text{m}^3$) for NMOR, from 0.04 to 0.39 $\mu\text{g}/\text{m}^3$ (average, 0.11 $\mu\text{g}/\text{m}^3$) for NDEA, and trace levels (less than 0.01 $\mu\text{g}/\text{m}^3$) for nitrosodi-N-butylamine (NDBA). The source of the nitrosamines in at least one car was shown to be the spare tire. Other sources contributing to the presence of NMOR and NDMA may include rubberized mats, rubber grommets, and rubber sealants.

NDMA pollution of indoor air from the burning of tobacco was investigated by Brunnemann and Hoffmann (1978). The appreciable amounts of NDMA detected in these environments were attributed largely to sidestream smoke.

A large portion of the NPYR produced during the frying of bacon escapes with the fumes (Sen *et al.*, 1976; also see review by Gray, *in press*). Fine (1980b) has calculated that a person inhaling the nitrosamine-laden air for 30 minutes in a typical domestic kitchen would be exposed to no more than 0.12 μg of NPYR.

In studies conducted in New York City, Boston, and northern New Jersey, Fine *et al.* (1977a) found little evidence to suggest that NDMA or other N-nitroso compounds were being formed in the atmosphere, even near amine factories. NDMA was found sporadically at only three of the 40 sites studied. These results suggest that airborne NDMA and other N-nitroso compounds do not present a wide-spread air pollution problem, but, rather, that they are localized pollutants associated with specific environments. One reason for this may be due to the rapid breakdown of N-nitroso compounds following exposure to light (Hanst *et al.*, 1977).

EXPOSURE OF HUMANS TO N-NITROSO COMPOUNDS

Individual Exposures, by Source

Occupational Settings. Some workers in rubber factories, rocket fuel factories, and leather tanneries in the United States can be exposed to relatively massive amounts of volatile nitrosamines, primarily

by inhalation. Table 7-11 summarizes the extent of these occupational exposures. The highest exposure apparently occurs in the leather-tanning industry where up to 440 μg of NDMA may be inhaled daily (Fine, 1980a).

The exposure of humans to NMOR in rubber factories was studied by McGlothlin *et al.* (1981). In this study, an area air sample contained 250 $\mu\text{g}/\text{m}^3$ of NMOR, and the highest personal (breathing zone) concentration was measured at 25 $\mu\text{g}/\text{m}^3$ (time-weighted average) for a worker in the feed mill and calender operation, a so-called "hot-process area" where rubber is heated by friction and compression. Daily exposure at this concentration of NMOR would equal 250 μg . In another study of exposures in the rubber industry, daily exposure to NDPhA ranged from 5 μg to 430 μg (Fajen *et al.*, 1979). Based on concentrations of NDMA and NMOR in rubber factories located in the Federal Republic of Germany, Preussmann *et al.* (1980) calculated that the average daily exposure to these nitrosamines was approximately 50 μg . More recently, Spiegelhalder and Preussmann (in press) reported that personal breathing zone samples in injection molding and curing areas of an industrial rubber products factory contained NMOR at an average concentration of 380 $\mu\text{g}/\text{m}^3$ and NDMA at an average concentration of 90 $\mu\text{g}/\text{m}^3$ (average over an 8-hour period). These concentrations would result in daily intakes of 3.8 μg NMOR and 0.9 μg NDMA. The only other data on occupational exposures to nitrosamines were reported by Fine *et al.* (1976b,c) in their studies of NDMA levels in a rocket fuel factory. Daily exposures of factory workers to NDMA from this source were calculated to be a maximum of 260 μg (average, 10 to 50 μg).

TABLE 7-11

Occupational Exposure to Airborne Nitrosamines^a

| Industry | Maximum Daily Exposure, $\mu\text{g}/\text{Person}/\text{Day}$ ^b | | |
|----------------------------|--|------|-------|
| | NDMA | NMOR | NDPhA |
| Leather tanning | 440 | 20 | |
| Rubber (tire curing) | | 250 | |
| Rubber chemical production | | | 430 |
| Rocket fuel production | 260 | | |

^aAdapted from Fine, 1980a.

^bIt is assumed that an average worker weighs 70 kg (1.85 m^2 body surface area) and breathes 20 liters/min during an 8-hour workday.

Tobacco and Tobacco Smoke. The largest nonoccupational exposure to preformed nitrosamines is derived from tobacco products and tobacco smoke.

Based on the levels of nitrosamines measured in snuff and chewing tobacco (see Table 7-4), exposure of humans to nitrosamines from these sources probably exceeds exposure from cigar and cigarette smoke. In addition to exposure to preformed tobacco-specific nitrosamines, in vitro and in vivo experiments have indicated that humans can also be exposed to tobacco-specific nitrosamines that are formed during snuff dipping (Hecht et al., 1974). A comparison of the saliva of snuff dippers and tobacco chewers shows that tobacco-specific nitrosamines are present in saliva in a wide range of concentrations. The variation is ascribed to differences in the product, in the manner of chewing, and in the composition of each person's saliva (Hoffmann and Adams, in press). Data on concentrations of tobacco-specific nitrosamines in the saliva of snuff dippers measured by Hoffmann et al. (in press) are presented in Table 7-12. Combined nitrosamine levels of 600 ng/g in the saliva seem fairly common. Thus, 100 ml of saliva from a snuff dipper would contain 60 µg of the tobacco-specific nitrosamines.

TABLE 7-12

Tobacco-Specific Nitrosamines in Saliva of Snuff-Dipping Women^a

NOTE: Some of the concentrations have been rounded off to two significant figures.

| <u>Age</u> | <u>Tobacco-Specific Nitrosamines,</u> <u>ng/g</u> | | |
|------------|--|------------|------------|
| | <u>NAT</u> | <u>NNN</u> | <u>NNK</u> |
| 37 | 17-510 | 30-130 | 13 |
| 40 | 29 | 22 | 2 |
| 41 | 210-470 | 110-140 | 21-26 |
| 43 | 14 | 27 | 20 |
| 44 | 320-370 | 320-420 | 62-96 |
| 45 | 7 | 5 | 8 |
| 52 | 13-46 | 26-57 | 11-23 |
| 53 | 150 | 120 | 200 |

^aData adapted from Hoffmann et al., in press.

However, before daily human exposure can be estimated, more information is needed on typical snuff-dipping habits, the average amount of snuff consumed per day, and the amount of saliva that is swallowed.

Tobacco smoke has been shown to contain eight nitrosamines: NDELA, four volatile nitrosamines (NDMA, NEMA, NDEA, and NPYR), and three tobacco-specific nitrosamines (NAT, NNN, and NNK). In estimating the exposure of humans to these compounds, several facts must be considered. First, the amount of tobacco-specific nitrosamines present in cigarettes far exceeds the amount of NDELA or of the volatile nitrosamines. Second, the cellulose acetate filter tips seem to trap a large portion of the volatile nitrosamines. Third, the amount of tobacco-specific nitrosamines is almost 10 times greater in the smoke of cigars than of cigarettes. Exposure of humans can be estimated by adding the concentrations given in Table 7-5 for all nitrosamines present in tobacco smoke. The nitrosamine intake is 0.87 μg for a U.S. cellulose-acetate filter tip cigarette, 0.76 μg for a U.S. nonfilter cigarette, 1.4 μg for a French cellulose-acetate filter tip cigarette, 4.3 μg for a French nonfilter cigarette, and as high as 11 μg for a small cigar. If these data are assumed to be typical of an average cigarette, then a pack of 20 U.S. filter cigarettes represents an intake of approximately 17 μg .

Food. Dietary exposures to preformed nitrosamines have been assessed for typical residents of the United Kingdom (Gough *et al.*, 1978; Webb and Gough, 1980), the Netherlands (Stephany and Schuller, 1980), and the Federal Republic of Germany (Spiegelhalder *et al.*, 1980a,b). A similar food-by-food assessment of dietary intake of nitrosamines has not yet been undertaken in the United States.

In the United Kingdom, the Laboratory of the Government Chemist (Gough *et al.*, 1978) conducted extensive analyses of foodstuffs typical of the diet of the U.K. population. Foods purchased in normal retail outlets were analyzed while within their shelf life or, when appropriate, after cooking. Tested foods included bacon, canned meats, fresh meat and meat products, fish and fish products, cheese, yogurt, desserts, canned fruits and jams, frozen and fresh vegetables, soups, beverages (but not beer), and baby foods. Complete meals were prepared under normal domestic conditions, and then the nitrosamine content of the prepared foods was measured. The investigators found that cured meats were the major source of volatile nitrosamines and that fish and cheese were the second and third largest sources. All other foods examined contained an average nitrosamine concentration less than 0.06 $\mu\text{g}/\text{kg}$.

The intake of the nitrosamines was estimated by determining the average intake and the nitrosamine content of the various food items consumed daily (Table 7-13). The investigators concluded that the likely daily intake of volatile nitrosamines from the normal diet (excluding beer) is 0.53 μg and that 0.43 μg of that total amount ($\sim 80\%$) is contributed by cured meats; however, this study did not include beer and whiskey (Webb and Gough, 1980).

TABLE 7-13

Estimated Daily Intake of Volatile Nitrosamines in Food
Consumed in the United Kingdom^a

NOTE: Some of the numbers in this table have been rounded off to two significant figures.

| <u>Food</u> | <u>Food Consumption, g/Person/Day</u> | <u>Total Nitrosamine Intake, $\mu\text{g}/\text{Person}/\text{Day}$</u> |
|-----------------|---|--|
| Cured meats | 49 | 0.43 |
| Fish | 20 | <0.01 |
| Cheese | 14 | <0.01 |
| All other foods | 1,400 | 0.08 |
| TOTAL | 1,500 | 0.53 |

^aFrom Gough et al., 1978.

The National Institute of Public Health in Bilthoven, the Netherlands, studied the nitrosamine content of 206 foods and beverages ingested by volunteers over a 24-hour sampling period (Stephany and Schuller, 1980). Based on the concentrations of nitrosamines found in various foods (Table 7-14), the average daily intake of NDMA was calculated to be 0.38 μg . In diets containing beer, beer was shown to be the major dietary source of NDMA, contributing 90% of the total NDMA intake of 1.1 μg . Because of the major contribution of beer and because males generally drink more beer than females, NDMA intake by the average male was approximately 4 times greater than that of the average female. However, since that study was undertaken, maltsters have modified their processes to reduce the nitrosamine contamination of malt.

The German Cancer Research Center conducted a survey of the nitrosamine levels in 2,826 commercially produced food products (Spiegelhalder et al., 1980a,b). Among the products tested were cured meats, cheeses, and numerous beer samples, along with more than 2,000 other types of food not normally associated with volatile nitrosamines. Data on these foods were then used to estimate typical concentrations to which humans are exposed. The calculations were based on the average per capita consumption representative of males in the Federal Republic of Germany. The average daily intakes of volatile nitrosamines for those males are shown in Tables 7-15 and 7-16. Beer contributed 0.7 μg to the per capita intake of NDMA; for meat and meat products, the intakes were 0.1 μg per capita for NDMA, 0.1 μg for NPYR, and 0.01 μg for NP1P. Cheese contributed 0.01 μg of

TABLE 7-14

NDMA Content of Various Kinds of Foods in the Netherlands^a

| Food | Number of Samples | NDMA Content, $\mu\text{g}/\text{kg}$ | | Percentage of Positive Samples ^b |
|------------|-------------------|---------------------------------------|---------------------|---|
| | | Mean | Range | |
| Beer | 57 | 1.2 | 0 ^c -5.7 | 72 |
| Whiskey | 7 | 0.3 | 0-0.9 | 86 |
| Cured meat | 38 | 0.5 | 0-3.6 | 71 |
| Veal | 22 | 0.1 | 0-0.4 | 29 |
| Seafood | 53 | 0.4 | 0-2.1 | 55 |
| Cheese | 84 | 0.1 | 0-1.1 | 45 |

^aFrom Stephany and Schuller, 1980.

^bSamples with content equal to or higher than the limit of detection (0.1 $\mu\text{g}/\text{kg}$).

^c0 = Content below limit of determination.

TABLE 7-15

Average Daily Intake of Nitrosamines by Males in the Federal Republic of Germany. Calculated from the Average per Capita Consumption^a

| Food | Product Consumption, g | Nitrosamine | Nitrosamine Intake, μg Per Capita |
|------------------------|------------------------|-------------|--|
| Beer ^b | 560 | NDMA | 0.7 |
| Meat and meat products | 210 | NDMA | 0.1 |
| | | NPYR | 0.1 |
| | | NPiP | 0.01 |
| Cheese | 30 | NDMA | 0.01 |
| Others | 1,500 | NDMA | 0.2 |
| | | NPYR | 0.03 |
| TOTAL | 2,300 | NDMA | 1.1 |
| | | NPYR | 0.13 |

^aData adapted from Spiegelhalder *et al.*, 1980b.

^bNDMA intake is corrected to account for the proportion of sales for different types of beer.

TABLE 7-16

Percentage Contributed by Different Types of Food to Total NDMA Intake (1.1 $\mu\text{g}/\text{person}/\text{day}$) by Males in the Federal Republic of Germany^a

| <u>Food</u> | <u>Total Percentage of NDMA Intake</u> | <u>Percentage of Total Food Consumption (by weight)</u> |
|------------------------|--|---|
| Beer | 64 | 24 |
| Meat and meat products | 10 | 9 |
| Cheese | 1 | 1 |
| Others | 25 | 66 |

^aFrom Spiegelhalder et al., 1980b.

NDMA, and all other products together were responsible for approximately 0.2 μg of NDMA and 0.03 μg of NPYR. The total daily intake by males was 1.1 μg of NDMA and 0.13 μg of NPYR.

At the time of this study, the largest contribution was clearly made by beer, which accounted for approximately 64% of the total NDMA intake (Table 7-16). But as stated earlier, maltsters have modified their manufacturing processes so that current NDMA levels in European beers average 1 $\mu\text{g}/\text{liter}$ (Havery et al., 1981).

Estimated Exposure of the U.S. Population to Nitrosamines

The committee has developed estimates of possible exposures to nitrosamines from the various sources in the environment discussed earlier in this chapter. Because of the variation in the concentrations of nitrosamines detected in each of the sources listed in Table 7-17, no attempt has been made to present an overall average concentration of these chemicals in each source. Instead, the committee has selected values that have been published by groups who have made comprehensive surveys of each environmental medium.

In addition to the uncertainties related to environmental concentrations of these chemicals, the committee made numerous assumptions concerning average consumption and usage of the various products (Table 7-17) in order to develop estimates of exposure of humans. As a result, the exposure levels given in Table 7-17 should not be interpreted as absolute numbers but, rather, should be used to understand the relative importance of the various environmental sources of nitrosamines. For example, the numbers in Table 7-17 indicate that tobacco smoke contributes far more to the exposure of humans to nitrosamines than any other source included in this table (occupational exposure would be higher, but it is not included).

Nonsmokers would be exposed to nitrosamines (in descending order) in beer (0.97 or 0.34 μg), automobile interiors (0.50 or 0.20 μg), in cosmetics (0.41 μg), in bacon (0.17 μg), and in Scotch whiskey (0.03 μg). However, these comparisons of relative exposures are made among different nitrosamines (that vary widely in their carcinogenic potential) and different exposure routes (e.g., inhalation, ingestion, and dermal contact). Thus, a further word of caution concerning the overinterpretation of these data seems necessary since it is presently unknown whether carcinogenic potency in animals (see Chapter 9) is similar to that in humans and whether one route of exposure is more important in the production of adverse health effects.

Two other sources of exposure were not included in this discussion -- occupational environments and endogenous formation of nitrosamines. Certain occupational environments have, however, been discussed in detail earlier and may result in extremely high exposures (e.g., 440 $\mu\text{g}/\text{day}$ in a U.S. tannery). The possible contribution to the exposure of humans resulting from the endogenous synthesis of nitrosamines is discussed in Chapter 8.

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Humans may be exposed to N-nitroso compounds as preformed nitrosamines in the environment via inhalation, ingestion, and dermal contact or following their formation in the body from various precursors. Because analytical methods for volatile nitrosamines are sufficiently sensitive, the levels of these compounds in many environmental sources have been determined. However, adequate methods for unstable and/or nonvolatile N-nitroso compounds have only recently been developed.

Nitrosamines have been detected in occupational settings, such as rubber factories and leather-tanning operations, and in tobacco, tobacco smoke, food, alcoholic beverages (e.g., beer),

TABLE 7-17

Estimated Exposures of Humans to Nitrosamines in the United States

| Source of Exposure | Nitrosamine | Primary Exposure Route | Concentration | Daily Intake, $\mu\text{g}/\text{person}$ |
|---------------------------------------|-------------|------------------------|--|---|
| Cigarette smoking ^a | NDEA | Inhalation | 1.0 ng/cig) | 17 |
| | NEMA | Inhalation | 0.5 ng/cig) | |
| | NDMA | Inhalation | 6.5 ng/cig) | |
| | NPYR | Inhalation | 7 ng/cig) | |
| | NDELA | Inhalation | 24 ng/cig) | |
| | NNN | Inhalation | 310 ng/cig) | |
| | NAT | Inhalation | 370 ng/cig) | |
| | NNK | Inhalation | 150 ng/cig) | |
| Automobile Interiors | NDMA) | Inhalation | 1.0 $\mu\text{g}/\text{m}^3$ ^b | 0.50 ^b |
| | NMOR) | | 0.34 $\mu\text{g}/\text{m}^3$ ^c | 0.20 ^c |
| | NDEA) | | | |
| Beer | NDMA | Ingestion | 2.8 $\mu\text{g}/\text{liter}$ | 0.97 ^d |
| | | | 1.0 $\mu\text{g}/\text{liter}$ | 0.34 ^e |
| Cosmetics ^f | NDELA | Dermal | 11 mg/kg | 0.41 |
| Cured meat; cooked bacon ^g | NPYR | Ingestion | 5 $\mu\text{g}/\text{kg}$ | 0.17 |
| Scotch whiskey ^h | NDMA | Ingestion | 0.97 $\mu\text{g}/\text{liter}$ | 0.03 |

^aAverage concentrations were taken from Hoffmann et al. (in press). See Table 7-5.

^bEstimate represents exposures from new automobile interiors. Average nitrosamine concentration was taken from Rounbehler et al., 1980. The committee assumed an average daily exposure of approximately 1 hour/day.

^cThe committee has assumed that the average daily exposure is approximately 1 hour/day and that it occurs in both new and older automobiles. Since the nitrosamine concentration is likely to be lower in older automobiles, the committee has reduced the average concentration to one-third of that in new automobiles.

TABLE 7-17 (Footnotes continued)

- ^dThe NDMA content was obtained by averaging the results of Goff and Fine (1979). This estimate does not reflect the reduction in NDMA content of beer as a result of recent changes in malting processes (see footnote e). In 1979, approximately 21 billion liters of domestically produced beer were sold in the United States (United States Brewer's Association, 1980). The committee has assumed 75% of the population consumed beer and the yearly per capita consumption was approximately 126 liters.
- ^eThe average NDMA content of U.S. beers is currently 1.0 $\mu\text{g/liter}$ (Havery *et al.*, 1981). The committee has used the above per capita consumption figure of 126 liter per year (see footnote d).
- ^fThe average NDELA level in cosmetics (11.3 mg/kg) was calculated by averaging the seven data points in Fan *et al.* (1977a). If a woman uses 2 g of cosmetics per day, then 22.6 μg NDELA would be applied to the skin and approximately 1.8% of this would penetrate (Edwards *et al.*, 1979).
- ^gIn 1979, approximately 1.5 billion pounds (~ 680 million kg) of bacon was produced in the United States (American Meat Institute, 1980). Assuming 25% of the U.S. population (55 million people) consumes bacon, daily per capita consumption would be 0.034 kg. The U.S. Department of Agriculture requires that bacon contain less than 10 $\mu\text{g/kg}$ nitrosamines. The committee has assumed that bacon contains one-half this amount (5 $\mu\text{g/kg}$).
- ^hThe average NDMA content of Scotch whiskey was calculated by averaging the data from seven samples analyzed by Goff and Fine (1979). It is assumed that the average drinker consumes 30 ml per day.

cosmetics, pharmaceuticals, pesticides, automobile interiors, water, and air. These data, coupled with assumptions concerning lifestyle and population behavior (e.g., number of cans of beer consumed per day, amount of cosmetic used, occupation, and hours in a car provide a basis for estimating exogenous exposure of humans to nitrosamines.

Because large quantities of nitrosamines are formed in certain occupational settings and are present in tobacco and tobacco smoke, humans may be exposed to high concentrations of nitrosamines from these sources. For example, a maximum exposure to NDMA was estimated to be 440 $\mu\text{g/day}$ in a leather-tanning facility. In one study of the U.S. rubber industry, a maximum exposure to NMOR of 250 μg was reported, and maximum exposure of humans to NDMA from a rocket fuel factory was calculated to be 260 $\mu\text{g/day}$. However, the figures for the tanning facility and the rocket fuel factory are maximum possible exposures estimated from area air samples. Exposure of workers could be

approximately 10% of these values if their typical activities over an 8-hour day are considered. Breathing zone samples would provide a more accurate indication of actual exposures.

Summing the levels of all nitrosamines present in tobacco smoke, total concentration is 0.87 μg per U.S. cellulose-acetate filter tip cigarette, 0.76 μg per U.S. nonfilter cigarette, 1.4 μg per French cellulose-acetate filter tip cigarette, 4.3 μg per French nonfilter cigarette, and as high as 11 μg for a small cigar. Smoking a pack of 20 U.S. cellulose acetate filter cigarettes would result in an intake of approximately 17 μg .

Assays of foodstuffs in the Netherlands and the Federal Republic of Germany have indicated that the largest single dietary source of nitrosamines is beer, which, in the German study, contributed 64% of the volatile nitrosamines in the diet. Since this study was completed, however, the concentrations of nitrosamines in beer have decreased considerably. Cured meat and meat products contribute the second highest amount of nitrosamines to the diet. According to results from the same German study, approximately 0.21 μg of nitrosamines is derived per person per day from this source (approximately 16% of the total dietary intake of nitrosamines).

Average relative exposures to nitrosamines by the U.S. population have been estimated by the committee for a variety of sources. With the exception of occupational exposures, which were not considered in the calculations, cigarette smoking contributes the greatest amount to total daily nitrosamine intake. Among the other sources considered (i.e., automobile interiors, beer, cosmetics, cooked bacon, and Scotch whiskey), Scotch whiskey contributed the lowest daily intake and the other four sources contributed intermediate amounts ranging from 0.16 to 0.97 $\mu\text{g}/\text{person}/\text{day}$.

The committee makes the following recommendations based on the data reviewed in this chapter:

1. The committee recommends that methods for the analysis of nonvolatile N-nitroso compounds be further developed and applied so that the total burden of these chemicals in the human population can be assessed.
2. The committee suggests that the origins of exposure to N-nitroso compounds in the various environmental media be determined and that methods to eliminate or reduce the production of these contaminants be developed. In this regard, the committee would like to call special attention to the potential health hazards posed by the presence of the carcinogenic contaminant, NDELA, in cutting fluids and cosmetics.

REFERENCES

- Acheson, E. D. 1976. Nasal cancer in the furniture and boot and shoe manufacturing industries. *Prev. Med.* 5:295-315.
- American Meat Institute. 1980. Meat Facts: A Statistical Summary About America's Largest Food Industry. American Meat Institute, Arlington, Virginia. 27 pp.
- Angeles, R. M., L. K. Keefer, P. P. Roller, and S. J. Uhm. 1978. Chemical models for possible nitrosamine artifact formation in environmental analysis. Pp. 109-115 in E. A. Walker, L. Gričiute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Bontoyan, W. R., M. W. Law, and D. P. Wright, Jr. 1979. Nitrosamines in agricultural and home-use pesticides. *J. Agric. Food Chem.* 27: 631-634.
- Bretschneider, K., and J. Matz. 1973. [In German; English summary.] [Nitrosamines (NA) in the atmospheric air and in the air at the places of employment.] *Arch. Geschwulstforsch.* 42:36-41.
- Bretschneider, K., and J. Matz. 1976. Occurrence and analysis of nitrosamines in air. Pp. 395-399 in E. A. Walker, P. Bogovski, and L. Gričiute, eds. *Environmental N-Nitroso Compounds: Analysis and Formation*, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.
- Bronaugh, R. L., E. R. Congdon, and R. J. Scheuplein. 1981. The effect of cosmetic vehicles on the penetration of N-nitrosodiethanolamine through excised human skin. *J. Invest. Dermatol.* 76:94-96.
- Brunnemann, K. D., and D. Hoffmann. 1978. Chemical studies on tobacco smoke LIX. Analysis of volatile nitrosamines in tobacco smoke and polluted indoor environments. Pp. 343-356 in E. A. Walker, L. Gričiute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Brunnemann, K. D., and D. Hoffmann. In press. Assessment of the carcinogenic N-nitrosodiethanolamine in tobacco products and tobacco smoke. *Carcinogenesis*.

- Brunnemann, K. D., L. Yu, and D. Hoffmann. 1977. Assessment of carcinogenic volatile N-nitrosamines in tobacco and in mainstream and sidestream smoke from cigarettes. *Cancer Res.* 37: 3218-3222.
- Brunnemann, K. D., W. Fink, and F. Moser. 1980. Analysis of volatile N-nitrosamines in mainstream and sidestream smoke from cigarettes by GLC-TEA. *Oncology* 37:217-222.
- Cardy, R. H., W. Lijinsky, and P. K. Hildebrandt. 1979. Neoplastic and nonneoplastic urinary bladder lesions induced in Fischer 344 rats and B6C3F hybrid mice by N-nitrosodiphenylamine. *Ecotoxicol. Environ. Saf.* 3:29-35.
- Christen, A. G., R. K. McDaniel, and J. E. Doran. 1979. Snuff dipping and tobacco chewing in a group of Texas college athletes. *Texas Dent. J.* 97:6-10.
- Cohen, J. B., and J. D. Bachman. 1978. Measurement of environmental nitrosamines. Pp. 357-372 in E. A. Walker, L. Griciute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Cohen, S. Z., G. Zweig, M. Law, D. Wright, and W. R. Bontoyan. 1978. Analytical determination of N-nitroso compounds in pesticides by the United States Environmental Protection Agency -- A preliminary study. Pp. 333-342 in E. A. Walker, L. Griciute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Edwards, G. S., M. Peng, D. H. Fine, B. Spiegelhalder, and J. Kann. 1979. Detection of N-nitrosodiethanolamine in human urine following application of a contaminated cosmetic. *Toxicol. Lett.* 4:217-222.
- Eisenbrand, G., B. Spiegelhalder, C. Janzowski, J. Kann, and R. Preussmann. 1978. Volatile and non-volatile N-nitroso compounds in foods and other environmental media. Pp. 311-324 in E. A. Walker, L. Griciute, M. Castegnaro, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Eisenbrand, G., B. Spiegelhalder, J. Kann, R. Klein, and R. Preussmann. 1979. Carcinogenic N-nitrosodimethylamine as a contamination in drugs containing 4-dimethylamino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (amidopyrine, aminophenazone). *Arzneim. Forsch.* 29:867-869.

- Fajen, J. M., D. P. Rounbehler, and D. H. Fine. In press. Summary report on N-nitroso compounds in the factory environment. Paper presented at the 7th International Meeting on Analysis and Formation of N-Nitroso Compounds, September 28-October 1, 1981, Tokyo, Japan. Meeting sponsored by the International Agency for Research on Cancer, Lyon, France.
- Fajen, J. M., G. A. Carson, D. P. Rounbehler, T. Y. Fan, R. Vita, U. E. Goff, M. H. Wolf, G. S. Edwards, D. H. Fine, V. Reinhold, and K. Biemann. 1979. N-Nitrosamines in the rubber and tire industry. *Science* 205:1262-1264.
- Fan, T. Y., and D. H. Fine. 1978. Formation of N-nitrosodimethylamine in the injection port of a gas chromatograph: An artifact in nitrosamine analysis. *J. Agric. Food Chem.* 26:1471-1472.
- Fan, S., D. H. Fine, R. Ross, D. P. Rounbehler, A. Silvergleid, and L. Song. 1976. Determination of N-nitroso pesticides in air, water, and soil. Paper presented at the 172nd American Chemical Society National Meeting, August 29-September 3, 1976, San Francisco, California. Abstract available from American Chemical Society, Washington, D.C.
- Fan, T. Y., U. Goff, L. Song, D. H. Fine, G. P. Arsenault, and K. Biemann. 1977a. N-Nitrosodiethanolamine in cosmetics, lotions and shampoos. *Food Cosmet. Toxicol.* 15:423-430.
- Fan, T. Y., J. Morrison, D. P. Rounbehler, R. Ross, D. H. Fine, W. Miles, and N. P. Sen. 1977b. N-Nitrosodiethanolamine in synthetic cutting fluids: A part-per-hundred impurity. *Science* 196:70-71.
- Fan, T. Y., I. S. Krull, R. D. Ross, M. H. Wolf, and D. H. Fine. 1978a. Comprehensive analytical procedures for the determination of volatile and non-volatile, polar and non-polar N-nitroso compounds. Pp. 3-17 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Fan, T. Y., R. Ross, D. H. Fine, L. H. Keith, and A. W. Garrison. 1978b. Isolation and identification of some thermal energy analyzer (TEA) responsive substances in drinking water. *Environ. Sci. Technol.* 12:692-695.
- Fiddler, W., J. W. Pensabene, R. C. Doerr, and C. J. Dooley. 1977. The presence of dimethyl- and diethyl-nitrosamines in deionized water. *Food Cosmet. Toxicol.* 15:441-443.
- Fiddler, W., J. W. Pensabene, and W. I. Kimoto. 1981. Investigations of edible oils for volatile nitrosamines. *J. Food Sci.* 46:603-605.

- Fine, D. H. 1980a. Exposure assessment to preformed environmental N-nitroso compounds from the point of view of our own studies. *Oncology* 37:199-202.
- Fine, D. H. 1980b. N-Nitroso compounds in the environment. *Adv. Environ. Sci. Technol.* 10:39-123.
- Fine, D. H., and D. P. Rounbehler. 1976. N-Nitroso compounds in water. Pp. 255-263 in L. H. Keith, ed. *Identification and Analysis of Organic Pollutants in Water*. Ann Arbor Science Press, Ann Arbor, Michigan.
- Fine, D. H., and D. P. Rounbehler. In press. Occurrence of N-nitrosamines in the work place: Some recent developments. In R. A. Scanlan and S. R. Tannenbaum, eds. *N-Nitroso Compounds*, ACS Symposium Series. American Chemical Society, Washington, D.C.
- Fine, D. H., D. P. Rounbehler, N. M. Belcher, and S. S. Epstein. 1976a. N-Nitroso compounds: Detection in ambient air. *Science* 192:1328-1330.
- Fine, D. H., D. P. Rounbehler, E. D. Pellizzari, J. E. Bunch, R. W. Berkley, J. McCrae, J. T. Bursey, E. Sawicki, K. Krost, and G. A. DeMarrais. 1976b. N-Nitrosodimethylamine in air. *Bull. Environ. Contam. Toxicol.* 15:739-746.
- Fine, D. H., D. P. Rounbehler, E. Sawicki, K. Krost, and G. A. DeMarrais. 1976c. N-Nitroso compounds in the ambient community air of Baltimore, Maryland. *Anal. Lett.* 9:595-604.
- Fine, D. H., J. Morrison, D. P. Rounbehler, A. Silvergleid, and L. Song. 1977a. N-Nitrosamines in the air environment. Pp. 168-181 in *Air Pollution Control Association, ed. Toxic Substances in the Air Environment*. Air Pollution Control Association, Pittsburgh, Pennsylvania.
- Fine, D. H., D. P. Rounbehler, T. Fan, and R. Ross. 1977b. Human exposure to N-nitroso compounds in the environment. Pp. 293-307 in H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds. *Origins of Human Cancer, Book A: Incidence of Cancer in Humans*. Cold Spring Harbor Conferences on Cell Proliferation, Vol. 4. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Fine, D. H., D. P. Rounbehler, A. Rounbehler, A. Silvergleid, E. Sawicki, K. Krost, and G. A. DeMarrais. 1977c. Determination of dimethylnitrosamine in air, water, and soil by thermal energy analysis: Measurements in Baltimore, Maryland. *Environ. Sci. Technol.* 11:581-584.

- Fishbein, L. 1980. Overview of some aspects of quantitative risk assessment. *J. Toxicol. Environ. Health* 6:1275-1296.
- Food and Drug Administration. 1980. Dimethylnitrosamine in malt beverages; Availability of guide. *Fed. Regist.* 45(113):39341-39342.
- Goff, E. U., and D. H. Fine. 1979. Analysis of volatile N-nitrosamines in alcoholic beverages. *Food Cosmet. Toxicol.* 17:569-573.
- Gough, T. A., M. F. McPhail, K. S. Webb, B. J. Wood, and R. F. Coleman. 1977a. An examination of some foodstuffs for the presence of volatile nitrosamines. *J. Sci. Food Agric.* 28:345-351.
- Gough, T. A., K. S. Webb, and M. F. McPhail. 1977b. Volatile nitrosamines from ion-exchange resins. *Food Cosmet. Toxicol.* 15:437-440.
- Gough, T. A., K. S. Webb, M. A. Pringuer, and B. J. Wood. 1977c. A comparison of various mass spectrometric and a chemiluminescent method for the estimation of volatile nitrosamines. *J. Agric. Food Chem.* 25:663-667.
- Gough, T. A., K. S. Webb, and R. F. Coleman. 1978. Estimate of the volatile nitrosamine content of UK food. *Nature* 272:161-163.
- Gray, J. I. In press. Formation of N-nitroso compounds in foods. In R. A. Scanlan and S. R. Tannenbaum, eds. *N-Nitroso Compounds*, ACS Symposium Series. American Chemical Society, Washington, D.C.
- Gray, J. I., D. M. Irvine, and Y. Kakuda. 1979. Nitrates and N-nitrosamines in cheese. *J. Food Prot.* 42:263-272.
- Hanst, P. L., J. S. Spence, and M. Miller. 1977. Atmospheric chemistry of N-nitroso dimethylamine. *Environ. Sci. Technol.* 11:403-405.
- Havery, D. C., D. A. Kline, E. M. Miletta, F. L. Joe, Jr., and T. Fazio. 1976. Survey of food products for volatile N-nitrosamines. *J. Assoc. Off. Anal. Chem.* 59:540-546.
- Havery, D. C., T. Fazio, and J. W. Howard. 1978. Trends in levels of N-nitrosopyrrolidine in fried bacon. *J. Assoc. Off. Anal. Chem.* 61:1379-1382.
- Havery, D. C., J. H. Hotchkiss, and T. Fazio. 1981. Nitrosamines in malt and malt beverages. *J. Food Sci.* 46:501-505.

- Hecht, S. S. 1981. Investigation of new nitrosamine contaminants of cosmetics -- Report on FDA contract studies. Paper presented at the SCC-FDA Scientific Seminar, Society of Cosmetic Chemists, Mid-Atlantic Chapter, April 15, 1981, held at the Food and Drug Administration, Washington, D.C.
- Hecht, S. S., R. M. Ornaf, and D. Hoffmann. 1974. Chemical studies on tobacco smoke. XXXIII. N'-Nitrosornicotine in tobacco: Analysis of possible contributing factors and biologic implications. J. Natl. Cancer Inst. 54:1237-1244.
- Hecht, S. S., C.-h. B. Chen, N. Hirota, R. M. Ornaf, T. C. Tso, and D. Hoffmann. 1978. Tobacco-specific nitrosamines: Formation from nicotine in vitro and during tobacco curing and carcinogenicity in strain A mice. J. Natl. Cancer Inst. 60:819-824.
- Hedler, L., and P. Marquart. 1975. Determination of volatile N-nitroso compounds, and particularly of nitrosodimethylamine, nitrosodiethylamine and nitrosodipropylamine, in soybean oil: Effect of storage period on recovery rates. Pp. 183-191 in P. Bogovski, and E. A. Walker, eds. N-Nitroso Compounds in the Environment, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Hedler, L., H. Kaunitz, P. Marquardt, H. Fales, and R. E. Johnson. 1972. Detection of N-nitroso compounds by gas chromatography (nitrogen detector) in soybean oil extract. Pp. 71-73 in P. Bogovski, R. Preussmann, and E. A. Walker, eds. N-Nitroso Compounds: Analysis and Formation, IARC Scientific Publication No. 3. International Agency for Research on Cancer, Lyon, France.
- Hedler, L., C. Schurr, and P. Marquardt. 1979. Determination of volatile N-nitroso compounds in various samples of edible vegetable oils and margarine (commercially available products). J. Am. Oil Chem. Soc. 56:681-684.
- Henderson, E. E., and M. Basilio. 1980. Evidence for in vivo damage of DNA by nitrosocimetidine. Proc. Am. Assoc. Cancer Res. 21:85.
- Hoffmann, D., and J. D. Adams. In press. Carcinogenic tobacco specific N-nitrosamines in snuff and in the saliva of snuff dippers. Cancer Res.
- Hoffmann, D., J. D. Adams, K. D. Brunnemann, and S. S. Hecht. 1979. Assessment of tobacco-specific N-nitrosamines in tobacco products. Cancer Res. 39:2505-2509.

- Hoffmann, D., J. D. Adams, J. J. Piade, and S. S. Hecht. 1980. Chemical studies on tobacco smoke LXVIII. Analysis of volatile and tobacco-specific nitrosamines in tobacco products. Pp. 507-516 in E. A. Walker, L. Gričič, M. Castegnaro, and M. Bőrzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Hoffmann, D., J. D. Adams, K. D. Brunnemann, and S. S. Hecht. In press. Formation, occurrence and carcinogenicity of *N*-nitrosamines in tobacco products. In R. A. Scanlan and S. R. Tannenbaum, eds. *N-Nitroso Compounds*, ACS Symposium Series. American Chemical Society, Washington, D.C.
- International Agency for Research on Cancer. 1978a. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France. 566 pp.
- International Agency for Research on Cancer. 1978b. *Environmental Carcinogens: Selected Methods of Analysis, Vol. 1. Analysis of Volatile Nitrosamines in Food*, IARC Scientific Publication No. 18. International Agency for Research on Cancer, Lyon, France. 212 pp.
- International Agency for Research on Cancer. 1980. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France. 841 pp.
- Janzowski, C., G. Eisenbrand, and R. Preussmann. 1978. Occurrence and determination of *N*-nitroso-3-hydroxypyrrolidine in cured meat products. *J. Chromatogr.* 150:216-220.
- Jensen, D. E., and P. N. Magee. 1981. Methylation of DNA by nitrosocimetidine *in vitro*. *Cancer Res.* 41:230-236.
- Kawabata, T., H. Ohshima, J. Uibu, M. Nakamura, M. Matsui, and M. Hamano. 1979. Occurrence, formation, and precursors of *N*-nitroso compounds in Japanese diet. Pp. 195-209 in E. C. Miller, J. A. Miller, I. Hirono, T. Sugimura, and S. Takayama, eds. *Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis*. Japan Scientific Society Press, Tokyo, Japan, and University Park Press, Baltimore, Maryland.
- Kimoto, W. I., C. J. Dooley, J. Carré, and W. Fiddler. 1980. Role of strong ion exchange resins in nitrosamine formation in water. *Water Res.* 14:869-876.
- Krull, I. S., T. Y. Fan, and D. H. Fine. 1978. Problem of artifacts in the analysis of *N*-nitroso compounds. *Anal. Chem.* 50:698-701.

- Krull, I. S., E. U. Goff, G. G. Hoffman, and D. H. Fine. 1979a. Confirmatory methods for the thermal energy determination of N-nitroso compounds at trace levels. *Anal. Chem.* 51:1706-1709.
- Krull, I. S., U. Goff, A. Silvergleid, and D. H. Fine. 1979b. N-Nitroso compound contaminants in prescription and nonprescription drugs. *Arzneim. Forsch.* 29:870-874.
- Lijinsky, W., M. D. Reuber, and W. B. Manning. 1980. Potent carcinogenicity of nitrosodiethanolamine in rats. *Nature* 288:589-590.
- Lijinsky, W., A. M. Losikoff, and E. B. Sansone. 1981. Penetration of rat skin by N-nitrosodiethanolamine and N-nitrosomorpholine. *J. Natl. Cancer Inst.* 66:125-127.
- Lu, S.-H., A.-M. Camus, L. Tomatis, and H. Bartsch. 1981. Mutagenicity of extracts of pickled vegetables collected in Linhsien County, a high-incidence area for esophageal cancer in Northern China. *J. Natl. Cancer Inst.* 66:33-36.
- Mangino, M. M., R. A. Scanlan, and T. J. O'Brien. In press. Nitrosamines in beer. In R. A. Scanlan and S. R. Tannenbaum, eds. N-Nitroso Compounds, ACS Symposium Series. American Chemical Society, Washington, D.C.
- McGlothlin, J. D., T. C. Wilcox, J. M. Fajen, and G. S. Edwards. 1981. A health hazard evaluation of nitrosamines in a tire manufacturing plant. Pp. 283-299 in American Chemical Society Symposium Series, Book No. 149. American Chemical Society, Washington, D.C.
- Mirvish, S. S., J. Sams, and S. Arnold. 1979. Spectrophotometric method for determining ureas applied to nitrosoureas, nitrosocyanamides and a cyanamide. *Fresenius Z. Anal. Chem.* 298:408-410.
- Modéer, T., S. Lavstedt, and C. Åhlund. 1980. Relation between tobacco consumption and oral health in Swedish schoolchildren. *Acta Odontol. Scand.* 38:223-227.
- New England Institute for Life Sciences. In press. N-Nitroso Compounds in the Factory Environment. Report prepared under Contract No. 210-77-0100 for the National Institute for Occupational Safety and Health, Center for Disease Control, Public Health Service, U.S. Department of Health, Education, and Welfare, Cincinnati, Ohio. New England Institute for Life Sciences, Waltham, Massachusetts.
- Nitrite Safety Council. 1980. A survey of nitrosamines in sausages and dry-cured meat products. *Food Technol.* 34(7):45-51, 53, 103.

- Ong, J. T. H., and B. S. Rutherford. 1980. Some factors affecting the rate of N-nitrosodiethanolamine formation from 2-bromo-2-nitropropane-1,3-diol and ethanolamines. *J. Soc. Cosmet. Chem.* 31: 153-159.
- Piade, J. J., and D. Hoffmann. 1980. Chemical studies on tobacco smoke LXVII. Quantitative determination of alkaloids in tobacco by liquid chromatography. *J. Liq. Chromatogr.* 3:1505-1515.
- Pindborg, J. J. 1980. Oral Cancer and Precancer. John Wright and Sons, Ltd., Bristol, United Kingdom. 177 pp.
- Preussmann, R. 1980. Letter to the Editor. *J. Am. Oil Chem. Soc.* 57:53.
- Preussmann, R., G. Eisenbrand, and B. Spiegelhalder. 1979. Occurrence and formation of N-nitroso compounds in the environment and *in vivo*. Pp. 51-71 in P. Emmelot and E. Kriek, eds. *Environmental Carcinogenesis*. Elsevier/North-Holland Biomedical Press, Amsterdam, the Netherlands.
- Preussmann, R., B. Spiegelhalder, and G. Eisenbrand. 1980. Reduction of human exposure to environmental N-nitroso-carcinogens. Examples of possibilities for cancer prevention. Pp. 273-285 in B. Pullman, P. O. P. Ts'o, and H. Gelboin, eds. *Carcinogenesis: Fundamental Mechanisms and Environmental Effects*. D. Reidel Publishing Company, the Netherlands.
- Preussmann, R., M. Habs, D. Schmähl, and G. Eisenbrand. In press a. Dose-response study on the carcinogenicity of N-nitrosodiethanolamine (NDELA) in male Sprague-Dawley rats. Paper presented at the 7th International Meeting on Analysis and Formation of N-Nitroso Compounds, September 28-October 1, 1981, Tokyo, Japan. Meeting sponsored by the International Agency for Research on Cancer, Lyon, France.
- Preussmann, R., B. Spiegelhalder, and G. Eisenbrand. In press b. Reduction of human exposure to environmental N-nitroso compounds. In R. A. Scanlan and S. R. Tannenbaum, eds. *N-Nitroso Compounds*, ACS Symposium Series. American Chemical Society, Washington, D.C.
- Ross, R. D., J. Morrison, D. P. Rounbehler, S. Fan, and D. H. Fine. 1977. N-Nitroso compound impurities in herbicide formulations. *J. Agric. Food Chem.* 25:1416-1418.
- Ross, R., J. Morrison, and D. H. Fine. 1978. Assessment of dipropyl-nitrosamine levels in a tomato field following application of Treflan EC. *J. Agric. Food Chem.* 26:455-457.

- Rounbehler, D. P., I. S. Krull, E. U. Goff, K. M. Mills, J. Morrison, G. S. Edwards, D. H. Fine, J. M. Fajen, G. A. Carson, and V. Rheinhold. 1979. Exposure to N-nitrosodimethylamine in a leather tannery. *Food Cosmet. Toxicol.* 17:487-491.
- Rounbehler, D. P., J. Reisch, and D. H. Fine. 1980. Nitrosamines in new motor-cars. *Food Cosmet. Toxicol.* 18:147-151.
- Rühl, C., J. D. Adams, and D. Hoffmann. 1980. Chemical studies on tobacco-specific N-nitrosamines in the smoke of selected cigarettes from the U.S.A., West Germany, and France. *J. Anal. Toxicol.* 4: 255-259.
- Saul, R. L., W. R. Bruce, and M. C. Archer. In press. An analytical method for N-nitrosamides. Paper presented at the 7th International Meeting on Analysis and Formation of N-Nitroso Compounds, Sept. 28-Oct. 1, 1981, Tokyo, Japan. Meeting sponsored by the International Agency for Research on Cancer, Lyon, France.
- Scanlan, R. A. 1975. N-Nitrosamines in foods. *Crit. Rev. Food Technol.* 5:357-402.
- Scanlan, R. A., J. F. Barbour, J. H. Hotchkiss, and L. M. Libbey. 1980. N-Nitrosodimethylamine in beer. *Food Cosmet. Toxicol.* 18:27-29.
- Schmähl, D. 1980. Risk assessment of N-nitroso compounds for human health. *Oncology* 37:193-307.
- Schmeltz, I., and D. Hoffmann. 1977. Nitrogen-containing compounds in tobacco and tobacco smoke. *Chem. Rev.* 77:295-311.
- Sen, N. P., W. F. Miles, B. Donaldson, T. Panalaks, and J. R. Iyengar. 1973. Formation of nitrosamines in a meat curing mixture. *Nature* 245:104-105.
- Sen, N. P., B. Donaldson, C. Charbonneau, and W. F. Miles. 1974. Effect of additives on the formation of nitrosamines in meat curing mixtures containing spices and nitrite. *J. Agric. Food Chem.* 22:1125-1130.
- Sen, N. P., S. Seaman, and W. F. Miles. 1976. Dimethylnitrosamine and nitrosopyrrolidine in fumes produced during the frying of bacon. *Food Cosmet. Toxicol.* 14:167-170.

- Sen, N. P., D. E. Coffin, S. Seaman, B. Donaldson, and W. F. Miles. 1977a. Extraction, clean-up and estimation as methyl ether of 3-hydroxyl-1-nitrosopyrrolidine, a non-volatile nitrosamine in cooked bacon at mass fractions of $\mu\text{g}/\text{kg}$. Pp. 179-185 in B. J. Tinbergen and B. Krol, eds. Proceedings of the Second International Symposium on Nitrite in Meat Products, September 7-10, 1976, Zeist, the Netherlands. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Sen, N. P., B. Donaldson, S. Seaman, B. Collins, and J. Y. Iyengar. 1977b. Recent nitrosamine analyses in cooked bacon. Can. Inst. Food Sci. Technol. J. 10:A13-A15.
- Sen, N. P., B. A. Donaldson, S. Seaman, J. R. Iyengar, and W. F. Miles. 1978. Recent studies in Canada on the analysis and occurrence of volatile and non-volatile N-nitroso compounds in food. Pp. 373-393 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. Environmental Aspects of N-Nitroso Compounds, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Sen, N. P., S. Seaman, and W. F. Miles. 1979. Volatile nitrosamines in various cured meat products: Effect of cooking and recent trends. J. Agric. Food Chem. 27:1354-1360.
- Sheets, T. J., and R. B. Leidy. 1979. Influence of insecticides and nematicides on the chemistry of tobacco. Recent Adv. Tobacco Sci. 5:83-131.
- Spiegelhalder, B., and R. Preussmann. In press. Nitrosamines and rubber. Paper presented at the 7th International Meeting on Analysis and Formation of N-Nitroso Compounds, Sept. 28-Oct. 1, 1981, Tokyo, Japan. Meeting sponsored by the International Agency for Research on Cancer, Lyon, France.
- Spiegelhalder, B., G. Eisenbrand, and R. Preussmann. 1979. Contamination of beer with trace quantities of N-nitrosodimethylamine. Food Cosmet. Toxicol. 17:29-31.
- Spiegelhalder, B., G. Eisenbrand, and R. Preussmann. 1980a. Occurrence of volatile nitrosamines in food: A survey of the West German market. Pp. 467-479 in E. A. Walker, L. Gričiute, M. Castegnaro, and M. Börzsönyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Spiegelhalder, B., G. Eisenbrand, and R. Preussmann. 1980b. Volatile nitrosamines in food. Oncology 37:211-216.

- Stephany, R. W., and P. L. Schuller. 1980. Daily dietary intakes of nitrate, nitrite and volatile N-nitrosamines in the Netherlands using the duplicate portion sampling technique. *Oncology* 37:203-210.
- U.S. Brewers Association. 1980. *Brewers Almanac: The Brewing Industry in the United States*. United States Brewers Association, Washington, D.C. 125 pp.
- U.S. Environmental Protection Agency. 1980. Pesticides contaminated with N-nitroso compounds; proposed policy. *Fed. Regist.* 45(124): 42854-42858.
- Walker, E. A., M. Castegnaro, L. Garren, G. Toussaint, and B. Kowalski. 1979. Intake of volatile nitrosamines from consumption of alcohols. *J. Natl. Cancer Inst.* 63:947-951.
- Walters, C. L., M. J. Downes, M. W. Edwards, and P. L. R. Smith. 1978. Determination of a non-volatile N-nitrosamine on a food matrix. *Analyst* 103:1127-1133.
- Webb, K. S., and T. A. Gough. 1980. Human exposure to preformed environmental N-nitroso compounds in the U.K. *Oncology* 37: 195-198.
- Webb, K. S., T. A. Gough, A. Carrick, and D. Hazelby. 1979. Mass spectrometric and chemiluminescent detection of picogram amounts of N-nitrosodimethylamine. *Anal. Chem.* 51:989-992.
- West, S. D., and E. W. Day, Jr. 1979. Determination of volatile nitrosamines in crops and soils treated with dinitroaniline herbicides. *J. Agric. Food Chem.* 27:1075-1080.
- White, R. H., D. C. Havery, E. L. Roseboro, and T. Fazio. 1974. Isolation of volatile N-nitrosamines in edible vegetable oils and cooked bacon fat. *J. Assoc. Off. Anal. Chem.* 57:1380-1382.
- Winn, D. M., W. J. Blot, and J. F. Fraumeni. 1981a. Letter to the Editor: Snuff dipping and oral cancer. *N. Engl. J. Med.* 305: 230-231.
- Winn, D. M., W. J. Blot, C. M. Shy, L. W. Pickle, A. Toledo, and J. F. Fraumeni, Jr. 1981b. Snuff dipping and oral cancer among women in the southern United States. *N. Engl. J. Med.* 304: 745-749.
- Wolf, M. H., W. C. Yu, and D. H. Fine. 1980. Analysis of N-nitroso compounds in pesticide information. Pp. 363-387 in G. Zweig and J. Sherma, eds. *Analytical Methods for Pesticides and Plant Growth Regulators, Vol. 11: Updated General Techniques and Additional Pesticides*. Academic Press, Inc., New York, London, Toronto, Sydney, and San Francisco.

Zweig, G., and W. Garner. In press. Policy and regulatory aspects of N-nitroso contaminants in pesticide products. In R. A. Scanlan and S. R. Tannenbaum, eds. N-Nitroso Compounds, ACS Symposium Series. American Chemical Society, Washington, D.C.

Zweig, G., S. Selim, R. Hummel, A. Mittelman, D. P. Wright, Jr., C. Law, Jr., and E. Regelman. 1980. Analytical survey of N-nitroso contaminants in pesticide products. Pp. 555-564 in E. A. Walker, L. Griciute, M. Castegnaro, and M. Börzsönyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.

CHAPTER 8

METABOLISM AND PHARMACOKINETICS OF NITRATE, NITRITE, AND N-NITROSO COMPOUNDS

| | <u>Page</u> |
|--|-------------|
| METABOLISM OF NITRATE AND NITRITE..... | 8-3 |
| Oral Cavity..... | 8-3 |
| Upper Gastrointestinal Tract..... | 8-8 |
| Absorption from the Gastrointestinal Tract..... | 8-12 |
| Transport and Chemical Reactions in the Blood..... | 8-13 |
| Excretion of Nitrate in Urine..... | 8-14 |
| Endogenous Synthesis of Nitrate and Nitrite..... | 8-15 |
| METABOLISM OF N-NITROSO COMPOUNDS..... | 8-17 |
| Pharmacokinetics..... | 8-17 |
| Activation and Deactivation..... | 8-19 |
| Macromolecular Interactions and Repair Mechanisms..... | 8-21 |
| <u>In Vivo</u> Formation of N-Nitroso Compounds..... | 8-22 |
| SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS..... | 8-32 |
| REFERENCES..... | 8-36 |

CHAPTER 8

METABOLISM AND PHARMACOKINETICS OF NITRATE, NITRITE, AND N-NITROSO COMPOUNDS

This chapter describes metabolic pathways that are pertinent to evaluating the contribution of nitrate, nitrite, and N-nitroso compounds to adverse health effects, which are reviewed in Chapter 9. The discussions focus on the metabolism of nitrate and nitrite (especially the reduction of nitrate to nitrite in the saliva) and the metabolism of N-nitroso compounds (especially those reactions that are important in the carcinogenic effect of these compounds). In addition, evidence for endogenous synthesis of nitrate, nitrite, and N-nitroso compounds is also reviewed. The committee then uses data from a study on endogenous synthesis of N-nitroso compounds in humans to estimate possible exposures from this source in several different population groups. These estimates of endogenous exposures are compared to estimates of exogenous exposures to N-nitroso compounds developed in Chapter 7.

METABOLISM OF NITRATE AND NITRITE

As pointed out in Chapter 5, the exposure of humans to nitrate and nitrite varies from individual to individual and is dependent on place of residence (which determines water supply and presence or absence of smog) and lifestyle (e.g., food consumption and smoking habits). Moreover, exposure to these two ions from exogenous sources varies considerably over time for a given individual who lives in one location and maintains reasonably constant dietary habits. This occurs because intakes of nitrate and nitrite generally fluctuate, occurring in "pulses"; for example, they are higher following ingestion of a nitrate-containing vegetable or a nitrite-containing cured meat product. Thus, average exposures will not reflect individual fluctuations and peak intakes. Similarly, the discussion of the metabolism of these chemicals is, of necessity, based on generalizations and will not reflect individual variation due to differences in physiology, age, and overall health.

Oral Cavity

Transport of Nitrate to Saliva. Ingested nitrate is absorbed from the gastrointestinal tract into the bloodstream, which carries it to the salivary glands (Spiegelhalder *et al.*, 1976). Because nitrate, thiocyanate, and iodide appear to share a common active transport system in the salivary glands of humans (Edwards *et al.*, 1954) and other animals (Brown-Grant, 1961; Burgen and Emmelin, 1961), they compete for uptake from the circulatory system. Relative affinities for the salivary transport system are highest (lowest K_m) for iodide; thiocyanate is intermediate; and nitrate has a low affinity (Brown-Grant, 1961; Burgen and Emmelin, 1961).

Although the kinetics of competitive inhibition in the salivary glands are not precisely understood (Edwards *et al.*, 1954), it seems reasonable to assume that concentrations of iodide and thiocyanate in the blood could inhibit transport of nitrate when levels of nitrate in the blood are low (Hartman, 1981). In fact, Spiegelhalder *et al.* (1976) found that a corresponding peak in salivary nitrate concentration was not consistently detected until a certain threshold of ingested nitrate had been surpassed. The proposed threshold dose of nitrate was approximately 54 mg of ingested nitrate (Figure 8-1). These investigators also found that at oral doses of nitrate above the threshold, roughly 25% of the ingested nitrate was recirculated into the saliva (Figure 8-1).

Studies in laboratory animals have shown a wide variation in the efficiency of salivary transport of nitrate, thiocyanate, and iodide ions (Brown-Grant, 1961; Burgen and Emmelin, 1961). These differences must be considered when extrapolating data from experiments in animals to humans.

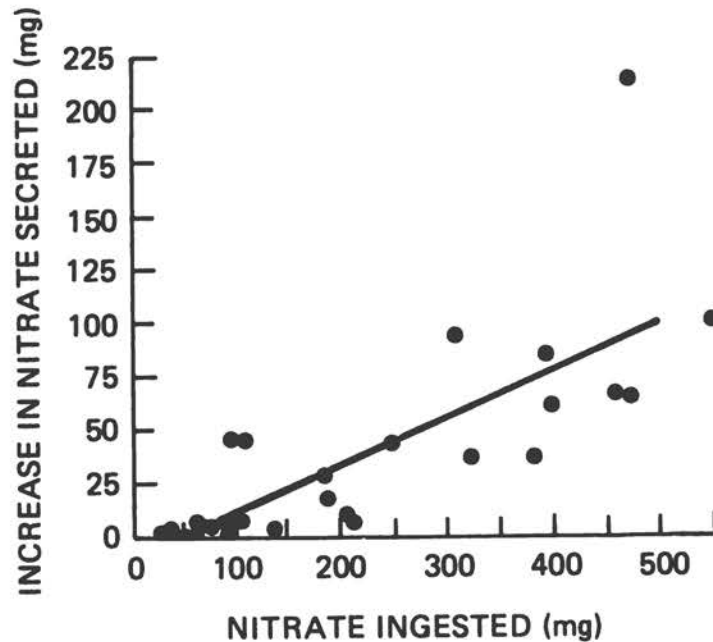


FIGURE 8-1. Ingestion of nitrate and salivary secretions of nitrate. From Spiegelhalder *et al.*, 1976.

The saliva of adult humans can contain up to 200 mg/liter concentrations of nitrate derived from food, water, and tobacco smoke (Boylard and Walker, 1974). The saliva of suckling infants may also contain appreciable concentrations of nitrate (Turek et al., 1980a,b), which may result from the absence of thiocyanate competition.

Bacterial Reduction of Nitrate to Nitrite. Tannenbaum et al. (1974) reported that levels of nitrite in the saliva of humans usually ranged from 6 to 10 mg/liter and that these levels did not change significantly following the consumption of a meal. More recently, however, Spiegelhalder et al. (1976) found that salivary nitrite concentration was directly proportional to the amount of nitrate ingested. For example, when vegetables with a high nitrate content were consumed, the nitrite concentration in saliva showed a corresponding peak that was approximately 5% of the amount of nitrate ingested. Tannenbaum et al. (1976) reinvestigated their earlier findings and found that high intakes of nitrate produced large increases in salivary nitrite concentrations. They decided that their previous conclusions (Tannenbaum et al., 1974) were oversimplified and may have been due to the fact that the diets of the subjects studied did not contain nitrate-rich food or water.

Measurements of nitrate and nitrite concentrations in the saliva of humans following the ingestion of high-nitrate-containing vegetables or sodium nitrate have demonstrated that nitrate is reduced to nitrite by bacteria present in human saliva and that some of these bacteria possessed nitrate reductase activity in vivo (Ishiwata et al., 1975a,b) and in vitro (Ishiwata et al., 1975c).

The nitrate reductase enzyme is a non-heme iron protein that contains various subunits and molybdenum (Bryan, 1981; Payne, 1981; Stouthamer, 1976; Yordy and Ruoff, 1981). It has been studied in several microorganisms including Escherichia coli, Klebsiella aerogenes, Proteus mirabilis, Bacillus stearothermophilus, Bacillus licheniformis, Haemophilus parainfluenzae, and Pseudomonas aeruginosa. The saliva of humans contains bacteria that are capable of reducing nitrate to nitrite. These bacteria belong to several genera, including Staphylococcus, Veillonella, Corynebacterium, and Fusobacterium (Tannenbaum et al., 1974). In one study, Brown et al. (1975) reported that the median counts of these bacteria in the stimulated saliva of six adults were as follows (all units are $\times 10^6/\text{ml}$): Veillonella = 4, Fusobacterium = 3, and Staphylococcus = 0.0035.

However, there is considerable variation in the amounts and kinds of oral bacteria in healthy individuals (Kraus and Gaston, 1956; Wilson and Miles, 1964). In many cases, humans appear to have the propensity for acquiring and maintaining certain microbial strains or mixtures of strains, and each strain has its own novel

metabolic spectrum and capacity. For example, some bacteria, such as Bacillus subtilis, competently reduce nitrate to nitrite but cannot utilize nitrite further, whereas other bacteria can contribute both to the formation and the degradation of nitrite (Selenka, 1970). Thus, interactions in a mixed bacterial flora can be quite complex.

The amount of nitrite produced from ingested nitrate in the saliva is determined not only by the number and kinds of microorganisms containing the nitrate reductase enzyme, but also by a number of other factors such as the amount of oxygen available to the bacteria. For example, when Klebsiella is shifted from anaerobic to aerobic conditions, there is a cessation in both the production of nitrite and the synthesis of nitrate reductase (Stouthamer, 1976). However, it has been shown that salivary reduction of nitrate to nitrite can occur under both aerobic and anaerobic conditions.

Nitrate reduction can also be enhanced or inhibited by other factors, such as salivary pH. The optimum pH for the conversion is 6.0 to 6.4. Nitrate reductase activity is often "inducible;" that is, it is present only after bacteria have been exposed to elevated nitrate concentrations for a certain period. This has led to the hypothesis that a continually elevated nitrate concentration in the saliva may select for bacteria capable of nitrate reduction (Hartman, 1981). Finally, compounds readily metabolized by bacteria are essential for maximal nitrate reductase activity once the enzyme complex is formed, although the optimal carbon source may vary among bacterial species (Stouthamer, 1976; Wallis, 1913). Thus, several factors in addition to the bacterial content of the oral cavity exert important influences on the rates at which nitrate is reduced to nitrite in the saliva.

Nitrate and Nitrite Content of Saliva. Persons on low-nitrate diets who are "pulsed" with significant amounts of nitrate exhibit a characteristic level of nitrite production, which amounts to roughly 5% of nitrate ingested (Spiegelhalder *et al.*, 1976; Figure 8-2), that is, the nitrite concentration (product) is roughly proportional to the nitrate concentration (substrate) of the saliva (Spiegelhalder *et al.*, 1976), and approximately 20% of the salivary nitrate is reduced to nitrite (Figure 8-3). Stephany and Schuller (1980) have also reported that the amount of ingested nitrate is directly proportional to salivary nitrite concentration. They calculated that the average conversion of nitrate to nitrite in the saliva of the average healthy adult is approximately 6.3% mol % (i.e., approximately 5% by weight) of total dietary nitrate intake during a 24-hour period.

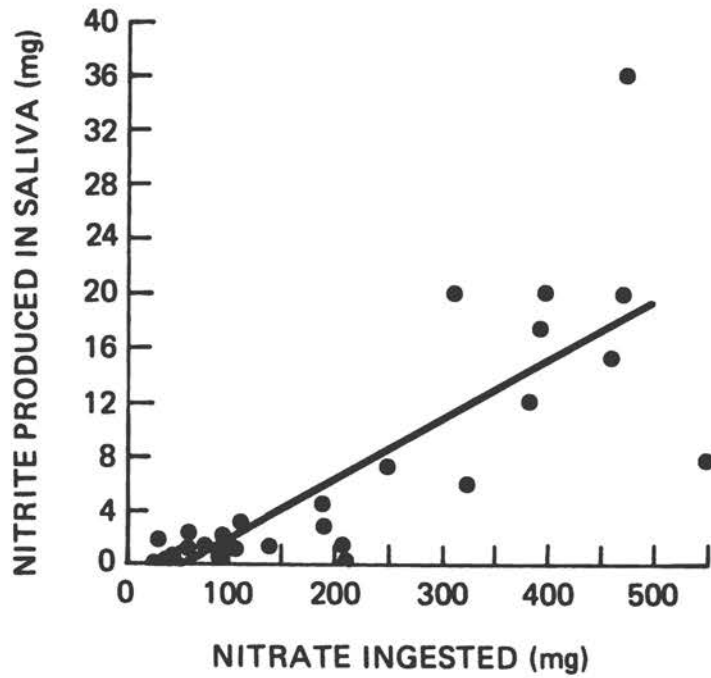


FIGURE 8-2. Nitrite in the saliva of persons on low-nitrate diets. These subjects were "pulsed" with different oral doses of nitrate. From Spiegelhalder *et al.*, 1976.

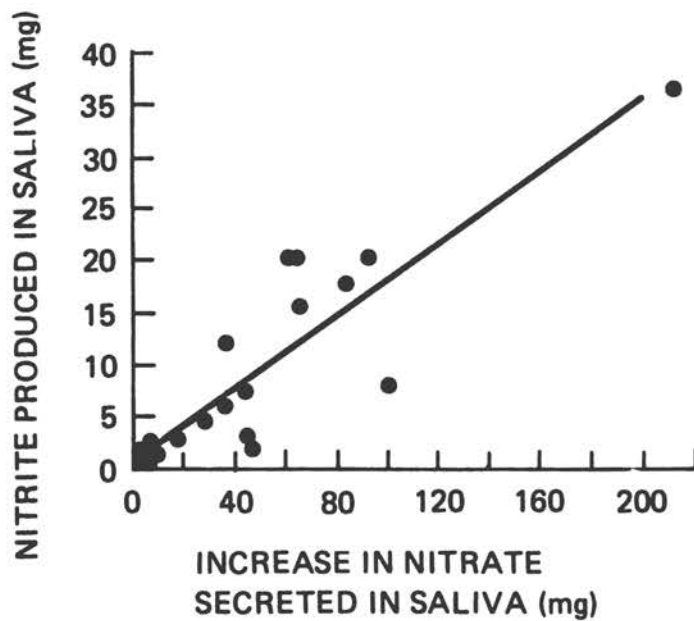


FIGURE 8-3. Relationship between the increase of salivary nitrate and the nitrite produced in saliva. From Spiegelhalder *et al.*, 1976.

Upper Gastrointestinal Tract

Sources of Nitrate. All ingested nitrate and nitrite ions enter the stomach, except for a small amount that is metabolized and assimilated by bacteria in the oral cavity and the esophagus. To this exogenous nitrate and nitrite load are added nitrate derived from recirculation of ingested nitrate through salivary excretion and nitrite produced by bacterial reduction of part of this nitrate in the oral cavity and the esophagus.

To these two sources of gastric nitrate should be added a third source: nitrate that is secreted directly into the stomach. Two physiologically different mechanisms have been proposed to explain how direct secretion may occur: (a) anion secretion (a nitrate ion displaces a chloride ion) and (b) active transport of nitrate into neutral and alkaline gastric secretions by a saturable carrier complex, in which iodide and thiocyanate ions compete for transportation. These two proposed mechanisms are based on studies of the gastric transport of thiocyanate (Logothetopoulos and Myant, 1956) and iodide (Davenport, 1943; Halmi and Stuelke, 1959; Honour et al., 1952; Howell and Van Middlesworth, 1956; Logothetopoulos and Myant, 1956; Mason and Bloch, 1950; Myant et al., 1950; Oeff et al., 1955; Ruegamer, 1963; Schiff et al., 1947).

Normally, anion secretion will not be an effective means of introducing nitrate into the lumen of the stomach since the molar concentration of the chloride ion in plasma normally exceeds the molar concentration of the nitrate ion by a factor of perhaps 1,000. Persons sustained on diets containing high levels of nitrate and those with impaired kidney function can accumulate up to 19 mM nitrate (Keith et al., 1930). However, this is still much less than the normal plasma chloride level of approximately 103 mM (Diem and Lentner, 1970).

On the other hand, active transport of nitrate into the gastric lumen could be an important physiological process, although it has not been considered thus far (Hartman, 1981). The above-cited studies of iodide transport indicate that active transport occurs throughout the stomach and is independent of gastric pH. Furthermore, the gastric transport of iodide in the rat is not an intrinsically regulated process. The amount transported can reach saturation and is dependent upon plasma iodide concentration (Halmi and Stuelke, 1959). In this case, transport of nitrate directly into gastric juices can account for the presence of appreciable quantities of gastric nitrate within minutes after the ion is injected intravenously (Balish et al., 1981; Witter et al., 1979a). The ratio of nitrate concentrations in the gastric juice compared to those in the plasma can reach 20:1 in rats ligated at the pyloric sphincter and given nitrate intravenously. Active transport appears saturated at plasma nitrate levels of 4.5 to 5.0 mM (Bloomfield et al., 1962).

Gastric secretion has not been directly studied in humans. In one study of esophageal cancer patients (whose saliva does not reach the stomach), Lowenfels et al. (1978) found excess gastric nitrite. It is possible that the nitrite was formed from gastric nitrate that had been actively transported into the stomach from the plasma.

Source of Nitrite. In order to estimate the total nitrite load in the upper gastrointestinal tract of adults with normal gastric acidity, one must consider both ingested nitrite and nitrite produced from salivary nitrate, which is recirculated following ingestion. Table 8-1 lists the estimates developed in Chapter 5 for the average daily nitrate and nitrite ingestion categorized by source for adults (see Tables 5-20 and 5-21). The amount of nitrite formed in the saliva from ingested nitrate is given in column 3 of Table 8-1. In calculating these figures, the committee assumed that approximately 5% (6.3 mol %) of the ingested nitrate is converted to nitrite (see footnote c). Total nitrite and the percent of total nitrite derived from each category are also given in the table.

According to these estimates, which are based on average intakes of nitrate and nitrite, the main dietary contributors to gastric nitrite load are vegetables (~72%), cured meats (~9%), and baked goods and cereals (~7%). However, none of these estimates include calculations of nitrate and nitrite loss due to bacterial or mammalian assimilation, nor do they consider the effect of possible endogenous synthesis of nitrate or nitrite (a possibility discussed later in this chapter).

Another source of nitrate in the upper gastrointestinal tract is reduction of nitrate by bacteria present in the achlorhydric stomach. Survival and proliferation of microorganisms in the human gastric lumen is dependent, among other things, on the pH (Bartle and Harkins, 1925; Franklin and Skoryna, 1971; Giannella et al., 1972; Knott, 1927; Lampé and Strassburger, 1943). Generally, a pH of approximately 4.5 to 5.0 is necessary to prevent substantial bacterial invasion by a mixed bacterial population that, in adult humans, includes a spectrum of nasopharyngeal and salivary bacteria (Bartholomew et al., 1980; Drasar et al., 1969; Franklin and Skoryna, 1971; Hawksworth et al., 1975; Lampé and Strassburger, 1943; Sander and Seif, 1969; Schweinsberg et al., 1975).

Gastric anacidity characterized by pH values often rising above pH 5 is relatively commonplace among the general population. This condition occurs more frequently and to a greater degree in older populations and may involve a majority of individuals over 70 years of age (Hartman, 1981). Even in persons with "normal" gastric acidity, the pH may occasionally rise sufficiently to permit bacterial

TABLE 8-1

Estimate of Per Capita Daily Nitrite Exposure in the Upper Gastrointestinal Tract of an Average Adult in the United States with Normal Gastric Acidity (pH below 4.0)

| Source | Exposure, mg/Person/Day ^a | | | Total Nitrite in Upper Gastrointestinal Tract | Percent Contribution of Nitrite from Each Source (Approximate) |
|-------------------------|--------------------------------------|------------------------------|-------------------------------|---|--|
| | Dietary Nitrite ^b | Dietary Nitrate ^b | Salivary Nitrite ^c | | |
| Cured meats | 0.30 | 1.2 | 0.06 | 0.36 | 9 |
| Fresh meats | 0.06 | 0.6 | 0.03 | 0.09 | 2 |
| Vegetables | 0.12 | 65 | 3.0 | 3.1 | 72 |
| Fruits, juices | 0.01 | 4.3 | 0.20 | 0.21 | 5 |
| Baked goods and cereals | 0.26 | 1.2 | 0.06 | 0.32 | 7 |
| Milk and milk products | 0.01 | 0.2 | 0.01 | 0.02 | < 1 |
| Water | 0.01 | 2 | 0.09 | 0.10 | 2 |
| TOTAL | 0.77 | 75 | 3.5 | 4.2 | |

^aWhere appropriate, values have been rounded off to two significant figures.

^bData from Tables 5-20 and 5-21.

^cCalculated by multiplying intake of nitrate by 6.3 mol % (0.0467) (Spiegelhalter *et al.*, 1976; Stephany and Schuller, 1980). See text for discussion of conversion of nitrate to nitrite in saliva.

invasion (Atkinson and Henley, 1955; Franklin and Skoryna, 1971), thereby providing an opportunity for extensive nitrite formation (Schweinsberg *et al.*, 1975).

Ruddell *et al.* (1980) observed an elevated gastric pH in patients with ulcers who had been treated with cimetidine. They also found an increase in gastric bacterial flora, which included large numbers of fecal organisms. Such bacteria are also present in the stomach of achlorhydric patients who are at increased risk for gastric cancer.

Most infants normally possess an elevated gastric pH, but it is especially high in infants fed cow's milk (Marriott and Davidson, 1923; Oliver and Wilkinson, 1933; Vanzant *et al.*, 1932). The presence of lactobacilli may be an important factor, at least in breast-fed infants, because lactobacilli do not reduce nitrate to nitrite and would maintain a pH lower than that amenable to gastric invasion by a mixed flora, including nitrate-reducing bacteria

(Bartholomew *et al.*, 1980; Wilson and Miles, 1964). This possibility is of some interest since infants can ingest large amounts of nitrate, on a mg/kg body weight basis (Chapter 5), and it has been well documented that early exposure to environmental substances is an important determinant of risk of gastric cancer much later in life (Hartman, 1981).

Gastric nitrite concentration appears to be influenced by the total content of nitrate-reducing bacteria rather than by a preponderance of any specific organism (Bartholomew *et al.*, 1980). The rate of nitrate reduction by bacteria in the stomach is also influenced by metabolizable carbon sources (Hartman, 1981).

As shown in Table 8-2, which presents a hypothetical example of the possible nitrite load in the stomach under conditions of hypoacidity and mixed bacterial infection, approximately half of the gastric nitrate may be reduced to nitrite in certain individuals. Thus, the bulk of gastric nitrite exposure in achlorhydric individuals may stem from the reduction of nitrate within the stomach itself.

TABLE 8-2

Hypothetical Example of Gastric Nitrite Load in Person with Gastric Hypoacidity and Mixed Bacterial Infection

| Source | Exposure, mg/Person/Day | | Percent Total Gastric Nitrite |
|------------------------------------|-------------------------|-----------------|-------------------------------|
| | Nitrate | Nitrite | |
| Ingested ^a | 75 | 0.77 | 1 |
| Recirculated Salivary ^b | 19 | 4 | 7 |
| Gastric ^c | 19 | 54 ^d | 92 |
| TOTAL | 109 ^e | 59 | -- |

^aNitrate and nitrite ingested for average U.S. population (Tables 5-20 and 5-21).

^bRecirculated nitrate equals 25% ingested nitrate (Speigelhalter *et al.*, 1976).

^cAssumes recirculation of nitrate to stomach via active transport equals salivary recirculation of 25%.

^dAssumes 50% of the total gastric nitrate is reduced to nitrite.

^eOver a 24-hour period, 75 mg ingested nitrate reaches stomach, plus 15 mg recirculated from saliva (19 - 4, which is converted to nitrite), plus 19 mg which is recirculated directly to the stomach.

Absorption from the Gastrointestinal Tract

The uptake of nitrate from the gastrointestinal lumen occurs primarily in the small intestine and appears to be an active process -- probably sharing carriers that also mediate iodide transport. There is little or no uptake of nitrate from the stomach. For example, as mentioned previously, nitrate is retained in the stomach of animals ligated at the pyloric sphincter (Witter *et al.*, 1979b). In humans, most of the gastric nitrate passes into the small intestine (Witter *et al.*, 1979b); however, small amounts of nitrate could penetrate through the gastric mucosa if nitrate behaves like iodide (Brown-Grant, 1961; Halmi, 1964).

In contrast, the transport of nitrate across the wall of the proximal small intestine is rapid and reasonably complete, at least in laboratory animals (Balish *et al.*, 1981; Hawksworth and Hill, 1971; Ishiwata *et al.*, 1977; Keith *et al.*, 1930; Witter and Balish, 1979; Witter *et al.*, 1979a). Studies in laboratory animals have also revealed that rats can excrete nitrate into the lumen of the mid-portion of the small intestine (Balish *et al.*, 1981; Witter and Balish, 1979; Witter *et al.*, 1979a). Therefore, the observation that nitrate is present in the lower small intestine of rats is no assurance that there has not been essentially complete absorption in the proximal small intestine. This ability to excrete nitrate and iodide (Acland and Iilman, 1959) into the small intestine is unique to rats and does not apply to humans or to other animals tested (Brown-Grant, 1961) -- a fact that must be considered when trying to extrapolate the results of studies on nitrate metabolism in rats to humans.

The penetration of the gastrointestinal mucosal barriers by nitrite has not been carefully measured at concentrations normally ingested. There have been reports that nitrite may pass through the mucosa following erosion and hemorrhage in cases of intense gastritis (Gwatkin and Plummer, 1946; Sollman, 1957). In humans, massive lethal doses of oral nitrite are predominantly retained in the stomach and its contents; very small amounts reach the intestine, and much less reaches the liver, kidney, or urine (Naidu and Venkatrao, 1945). Much lower levels of ingested nitrite can prolong the emptying time of the normal human stomach (Sleeth and Van Liere, 1941), and gastric paralysis appears almost complete at lethal doses of nitrite (Naidu and Venkatrao, 1945).

The rate at which nitrite disappears from the stomachs of laboratory animals is greater than would be expected from the kinetics of gastric emptying (Friedman *et al.*, 1972; Ishiwata *et al.*, 1977; Mirvish *et al.*, 1975), and it occurs in mice ligated at the gastroduodenal junction (Friedman *et al.*, 1972). Some of this nitrite loss can be accounted for by chemical oxidation of nitrite to nitrate, a process that occurs even in germ-free rats (Friedman

et al., 1972; Witter and Balish, 1979). Because some nitrite is probably bound to insoluble material in the gastric lumen, it cannot be detected. The disappearance of gastric nitrite in vivo in conventional animals also follows second-order kinetics, indicating involvement of dinitrogen trioxide (Friedman et al., 1972). In this process, there is active gastric absorption of dinitrogen trioxide (Friedman et al., 1972), but much, if not all, of the loss could be equally due to chemical reactivity of dinitrogen trioxide with components of gastric chyme, such as urea and mucins, as well as with surfaces of gastric epithelial cells. The importance of these reactions in the in vivo formation of N-nitroso compounds will be discussed later in this chapter.

Some nitrite is carried into the small intestine where chemical oxidation to nitrate may continue, but again little or no absorption of nitrite is evident (Witter and Balish, 1979).

Transport and Chemical Reactions in the Blood

No appreciable reduction of nitrate to nitrite has been observed in the mammalian circulatory system (Parks et al., 1981). Rather, plasma nitrate appears to circulate and to participate in rapid dissemination processes that often mimic the flow of iodide. Relatively low plasma levels of nitrate are maintained by a presumably reversible, rapid dissemination in tissues that occurs within minutes. This fast-equilibrating nitrate compartment represents approximately 28% of the body weight of dogs (Greene and Hiatt, 1954) and is the same relative size as the rapidly equilibrating iodide compartment of humans (Myant et al., 1950). Thus, pulses of nitrate introduced orally or administered intravenously are rapidly disseminated in the mammal, preventing accumulation in the blood even before nitrate reaches the urine (Balish et al., 1981; Parks et al., 1981; Witter and Balish, 1979; Witter et al., 1979a,b,c).

Nitrate is also removed from plasma in the kidneys, ending up largely as nitrate in urine. Thus, urinary excretion is another important mechanism for the maintenance of low plasma nitrate levels.

Nitrite reaching the circulatory system by slow diffusion or through lesions in mucosal barriers rapidly reacts with oxyhemoglobin to form methemoglobin (Chapter 9). The rate at which nitrite reacts with oxyhemoglobin varies widely among species (Parks et al., 1981; Rath and Krantz, 1942; Smith and Beutler, 1966). Thus, care must be used when extrapolating to humans data obtained from experiments in animals. For example, the formation of methemoglobin in pigs is much slower (Smith et al., 1978) than in other species examined (Smith and Beutler, 1966). If nitrite does not react immediately with hemoglobin in the maternal circulation, an effective portion of a heavy maternal nitrite load could be transmitted across the

placenta to the fetus and could cause fetal methemoglobinemia (Gruener et al., 1973) and the morphological or neoplastic transformation of fetal cells (Inui et al., 1979a,b), as observed in laboratory animals.

In all the species studied, the action of enzymes in the red blood cell restores hemoglobin function and releases free nitrate in the process (Smith and Beutler, 1966).

Excretion of Nitrate in Urine

In contrast to the rapid oxidation of ingested nitrite to nitrate, the dissemination of nitrate into the major tissue compartments, and recirculation of nitrate by active transport systems, all of which occur within minutes, there may be more slowly operating processes that affect nitrate flow, for example, in more slowly equilibrating nitrate and iodide tissue compartments such as the cerebrospinal fluid (Hiatt, 1940; Myant et al., 1950).

In the rat, additional nitrate is withdrawn from the system by its active secretion into the mid-portion of the small intestine; failure of efficient transport from the lower gastrointestinal tract then allows excretion in feces or assimilation by bacteria in the large bowel (Balish et al., 1981; Green et al., 1981; Witter and Balish, 1979; Witter et al., 1979a). In germ-free rats, approximately 16% of ingested nitrate is excreted in the feces (Green et al., 1981). However, as pointed out above, active transport of nitrate from the circulatory system into the intestine is unlikely to occur in the human (Brown-Grant, 1961). Essentially all of the nitrate ingested by humans, especially at higher levels, appears in the urine (Hawksworth et al., 1975).

The effect of increased and decreased nitrate ingestion on the urinary excretion of nitrate has been studied in humans (Hill, 1979; Keith et al., 1930; Mitchell et al., 1916; Tannenbaum et al., 1978) and in laboratory animals (Balish et al., 1981; Green et al., 1981; Keith et al., 1930). Urinary excretion of nitrate after ingestion of large oral doses of nitrate has also been studied in humans (Hill, 1979; Ishiwata et al., 1978) and in laboratory animals (Hawksworth and Hill, 1971; Hill, 1979; Wang et al., 1981). In humans, total urinary excretion of nitrate takes several days (Hill, 1979), although the bulk is eliminated in a shorter period. Excretion of nitrate from humans is reported to follow first-order kinetics, having an average elimination half-life of 5 hours (Green et al., 1981).

Nitrite is not a normal constituent of urine, but tens of milligrams of nitrite can be formed in urine daily by bacterial action during urinary tract infections (Radomski et al., 1978).

It is logical to assume that the amount of nitrite produced during bacterial infection would be positively related to the amount of substrate (nitrate) available for reduction and, thus, to the amount of nitrate and nitrite ingested by the individual. Implications that nitrate reduction is involved in the induction of bladder cancer are discussed later in this chapter.

Endogenous Synthesis of Nitrate and Nitrite

In addition to acquiring nitrate and nitrite from exogenous sources, endogenous formation of these ions may also occur. Two hypotheses have been advanced to explain the mechanisms of endogenous synthesis -- heterotrophic bacterial nitrification and synthesis in mammalian tissues.

Heterotrophic Bacterial Nitrification. Tannenbaum and coworkers (1978) described the endogenous synthesis of nitrite and nitrate in humans. Using a modified Griess procedure, they measured components of urine and the diet for nitrate and nitrite content in order to conduct nitrate balance studies. Their results indicated that there were wide fluctuations in urinary nitrate on a day-to-day basis and that urinary excretion of nitrate greatly exceeded the intake of nitrate. Similar observations were reported by Mitchell et al. (1916). In addition, analysis of fecal samples conducted by Tannenbaum et al. (1978) showed the presence of both nitrate and nitrite, whereas ileostomy effluents contained nitrite, but no detectable nitrate.

In order to explain these puzzling results, Tannenbaum et al. (1978) concluded that "heterotrophic nitrification of ammonia or organic nitrogen compounds takes place in the upper, aerobic portion of the intestine" in much the same way that such processes occur in other ecosystems such as those of sewage, soil, lakes, and rivers. Continuing this rationale, they postulated that the nitrification process could lead to the formation of nitrate/nitrite in the more anaerobic large intestine and that N-nitroso compounds may be synthesized in the acidic environment of the cecum and colon.

Another explanation of the findings reported by Tannenbaum and coworkers was offered by Witter et al. (1979b), who studied the distribution of $^{15}\text{NO}_3^-$ in humans and rats. Their results indicated that approximately 25% of gavaged nitrate and nitrite could pass directly into the lower intestinal tract and could account for the nitrate and nitrite in fecal samples and the nitrite in ileostomy samples detected by Tannenbaum et al. (1978). Since humans and rats have the capability of temporarily storing nitrate and nitrite in the extravascular spaces of the body (as measured by studies with labeled nitrate), a slow washing of nitrate from the body could explain the excess nitrate excretion found by Tannenbaum et al. (Witter et al., 1979b,c).

In explaining his model, Tannenbaum (1979) pointed out that the clearance of nitrate from blood after an oral dose results in peak concentrations in urine and saliva 2 to 5 hours after administration and a clearance phase with a half-life of approximately 8 hours. Furthermore, if no additional nitrate enters the body, the nitrate could be cleared within 48 hours, independent of the pool concentration, since the clearance of nitrate follows first-order kinetics.

Witter *et al.* (1979c) noted that nitrate and nitrite ingested in foods can be difficult to detect with the Griess test, bringing into question the accuracy of dietary levels measured by Tannenbaum *et al.* (1978). These workers also pointed out that Tannenbaum *et al.* (1978) had not considered the contribution of endogenous nitrate from nitrogen oxides. Witter *et al.* (1979c) calculated that as much as 500 mg of nitrate could be formed per month if 6 liters of air containing 1 ppm ($\sim 1,880 \mu\text{g}/\text{m}^3$) nitrogen dioxide are inhaled per minute.

Witter *et al.* (1979b,c) questioned the heterotrophic nitrification hypothesis on the basis that the intestinal tract is probably predominantly anaerobic. Furthermore, oxidation of ammonia to nitrate would require vast numbers of organisms, and the upper intestine contains few bacteria. Since heterotrophic nitrification was expected to occur at this site, it appears that the conditions might not be suitable for such a process. An alternative explanation for ileal nitrite levels reported by Tannenbaum *et al.* (1978) would be bacterial reduction of nitrate to nitrite rather than oxidation of ammonia to nitrite (Witter *et al.*, 1979b,c).

Mammalian Synthesis. Nitrate balance studies were conducted by Tannenbaum's group (Green *et al.*, 1981) in germ-free and conventional Sprague-Dawley rats to test the hypothesis that nitrate is synthesized endogenously by heterotrophic bacterial nitrification. The basal diet of these animals was supplemented with $\text{Na}^{15}\text{NO}_3$ so that the pools of nitrate could be distinguished using mass spectrometric measurements of the ^{15}N and ^{14}N abundance ratios. Results of analysis of nitrate in the diet and in the urine indicated that at various levels of nitrate ingestion, the urinary output of sodium nitrate exceeded that of the dietary intake for both germ-free and conventional animals. Since both animal groups excreted similar levels of nitrate, the role of the bacterial flora in the synthesis of nitrate was eliminated. Thus, the authors postulated that mammalian synthesis, rather than their original suggestion of heterotrophic nitrification, was the most likely source of excess nitrate.

Witter *et al.* (1981) measured dietary and urinary nitrate by high performance liquid chromatography (HPLC) and found that the intestinal flora of conventional rats on a chow diet actually decreased the urinary excretion of nitrate, whereas there was an excess of nitrate in the urine of germ-free animals. When animals were maintained

on a glucose diet (absence of dietary nitrate), an increase in urinary nitrate was found in both groups. The authors also concluded that nitrate is synthesized by mammalian and not by microbial processes.

Saul et al. (1981) reported that nitrate and nitrite are destroyed by fecal microorganisms in vitro. Using an analytical procedure with good (although variable) recoveries (approximately 70-80%), they found that nitrate and nitrite levels in feces and ileostomy fluid were much lower than those published by Tannenbaum et al. (1978). They concluded that the maximum fecal excretion of nitrate and nitrite is much lower than the normal dietary intake and that during in vitro incubations with added nitrite, no oxidation to nitrate occurred. Although there are many possible explanations why nitrite (or lower oxidation states of nitrogen) cannot be converted to nitrate by intestinal microorganisms, it appears that conditions in the lower gastrointestinal tract favor reduction of nitrate and nitrite (Saul et al., 1981). Therefore, heterotrophic nitrification appears to be an inadequate explanation of the results obtained in the nitrate balance studies of Tannenbaum et al. (1978), and mammalian synthesis of nitrate appears to be more plausible (Green et al., 1981; Parks et al., 1981).

METABOLISM OF N-NITROSO COMPOUNDS

The metabolism of nitrosamines has been exhaustively reviewed (Magee, 1980; Magee and Barnes, 1967; Magee et al., 1976; Montesano and Bartsch, 1976; Pegg, 1980). It is not the purpose of this report to recapitulate all the published data but, rather, to summarize the general principles of nitrosamine metabolism, with emphasis on recent studies, in order to aid in the evaluation of the potential risk of these carcinogens to humans.

Of the large number of nitrosamines that have been found to be carcinogenic in laboratory animals, only a few, especially nitrosodimethylamine (NDMA) and nitrosodiethylamine (NDEA), have been studied in any great detail. However, alkylation, mutagenesis, teratogenesis, and carcinogenesis studies with a number of nitrosamides can also provide insight into the potential consequences of exposure to nitrosamines since the alkylating species, which are considered to cause the pathologic changes, are similar for nitrosamines and nitrosamides. The major difference is that nitrosamides, in contrast to nitrosamines, are quite unstable and do not require metabolic activation.

Pharmacokinetics

As a result of his early biochemical studies, Magee concluded that intravenously administered NDMA rapidly equilibrates throughout the body of rats (Magee, 1956). This conclusion was confirmed and extended by an autoradiographic study of transected mice. That study was designed to determine the distribution of radioactivity in the organs of mice that had received intravenous injections of ^{14}C -labeled

NDMA (Johansson and Tjälve, 1978). Animals were examined from 1 minute to 5 days after injection. In preliminary studies, these investigators confirmed that pyrazole, ethanol, and nialamide blocked the in vivo metabolism of NDMA. In addition, the unmetabolized, volatile NDMA was evaporated from the transected tissue block by lyophilization. Thus, the exposure of x-ray film to the lyophilized block revealed only the bound (nonvolatile) products of NDMA metabolism, whereas exposure of the film to the tissue at -80°C revealed both metabolized (nonvolatile) and unmetabolized (volatile) NDMA.

Autoradiography of the pretreated mice at -80°C demonstrated that radioactivity was uniformly distributed throughout the organs after 30 minutes and that there was no radioactivity in the tissues where evaporation had occurred. This confirmed that unmetabolized, volatile NDMA was distributed equally throughout the body tissues. In contrast, in animals that had not been pretreated, selective labeling of certain organs, including liver, pancreas, salivary gland, intestinal mucosa, and bone marrow, was evident after 30 minutes. The investigators also demonstrated that as soon as 1 minute after injection of the ^{14}C -labeled NDMA, radioactivity was concentrated mainly in the liver and kidney and there was some diffuse background activity in other tissues in samples maintained at -80°C . Furthermore, even after the transected tissue blocks were freeze-dried and evaporated, the radioactivity persisted in the liver and renal cortex; however, the diffuse radioactivity in other tissues had disappeared.

These results add strong support to the notion that the striking organotropic, toxic, and carcinogenic effects of nitrosamines are not due to the preferential uptake of the unmetabolized carcinogen but, rather, to preferential metabolism by specific organs. Furthermore, since the organ distribution of ^{14}C at 30 minutes after injection of ^{14}C -labeled NDMA resembled that observed at 30 minutes following injection of ^{14}C -labeled formaldehyde (with the exception of the liver, where concentrations were much higher in the NDMA-injected mice), the degraded products of ^{14}C -labeled NDMA are apparently incorporated into the 1-carbon pool of protein-synthesizing or replicating cells. In both instances, radioactivity between 30 minutes and 24 hours after injection was high in organs that had the highest rates of cell replication, including bone marrow and intestinal mucosa.

In contrast to these findings from intravenous administration of NDMA, there is good evidence that certain ingested nitrosamines and those formed in vivo in the upper gastrointestinal tract are absorbed rapidly from the duodenum (Hashimoto et al., 1976) and subsequently carried by the portal circulation to the abdominal viscera. When low doses of nitrosamines are ingested, the liver appears to behave as a "first line of defense" by markedly reducing the concentration of nitrosamines in the systemic circulation. This inference is drawn from a comparison of the degree of alkylation of kidney and liver DNA following oral or intravenous administration of NDMA (Pegg, 1980). Over

a broad dosage range (1 to 100 $\mu\text{g}/\text{kg}$ body weight), Pegg observed that the liver to kidney ratio for alkylated products was 4 to 7 times greater after oral doses than after intravenous administration. In one sense, this is reassuring, since O^6 -methylguanine excision and repair rates appear to be higher in the liver than in other organs of the rat (Brash and Hart, 1978). On the other hand, this preferential hepatic metabolism following oral intake suggests that the total body burden of nitrosamines may be higher than estimates based on peripheral venous blood concentrations (Pegg, 1980).

Activation and Deactivation

In common with a broad range of other carcinogenic agents (Miller and Miller, 1971b), the ultimate toxic and carcinogenic species resulting from the metabolism of nitrosamines are electrophiles, which are capable of covalently reacting with cellular macromolecules including DNA, RNA, and protein (Pegg, 1977). However, although tumor induction occurs only in organs in which alkylation takes place (Magee and Barnes, 1967; Magee *et al.*, 1976; Montesano and Bartsch, 1976), the nature of the alkylating molecule is not known for any given nitrosamine. The primary reaction required for the activation of nitrosamines is considered by most investigators to involve an enzyme-mediated α -hydroxylation requiring reduced nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen. β -Hydroxylation of nitrosodialkylamines yielding a nitrosomethylalkylamine may also occur prior to α -hydroxylation and may account for methylation by longer chain nitrosodialkylamines (Krüger, 1972; Lawson *et al.*, 1981a,b).

Recent studies with nitrosomethyl(acetoxymethyl)amine strongly support the role of α -hydroxylation (Pegg, 1980). After intravenous administration of this nitrosamine, the spectrum of DNA-alkylated products is identical to that observed after NDMA activation (Kleihues *et al.*, 1979). Since neoplasms are mainly induced close to the sites of injection of nitrosomethyl(acetoxymethyl)amine (Berman *et al.*, 1979; Habs *et al.*, 1978; Kleihues *et al.*, 1979; Wiessler and Schmähl, 1976), and activation is solely dependent on the activity of a ubiquitous esterase, it appears that the only requisite for activation of the carcinogen is generation of an α -hydroxylated product, which then undergoes spontaneous decomposition and leads to alkylation. Mochizuki *et al.* (1980) recently succeeded in synthesizing the α -hydroxy derivatives of nitrosodialkylamines and found that they were not as unstable as previously believed. Furthermore, these derivatives were recently shown to be mutagenic (Maekawa *et al.*, 1981). Formaldehyde, generated in the course of subsequent oxidative demethylation, is entirely degraded to carbon dioxide. However, it may take a long time for carbon dioxide to form, especially since other, rate-limiting reactions may occur during the course of degradation (den Engelse *et al.*, 1975). Thus, it does not appear reasonable to expect quantitative recovery of carbon dioxide in order

to validate the postulated pathway. On the other hand, one would expect near quantitative recovery of nitrogen. In fact, this has been documented in at least two different laboratories (Magee, 1980; Milstein and Guttenplan, 1979).

Speculations concerning the intermediate steps in nitrosamine metabolism (Heath, 1962; Hultin *et al.*, 1960) continue to generate a great deal of research. Rose (1958) originally suggested that the major toxic derivative of NDMA metabolism was diazomethane. However, this possibility was subsequently ruled out by experiments in which fully deuterated NDMA was used (Lijinsky *et al.*, 1968). Since the alkylated hepatic nucleic acid derivatives contained three deuterium atoms, they could not possibly have arisen from diazomethane. Therefore, by a process of exclusion, it appears that either a methyl diazohydroxide ion or a methyl diazonium ion reacts with cell macromolecules. This reaction is believed to be involved in the process leading to cancer.

Czygan *et al.* (1973) discovered that the mutagenic action of NDEA was dependent on oxidative demethylation -- the result of a cytochrome-P450 metabolizing system. Therefore, it seems likely that the oxidative dealkylation necessary for the toxic and carcinogenic action of the nitrosamines is similarly dependent on cytochrome P450. However, another pathway, possibly dependent on amine oxidase, may also be involved (Lake *et al.*, 1975, 1976; Phillips *et al.*, 1977), since a number of anomalous aspects of the metabolism of NDMA cannot be easily explained by the dependency on a cytochrome-P450 system. These unexplained observations include inhibition rather than induction of metabolism by such classical inducers as phenobarbitone, polycyclic hydrocarbons, and polychlorinated biphenyls (Arcos *et al.*, 1975, 1977); atypical spectra when bound to cytochrome P450; weak inhibition by SKF 525; and stimulation of demethylation by metyrapone (Lake *et al.*, 1976). A number of high and low K_m dealkylating enzymes also have recently been described. Their identification has helped to explain some of the discrepancies in the literature, since "classical inducers" of cytochrome P450 generally inhibit the low K_m enzymes and stimulate the high K_m enzymes (Arcos *et al.*, 1975, 1977).^m It now appears that only the low K_m enzymes are of physiologic importance since the maximum nonlethal concentration of NDMA in body water is $10^{-3}M$ (Johansson and Tjälve, 1978). Accordingly, earlier studies of demethylase activity, in which high concentrations of substrate were used, do not reflect *in vivo* metabolism (Magee, 1980). The stimulation of NDMA demethylase by pretreatment with ethanol was described recently by Garro *et al.* (1981). This unconfirmed finding is potentially important since it was documented at both high and low concentrations of substrate.

Macromolecular Interactions and Repair Mechanisms

The recognition that administration of nitrosamines results in the alkylation of DNA and the generation of at least 11 different nucleoside and alkyl-phosphotriester adducts (Pegg, 1977; Singer, 1979) is consistent with the somatic mutation hypothesis of carcinogenesis (Boveri, 1914; Burdette, 1955; Miller and Miller, 1971a). The major alkylated base produced by the administration of NDMA is 7-methylguanine (Magee and Farber, 1962). However, the formation of this adduct does not correlate with the relative carcinogenicity of NDMA, nitrosomethylurea, and methylmethane sulfate for the kidney (Swan and Magee, 1968). Furthermore, the degree of miscoding due to 7-methylguanine did not correlate with the degree of mutagenicity in bacteriophage following exposure to methylating and ethylating agents (Loveless and Hampton, 1969). Alkylation of DNA-derived guanine in the O⁶ position is a more likely cause of mispairing and mutation since this position is involved in base pairing.

In several classic studies, investigators have found that the persistence of O⁶-methylguanine in rats, probably as a consequence of impaired excision repair, seemed to correlate with the distribution of neoplasms following administration of nitrosourea (Goth and Rajewsky, 1974a,b; Kleihues and Margison, 1974). However, tests in some other species have indicated that there are exceptions to this correlation. For example, the susceptibility to development of neoplasms in the brain and liver of two different strains of mice did not seem to depend only on the formation and persistence of O⁶-ethylguanine in DNA (Buecheler and Kleihues, 1977). Furthermore, O⁶-methylguanine persists in gerbil brains after treatment with nitrosomethylurea, even though these animals do not develop brain tumors (Kleihues et al., 1976). Additional adducts such as O⁴-alkylthymine, N'-alkylureas, and N-3-alkylpyrimidines are also suspect (Kröger and Singer, 1979; Lawley, 1974, 1976; Singer, 1979). Alkylphosphotriesters may affect chromatin structure (Cooper and Itzhaki, 1975), but their prevalence suggests they are unlikely to be strong mutagens. In the future, quantitation of adduct formation will be aided by the continued development of more refined radioimmunoassay procedures (Poirier, 1981; Poirier and Yuspa, 1981). These methods may ultimately become valuable in the assessment of human risk since the covalent binding of carcinogenic compounds can be studied in organ cultures of tissues derived from humans at autopsy (Harris, 1981).

Finally, it appears advisable to maintain proper perspective concerning adduct formation, repair mechanisms, and cell replication in assessing risks for humans. Frei (1976) has aptly referred to a "contest or race" between DNA replication and DNA repair that is of fundamental importance in the carcinogenic process. According to this concept, conditions that accelerate replication or delay repair may foster miscoding and, presumably, increase the number of mutations and the degree of carcinogenicity, whereas conditions that delay

replication or increase repair efficiency will have the reverse effect. Furthermore, when considering the risk to humans exposed to nitrosamines, two additional factors should be kept in mind. First, it now appears that the O⁶-methylguanine that forms after exposure to NDMA is more efficiently repaired after exposure to low doses of carcinogens than after exposure to high doses (Pegg and Balog, 1979). Second, when assessing the results of experiments in animals, one should bear in mind that repair mechanisms are generally more efficient in the human than in other species.

In Vivo Formation of N-Nitroso Compounds

Humans may be exposed to N-nitroso compounds formed endogenously from a variety of precursors. These precursors may be amines and other nitrosatable substances and nitrosating agents inhaled in air, ingested in food, or formed in vivo from more elementary precursors. Evidence for the endogenous formation of N-nitroso compounds has come primarily from studies in laboratory animals. In contrast, the data pertaining to endogenously formed nitrosamines in humans are poor. Much of the information is still being subjected to scientific scrutiny, primarily to assess the efficacy of the analytical methods that have been used to gather the data. However, an experimental methodology for evaluating in vivo nitrosation in humans designed by Ohshima and Bartsch (1981) seems to have overcome all the earlier deficiencies. Their technique is discussed below.

Experiments with animals. Carcinogenicity, mutagenicity, and acute toxicity assays have been used as endpoints to detect in vivo nitrosation. In addition, nitrosamines in biological samples have been measured following ingestion of nitrite and amines.

In 1969, Sander and Bürkle induced esophageal and hepatic tumors in rats by feeding them N-methylbenzamine or morpholine plus nitrite. Tumors characteristic of the corresponding N-nitroso compound were also induced in rats fed nitrite plus methylurea, ethylurea, 1,3-dimethylurea, 2-imidazolidone, and N-methylaniline. Lung adenomas were induced in mice by long-term administration of nitrite plus morpholine, piperazine, N-methylaniline, methylurea, and ethylurea, but not with dimethylamine (Greenblatt et al., 1971; Mirvish et al., 1972). In a study of mice fed 6 g of piperazine plus nitrite for 1 to 40 weeks, the number of lung adenomas per mouse was approximately proportional to the concentration of piperazine in the food and the square of the concentration of nitrite, which was added to the drinking water (Greenblatt and Mirvish, 1973). The tumors in these studies were presumed to be caused by the endogenous formation of nitrosamines.

Whong et al. (1979) detected in vivo nitrosation in mice and rats in experiments using an intrahepatic, host-mediated mutagenicity assay with Salmonella typhimurium as the detecting organism. The sensitivity of the assay for NDMA was 0.2 mg/kg in mice and 0.6 mg/kg in rats.

Acute toxic effects have also been observed in animals exposed to nitrite plus an amine. For example, liver necrosis, attributed to intragastric formation of NDMA, was observed when large doses of dimethylamine and sodium nitrite were administered to rats by gavage (Asahina et al., 1971). Similarly, rats gavaged with aminopyrine plus nitrite produced liver necrosis (Lijinsky and Greenblatt, 1972). Further evidence that liver damage was due to the formation of NDMA was provided by the studies of Montesano and Magee (1971), who detected [7-¹⁴C]methylguanidine in the nucleic acids of the stomach, intestines, and liver, and attributed its presence to the formation of [¹⁴C]nitrosomethylurea.

Chemical analysis has also been used to determine the extent of in vivo nitrosation. Sander et al. (1968) found up to 30% yields of nitrosodiphenylamine and 4% yields of nitrosomethylaniline after gavage of the corresponding amines plus nitrite to rats (Sander and Schweinsberg, 1972). In vivo formation of nitrosoethylamine, nitrosopiperidine, and nitrosoproline was also confirmed by chemical analysis of the stomach contents of laboratory animals following administration of an appropriate amine plus nitrite (Alam et al., 1971a,b; Braunberg and Dailey, 1973).

Mirvish and Chu (1973) found nitrosomethylurea and nitrosoethylurea in the stomach of starved rats gavaged with urea plus nitrite. More recently, Mirvish et al. (1980) measured gastric nitrosomethylurea, formed from ³H-methylurea and nitrite. They found that the formation of nitrosourea was greater when rats were fed a semisynthetic diet with a low protein content than when they were fed a similar diet with a high protein content. This observation was correlated with the finding from an epidemiological study (Modan et al., 1974) that gastric cancer is associated with high-starch, low-protein diets (see Chapter 9).

In studies of the in vivo nitrosating potential of nitrogen oxides, Iqbal et al. (1980) gavaged mice with morpholine (2 mg/mouse) and exposed them to atmospheric nitrogen dioxide in concentrations up to 50 ppm for as long as 4 hours. They observed that nitrosomorpholine (NMOR) (up to 2.3 µg/mouse) was synthesized in vivo. Mirvish et al. (in press) reexamined the same system. They confirmed the results of Iqbal et al. using the same method, but did not obtain NMOR when a "stopping solution" containing ammonium sulfamate, ascorbic acid, and sulfuric acid was included in the preparation of the homogenate. This indicated that the nitrosamine formation occurred during the preparation of the whole-mouse homogenate in a methanol-water solution. This nitrosation may have been due to the in vivo formation of a nitrosating agent from the nitrogen dioxide -- an agent that did not nitrosate morpholine in vivo, but that did effect this reaction in the homogenate. The nature of this nitrosating agent, however, is currently unknown.

Very recently, however, Oda et al. (1981) reported that inhalation of nitrogen dioxide leads to the appearance of relatively large amounts of nitrate and nitrite in the blood of rats, and Pryor and Lightsey (1981) reported that nitrogen dioxide reacts with unsaturated fats to produce nitrite. This latter reaction could also occur in vivo. The potential importance of these studies on the nitrosating potential of nitrogen oxides is indicated by the study of Van Stee et al. (1980), who reported a small but statistically significant increase in the yield of lung adenomas in mice exposed chronically to atmospheric nitrogen dioxide at 1 to 2 ppm and to morpholine in drinking water at 1 g/liter.

Detection of Endogenously Formed N-Nitroso Compounds in Humans.

Previous attempts to demonstrate possible in vivo formation of N-nitroso compounds in humans have focused on the detection of nitrosamines in feces, urine, or the blood. Although Wang et al. (1978) reported that relatively high levels of nitrosamines were present in feces of humans, further work has shown this result to be in error (Eisenbrand et al., 1981). These investigators discovered that a marker amine produced a nitrosamine when added to fresh feces; for example, morpholine produced NMOR. Thus, they suspected that the high levels detected in the earlier study could have resulted from the artifactual formation of nitrosamines during storage or during the analytical procedures.

Secondary amines, especially dimethylamine, piperidine, and pyrrolidine, are found in normal urine in levels as high as 6 mg/day (Drasar and Hill, 1974). These amines are presumably formed by bacterial action on breakdown products of food in the intestine (Asatoor and Simenhoff, 1965). Nitrosamines in concentrations of 2 to 3 $\mu\text{g/liter}$ have been found in the urine of patients with Proteus mirabilis and Escherichia coli infections (Brooks et al., 1972; Radomski et al., 1978). Unfortunately, there were no adequate controls for possible artifactual formation of NDMA during analytical chemistry procedures. In the study by Brooks et al., urine was acidified to pH 2 and then extracted with chloroform. If amines and nitrite were present in the urine, NDMA would have been formed at this stage. In the study by Radomski et al., the urine was frozen prior to analysis without the inclusion of an artifact inhibitor.

Although several other workers have reported the presence of nitrosamines in the normal urine of healthy volunteers (El-Merzabani et al., 1979; Hicks et al., 1977; Kakizoe et al., 1979), they may have underestimated the analytical problems encountered when determining volatile nitrosamines in urine at the 0.02 to 2 $\mu\text{g/liter}$ level. Eisenbrand et al. (1981) detected nitrosamines in urine of a volunteer who had consumed a liter of beer containing 60 μg of NDMA; however, they could not detect nitrosamines at 0.1 $\mu\text{g/liter}$ in normal urine.

Nitrosamines have been detected in blood at levels less than 1 $\mu\text{g/liter}$ following a meal (Fine et al., 1977; Kowalski et al., 1980; Lakritz et al., 1980). All three groups of investigators occasionally reported the presence of nitrosamines in the blood prior to consumption of a meal. Fine et al. and Kowalski et al. demonstrated that the level of nitrosamines increased immediately following ingestion of a meal. Lakritz et al. (1980) did not make similar measurements. As with urine and feces, it is extraordinarily difficult to be certain that no artifacts have been formed in blood. None of these three studies included the critical artifact test of assessing the amount of a marker amine that is converted to a nitrosamine. Eisenbrand et al. (1981) have not been able to reproduce these data when they incorporated appropriate controls. Thus, the initial finding of nitrosamines in blood by Fine and his colleagues could have resulted from the ingestion of preformed nitrosamines presumed to be present in the beer consumed with the meal rather than from endogenous formation (Eisenbrand et al., 1981).

Recently, Ohshima and Bartsch (1981) have developed a technique for estimating the extent of in vivo nitrosamine formation in humans who have ingested proline with nitrate by assaying urine samples, collected for 24 hours following ingestion, for nitroso-proline (NPRO), a nonvolatile nitrosamine. They believe that their approach is sensitive and practical for two reasons: NPRO is not carcinogenic in animals and it is not readily metabolized (80% is excreted in the urine within 24 hours). They reported a background NPRO level of less than 3 $\mu\text{g/liter}$ of urine (3 $\mu\text{g/person/day}$), which was not significantly increased by ingesting proline alone or nitrate alone. However, ingestion of red beet juice (containing up to 325 mg of nitrate) followed 30 minutes later by proline (500 mg) produced readily detectable levels of NPRO (0.016 to 0.030 mg).

The data reported by Ohshima and Bartsch compare well with the estimates of in vivo formation of NPRO derived by Fine et al. (1981) from the kinetics data on the rate at which NPRO is formed from proline and nitrite in vitro (Mirvish et al., 1973). At the low rates of conversion observed by Ohshima and Bartsch (0.002% of the nitrate and 0.004% of the proline), the rate of reaction can be assumed to be linear over the time of the reaction. The amount of NPRO formed during the time interval Δt can be determined by the following equation:

$$\Delta [\text{NPRO}] = \Delta t k_2 [\text{amine}][\text{nitrite}]^2 \quad (1)$$

The total amount of NPRO formed is the sum of the amounts formed during each time interval. The reaction is assumed to proceed in time intervals (Δt) of 1 hour over the 5 hours it takes for the stomach to empty. For 500 mg of ingested proline, a stomach volume of 900 ml, and a k_2 of $0.037 \text{ mol}^{-2} \text{ sec}^{-1}$ at a pH of 2.5 (Mirvish, 1975), the equation can be summarized as:

$$\sum_{1-5 \text{ hr}} (\text{NPRO, in } \mu\text{g}) = \sum_{1-5 \text{ hr}} 0.04865 (\text{nitrite, in mg})^2 \quad (2)$$

The data of Ohsima and Bartsch are compared in Figure 8-4 with the amount of NPRO calculated by these workers. The center line represents results of calculations based on 1.0 mg of nitrite produced in 50 ml of saliva each hour per 100 mg of nitrate ingested (Spiegelhalter *et al.*, 1976). Reasonable lower and upper bounds for salivary nitrite levels, considering person-to-person and day-to-day

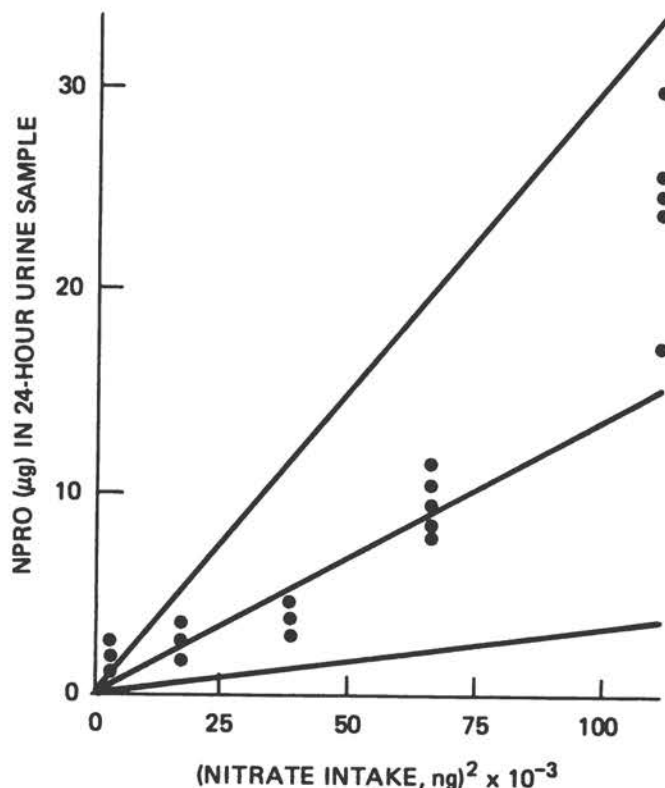


FIGURE 8-4. Plot of NPRO produced by endogenous synthesis versus the square of the nitrate concentration in the diet. The data points (●) are from Ohsima and Bartsch (1981). The straight lines were calculated using equation (2) for three levels of conversion of ingested nitrate to salivary nitrite. The top line represents 1.5 mg of salivary nitrite per 100 g of ingested nitrate, the middle line represents 1.0 mg, and the lower line, 0.5 mg. From Fine *et al.*, 1981.

variations, are shown by the lower and upper lines of Figure 8-4 as 50% and 150% of the basal value. The model agrees with the experimental results of Ohshima and Bartsch within the limits of experimental error, thereby validating the effectiveness of the model to predict the extent of endogenously formed NPRO. Additional confidence in the model is provided by Tannenbaum (personal communication, 1981), who used the approach of Ohshima and Bartsch and obtained essentially similar results.

These findings and their remarkable correlation with in vitro kinetics have several important implications. First, it seems likely that both volatile and nonvolatile nitrosamines are formed in humans. Second, the extent of this in vivo formation, at least in the stomach, can be readily calculated from the nitrosation kinetics of the amine at acid pH. Third, the calculations can be applied to both volatile and nonvolatile nitrosamines. Fourth, the experimental findings of the percentage of nitrate converted to nitrite can be used to estimate the body burden of nitrite from ingested nitrate.

Table 8-3 shows the amounts of endogenously produced NPRO calculated by using the data for nitrate and nitrite in Tables 5-20 and 5-21 and assuming that the daily amine intake is 4,000 mg. (Actual intake of nitrosatable amines is unknown and could, of course, be significantly different from this estimate.) For the purposes of calculation, intake of nitrate, nitrite, and amino compounds is assumed to be equal at major meal times, i.e., 2,000 mg of proline per meal twice daily. It is also assumed that dietary amines are nitrosated at the same rate as proline. This is an oversimplification since Mirvish (1975) showed that the rate at which proline is nitrosated under acidic conditions lies between that of dimethylamine (which is 22 times slower than proline) and morpholine (which is 11 times faster than proline). The rates for amines such as aminopyrine and piperazine are 2,000 times faster than that of proline. Another assumption, which may or may not be valid, is that the stomach is the primary site for nitrosation reactions in vivo.

Despite the inexact nature of the calculations presented, the committee believes that they do permit the comparison of relative exposures that stem from different lifestyles. Five different lifestyles are considered: (1) the average intake for the general population; (2) a high-cured-meat diet, assuming 4 times the average intake of cured meats; (3) a vegetarian diet with 4 times the normal vegetable intake, excluding meat; (4) average intake including 160 mg nitrate/person/day from nitrate-contaminated drinking water; and (5) a high-cured-meat, high-nitrate-water diet. The five categories are arbitrary and are not meant to be accurate estimates. They were selected to illustrate the impact of lifestyle on endogenous synthesis of nitrosamines, and they can be modified to include other lifestyle factors to develop an estimate for other populations.

TABLE 8-3

Amount of Nitrosamines Produced In Vivo from Proline and
Various Levels of Nitrate and Nitrite

| Compound | Intake, mg/Person/Day, by Type of Diet | | | | |
|---|--|------------------------------|-------------------------|---------------------------------|--|
| | Average | High Cured Meat ^a | Vegetarian ^b | Nitrate-Rich Water ^c | High Cured Meat ^a and High-Nitrate Water ^c |
| Exogenous nitrate ^{a,b,c} | 75 | 78 | 268 | 233 | 236 |
| Exogenous nitrite ^{a,b,c} | 0.77 | 1.7 | 0.77 | 0.77 | 1.7 |
| Nitrosoproline Produced Endogenously, μg | | | | | |
| Endogenous NPRO with ascorbic acid ^{d,e} | 0.32 | 0.63 | 2.4 | 10 | 12 |
| Endogenous NPRO, without ascorbic acid ^{d,f} | 2.2 | 3.13 | 22 | 17 | 20 |

^aAssuming 4 times the normal amount of cured meats ingested. From Tables 5-20 and 5-21.

^bAssuming 4 times the normal amount of vegetables ingested and no meat. From Tables 5-20 and 5-21.

^cFrom Table 5-20. Nitrate-rich water estimated as 100 mg/liter and assuming daily intake to be 1.6 liters or 160 mg of nitrate.

^dAssumes one-half of the vegetables and all fruit eaten raw. Thus, coingested ascorbic acid combines with half of the nitrite produced from 75 mg nitrate, except for the vegetarian diet, where it would react with half of the nitrate from 268 mg of nitrate.

^eAssumes 2,000 mg of proline ingested per meal twice a day and that half of the daily nitrate and nitrite are ingested at each meal. The rate is calculated from in vitro kinetics in a stomach with a 900 ml capacity. Equation reduces to $\mu\text{g NPRO} = 0.04865 (\text{mg NO}_2^-)^2$.

^fEndogenous NPRO produced, calculated by equation in footnote e, assuming that no ascorbic acid is available to react with nitrite.

The lower NPRO levels shown in the table were calculated by assuming that there was enough ascorbic acid in the vegetables and fruit to react with half of the nitrite in the stomach. The higher NPRO values were calculated by assuming that all the nitrite present in the stomach was free to react, without competition from ascorbic acid. A situation like this could occur when foods are overcooked. These calculated daily NPRO exposures indicate that the ratio of ascorbic acid to nitrate in vegetables is of critical importance. Ohshima and Bartsch observed complete inhibition of endogenous NPRO synthesis when the ascorbic acid/nitrate molar ratio exceeded 1.0. Although the molar ratio is less than 0.1 for vegetables such as lettuce, beets, and celery, it exceeds 3.0 for vegetables such as brussels sprouts and peas. Thus, large variations in stomach nitrite levels and, hence, the extent of endogenous synthesis are to be expected, depending on the type and quantity of the vegetables and the method by which they are prepared and cooked (see Chapter 5, Table 5-9).

The committee has compared its estimates of endogenous exposures to nitrosamines to possible exogenous exposures in Table 8-4. The estimates used in Table 8-4 for exogenous exposure to nitrosamines from the diet, cosmetics, automobile interiors, tobacco products, and occupational settings were taken from Chapter 7. The comparison of these exposures to endogenous exposures and to nitrosamines are based on several assumptions: first, that the total dietary intake of amines is 4,000 mg per day; second, that all dietary amines behave like proline; and third, that endogenous nitrosation is restricted to the stomach. A further limitation to this comparison is that, apart from tobacco products, the data base for exogenous exposures is limited to volatile nitrosamines, whereas estimates of endogenous exposures include both volatile and nonvolatile nitrosamines.

The estimates in Table 8-4 imply that the endogenous synthesis of nitrosamines for the average nonsmoker contributes from 15% (reasonable ascorbic acid intake in vegetables and fruit) to 58% (no ascorbic acid whatsoever in the diet) of total nitrosamine exposure. This finding is substantially different from the earlier estimates that endogenous synthesis greatly exceeds exogenous exposures (Tannenbaum, 1980). The greatest exogenous exposures to nitrosamines would occur in workers in certain industries, namely rubber factories and leather tanneries, and in smokers. The greatest endogenous exposures would occur in individuals consuming high-nitrate water and large quantities of cured meats.

Bacteria-Mediated Catalysis of Nitrosation Reactions. In vivo formation of N-nitroso compounds can be increased or decreased by a number of agents. For example, under certain conditions, some phenols may catalyze the nitrosation reaction, whereas ascorbic acid and α -tocopherol may inhibit it. Studies of the mechanisms and environmental distribution of the modifiers of nitrosation reactions are discussed in Chapters 4 and 6, respectively. Other studies indicate that bacteria may also catalyze the in vivo formation of N-nitroso compounds and epidemiological studies have suggested that bacterial catalysis of nitrosation reactions may be involved in the induction of stomach and bladder cancers in humans.

Three years after Sander (1968) demonstrated that bacteria could catalyze the nitrosation reaction, Hawksworth and Hill (1971) reported that five strains of E. coli formed nitrosamines when incubated aerobically with the secondary amines diphenylamine, dimethylamine, diethylamine, piperidine, pyrrolidine, and N-methylaniline. They also demonstrated that nitrosamines were formed only in the presence of bacteria and that the reaction was not due to acid catalysis since formation of nitrosamines occurred at pH 6.5. Subsequently, however, other investigators have demonstrated that bacterial enhancement of nitrosation is greater at high pH than at low pH (Collins-Thompson et al., 1972; Drasar and Hill, 1974; Kunisaki and Hayashi, 1979). This results from bacterial production of acid, which then permits an increase in the rate of nonenzymatic nitrosation in the culture medium.

TABLE 8-4

Exposure of Humans to N-Nitroso Compounds from Exogenous and Endogenous Sources^a

| Source | Exposure, $\mu\text{g}/\text{Person}/\text{Day}$ | |
|---|--|-------------------|
| | Average | High |
| <u>Exogenous Exposure:</u> ^b | | |
| Cosmetics (volatile) | 0.41 | 0.82 ^c |
| Car interiors (volatile) | 0.20 | 0.50 |
| Dietary (volatile) | 1.1 ^d | |
| Beer | 0.97 | 3.9 ^e |
| Bacon | 0.17 | 0.68 ^f |
| Tobacco smoke (volatile and nonvolatile) | ~17 | ~35 ^g |
| <u>Occupational Exposure:</u> ^h | | |
| | <u>Range</u> | |
| Leather tanning (volatile) | 20 - 180 | 440 |
| Rubber factory (volatile) | 50 - 130 | 250 |
| Rocket fuel factory (volatile) | 10 - 50 | 260 |
| <u>Endogenous Exposure (Dietary):</u> ⁱ | | <u>Average</u> |
| Average (U.S.) (volatile and nonvolatile) | 0.32 - 2.2 | 1.3 |
| High cured meat (volatile and nonvolatile) | 0.63 - 3.3 | 2.0 |
| Vegetarian (volatile and nonvolatile) | 2.4 - 22 | 12 |
| High-nitrate water (volatile and nonvolatile) | 10 - 17 | 14 |
| High cured meat and high-nitrate water (volatile and nonvolatile) | 12 - 20 | 16 |

^aEstimates for exogenous exposure based mainly on data for volatile nitrosamines (apart from tobacco smoke). The extent of exposure to exogenous nonvolatile nitrosamines is still unknown.

^bTaken from Table 7-17.

^cAssumes twice the amount of cosmetics used.

^dTaken from Table 7-15.

^eAssumes 4 times the amount of beer consumed.

^fAssumes 4 times the amount of bacon consumed.

^gAssumes exposure to twice the amount of tobacco smoke.

^hTaken from Table 7-11, and Fine, 1980.

ⁱTaken from Table 8-3.

Klubes and Jondorf (1971) found labeled NDMA after cecal contents from rats had been incubated anaerobically with ^{14}C -labeled dimethylamine and sodium nitrite at neutral pH. Subsequently, Klubes *et al.* (1972) observed that the formation of nitrosamine increased with nitrite concentration, but that it was inhibited when the intestinal contents were autoclaved or boiled or when the broad-spectrum antibiotic neomycin was added to the incubation. Both *Bacteroides* and *Clostridia* would not be affected by neomycin and, presumably, are not involved in catalyzing nitrosation. The addition of riboflavin increased the formation of nitrosamines by approximately 30% by stimulating the enzyme systems of the anaerobic bacteria. The authors also pointed out that the mixed fecal cultures used in their studies produced higher levels of nitrosamines than did the pure cultures used by Hawksworth and Hill (1971) under aerobic conditions.

Other examples of bacteria-mediated nitrosation have been reported by Archer *et al.* (1978), who found that the rates at which certain dialkyl nitrosamines were formed were accelerated at pH 3.5 in the presence of various microorganisms such as gram-positive and gram-negative bacteria as well as yeast. However, in these studies, rate enhancements were similar for both boiled and intact cells, suggesting that there was a hydrophobic interaction of precursor amine with a cellular constituent, such as a component of the cell wall. In support of this idea, the authors found that the particulate fraction of sonicated cells was responsible for 80% of the catalysis. Further evidence that a hydrophobic interaction was responsible came from structure-activity studies where rate enhancement increased with increasing alkyl chain length of the amine (Archer *et al.*, 1978).

Hill *et al.* (1973) summarized clinical situations in which bacteria-catalyzed nitrosamine formation was implicated in disease causation. Likely sites of such catalysis include the infected urinary bladder and the achlorhydric stomach (Hill, 1979). For example, evidence that bacteria-mediated nitrosamine formation can occur in the infected bladder has been provided by studies in rats (Hawksworth and Hill, 1974). These authors also found that labeled NDMA injected into the bladder was not totally excreted in the urine but was absorbed into the circulating blood. Label was found primarily in the liver, kidney, lung, stomach, and small intestine. In another study, Fong *et al.* (1980) induced bladder infections in rats with *E. coli* and exposed the infected rats to nitrate and aminopyrene. Mutagens requiring metabolic activation were detected in these animals, but not in infection-free control animals given nitrate and aminopyrene. There was also an apparent increase in the number of tumors in the infected animals.

In humans, the possible importance of mixed bacterial infections in the induction of bladder cancer has been discussed by Hill (1979),

who suggested that mixed infections, as opposed to simple infections with one major organism causing the infection, may result in the more frequent formation of carcinogens. Epidemiological studies of bladder cancer in humans are discussed in detail in Chapter 9.

Gastric achlorhydria creates conditions that favor a profuse gastric flora because of the elevated pH (Tannenbaum *et al.*, 1977). Thus, individuals with that condition may be at high risk of gastric cancer since the bacteria could catalyze the formation of N-nitroso compounds from ingested secondary amines/amides and nitrate/nitrite (Hill *et al.*, 1973). In addition, bacteria may aid in the nitrosation of drugs in the saliva (Spiegelhalter *et al.*, 1976). Mirvish (1975), Lijinsky and Epstein (1970), and Rao (1980) provided lists of nitrosatable drugs currently in use (Chapter 6). Some of the nitrosamines resulting from nitrosation of those drugs are carcinogenic in animals.

Bacteria-mediated catalysis of nitrosation reactions can also occur exogenously. Ayanaba and Alexander (1973) described studies in which microorganisms isolated from soil or sewage formed NDMA during incubations with trimethylamine and nitrate at neutral pH. These results suggest that the presence of nitrosamines or their precursors in sewage or soil may be a potential environmental hazard to humans.

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

In persons with normal gastric acidity, nitrate is converted to nitrite mainly in saliva by the action of oral microflora. Ingested nitrate is absorbed from the gastrointestinal tract and is transported via the plasma to the salivary glands. Nitrate, thiocyanate, and iodide appear to share a common active transport system in the salivary glands of animals and, thus, compete for uptake from the circulatory system. Nitrate secreted by the salivary glands is approximately 25% of the nitrate ingested and approximately 20% of salivary nitrate is reduced to nitrite. Thus, 5% of ingested nitrate is reduced to nitrite in the saliva. Using this conversion factor, the committee estimated that approximately 72% of the nitrite load to which the upper gastrointestinal tract is exposed is derived from ingested vegetables, and about 9% from cured meats. When gastric pH approaches neutrality, the presence of microorganisms could also permit extensive reduction of nitrate in the stomach, resulting in a greatly increased gastric nitrite load.

Although the active transport of nitrate from the circulatory system into the stomach may occur in humans, it is an unlikely process in the intestine. Most of the ingested nitrate, especially

at high intake levels, appears in the urine. In humans, total urinary excretion of nitrate takes several days, and the average elimination half-life is 5 hours. Nitrite is not a normal constituent of urine, but it has been found in the urine of patients with urinary tract infections.

Nitrate balance studies in humans have indicated that the quantity of nitrate excreted in the urine can exceed the amount ingested when these amounts are small. Heterotrophic nitrification of ammonia or organic nitrogen has been postulated as a mechanism to account for this difference; however, subsequent studies with labeled nitrate in rodents indicate that such microbial reactions are not likely. Moreover, the analytical method used might have led to an underestimate of the nitrate content of ingested food, water, and air. Recent studies suggest that mammalian synthesis of nitrate partially explains the excess nitrate excreted in the urine.

The organotropic, toxic, and carcinogenic effects of nitrosamines probably result from preferential metabolism by specific organs. However, although tumor induction occurs only in organs in which alkylation takes place, the nature of the alkylating molecule is not known for a single nitrosamine. The primary reaction required for activation may involve an enzyme-mediated α -hydroxylation. Studies of the mechanisms of activation also suggest that oxidative dealkylation is necessary for the toxic and carcinogenic action of nitrosamines and that cytochrome P450 metabolizing enzymes are involved.

Efforts to explain the carcinogenicity of various nitrosamines have focused on alkylation of DNA, and some 11 different nucleoside and alkylphosphotriester adducts have been found. However, the balance among adduct formation, repair mechanisms, and cellular replication may be the most important determinant of the carcinogenic process. Conditions that accelerate replication or delay repair could enhance miscoding, thereby increasing the number of mutations and enhancing the carcinogenic effect, whereas conditions that delay replication or enhance repair efficiency would have the reverse effect. When considering the importance of repair mechanisms, one must take into account the fact that humans repair damaged DNA more efficiently than do other species and that repair is more efficient at low doses of carcinogen.

In vivo formation of N-nitroso compounds has been studied extensively in laboratory animals. Sodium nitrite and an amine coadministered to animals have led to the formation of nitrosamines, which produce toxic effects (tumors or acute damage to target organs). In humans, the evidence is sparse. Nitrosamines have been measured in a variety of biological samples (e.g., feces, urine, and blood); however, because of the low levels of nitroso compounds that would be expected in specimens from humans and because of artifact formation during such assays (Chapter 7), the validity of these results remains open to question.

One study, which has recently been repeated, has provided direct evidence of increased levels of nitrosamines in the urine of human subjects following ingestion of proline and nitrate. This suggests that in vivo synthesis of these compounds does occur in humans. Based on these preliminary findings and data on average ingestion of nitrate and nitrite from Chapter 5, the committee has calculated rough estimates of the amounts of N-nitroso compounds formed in vivo based on the limited data available on exposure to nitrate and nitrite and endogenous formation of N-nitroso compounds in humans. For the average population, the amount of preformed nitrosamines in the diet is roughly equivalent to the amount formed in vivo from the intake of nitrate and nitrite. However, for special population groups, such as those ingesting high-nitrate water, the increased intake of nitrate could lead to a corresponding increase in the amount of nitrosamines formed in vivo.

Bacteria-mediated formation of nitrosamines may be important in certain target tissues, such as the achlorhydric stomach and the infected bladder, which are colonized by bacteria. Although the precise role of bacteria in nitrosation reactions is not known, it has been suggested that a hydrophobic interaction of a precursor amine with a bacterial cellular constituent (e.g., cell wall) may catalyze the reaction if the amine is lipophilic.

Although much is known about the metabolism of nitrate, nitrite, and N-nitroso compounds, many remaining uncertainties will have an important impact on the overall conclusions on the possible health effects of exogenous exposures to these compounds in humans -- a topic that will be considered in detail in the following chapter. These uncertainties include the extent and importance of the endogenous synthesis of nitrate, nitrite, and N-nitroso compounds, as compared to exogenous exposure. In addition, more detailed information is needed on the metabolism of N-nitroso compounds, including metabolic activation, deactivation, and cellular repair mechanisms for genotoxic effects in the human. Thus, although the data presented in this chapter may indicate the possible significance of the endogenous synthesis of N-nitroso compounds following exposure to exogenous nitrate/nitrite, many uncertainties remain so that the overall significance to human health of the experimental findings is still unclear.

Based on the data presented in this chapter, the committee recommends the following:

1. Additional studies should be conducted to increase understanding of the metabolism of nitrate in humans and to clarify the role of mammalian synthesis of nitrate and nitrite. Also requiring clarification is the role of bacteria in the reduction of nitrate to nitrite and in the formation of N-nitroso compounds, especially in certain clinical conditions such as gastric achlorhydria and bladder infections.

2. The nature and extent of in vivo nitrosation by nitrite and nitrogen oxides also require further evaluation. In addition, the nitrosation-inhibiting effects of normal dietary constituents should be studied further to determine the extent of their effects in vivo. Specifically, further research is needed to determine the amount of nitrite that is destroyed in the human stomach and the extent to which nitrosation reactions are modified by the various inhibitors. Attention should also be directed toward in vivo interactions among inhibitors, catalysts, and other food-derived substances.

REFERENCES

- Acland, J. D., and O. Illman. 1959. Studies on iodide transport against a concentration gradient by the small intestine of the rat in vitro. *J. Physiol.* 147:260-268.
- Alam, B. S., I. B. Saporoschetz, and S. S. Epstein. 1971a. Formation of N-nitrosopiperidine from piperidine and sodium nitrite in the stomach and the isolated intestinal loop of the rat. *Nature* 223:116-118.
- Alam, B. S., I. B. Saporoschetz, and S. S. Epstein. 1971b. Synthesis of nitrosopiperidine from nitrate and piperidine in the gastrointestinal tract of the rat. *Nature* 232:199-200.
- Archer, M. C., H. S. Yang, and J. D. Okun. 1978. Acceleration of nitrosamine formation at pH 3.5 by microorganisms. Pp. 239-246 in E. A. Walker, M. Castegnaro, L. Gričiute, and R. E. Lyle, eds. *Environmental Aspects of N-Nitroso Compounds*, IARC Scientific Publication No. 19. International Agency for Research on Cancer, Lyon, France.
- Arcos, J. C., G. M. Bryant, N. Venkatesan, and M. F. Argus. 1975. Repression of dimethylnitrosamine-demethylase by typical inducers of microsomal mixed-function oxidases. *Biochem. Pharmacol.* 24:1544-1547.
- Arcos, J. C., D. L. Davies, Ch. E. L. Brown, and M. F. Argus. 1977. Repressible and inducible enzymic forms of dimethylnitrosamine-demethylase. *Z. Krebsforsch.* 89:181-199.
- Asahina, S., M. A. Friedman, E. Arnold, G. N. Millar, M. Mishkin, Y. Bishop, and S. S. Epstein. 1971. Acute synergistic toxicity and hepatic necrosis following oral administration of sodium nitrite and secondary amines to mice. *Cancer Res.* 31:1201-1205.
- Asatoor, A. M., and M. L. Simenhoff. 1965. The origin of urinary dimethylamine. *Biochim. Biophys. Acta* 111:384-392.
- Atkinson, M., and K. S. Henley. 1955. Levels of intragastric and intraduodenal acidity. *Clin. Sci.* 14:1-14.
- Ayanaba, A., and M. Alexander. 1973. Microbial formation of nitrosamines in vitro. *Appl. Microbiol.* 25:862-868.
- Balish, E., J. P. Witter, and S. J. Gatley. 1981. Distribution and metabolism of nitrate and nitrite in rats. Pp. 305-317, 337-341 in W. R. Bruce, P. Correa, M. Lipkin, S. R. Tannenbaum, and T. D. Wilkins, eds. *Gastrointestinal Cancer: Endogenous Factors*, Banbury Report 7. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

- Bartholomew, B. A., M. J. Hill, M. J. Hudson, W. S. J. Ruddell, and C. L. Walters. 1980. Gastric bacteria, nitrate, nitrite and nitrosamines in patients with pernicious anaemia and in patients treated with cimetidine. Pp. 595-608 in E. A. Walker, M. Castegnaro, L. Gričiute, and M. Börzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Bartle, H. J., and M. J. Harkins. 1925. The gastric secretion: Its bactericidal value to man. *Am. J. Med. Sci.* 169:373-388.
- Berman, J. J., J. M. Rice, M. L. Wenk, and P. P. Roller. 1979. Intestinal tumors induced by a single intraperitoneal injection of methyl(acetoxymethyl)nitrosamine in three strains of rats. *Cancer Res.* 39:1462-1466.
- Bloomfield, R. A., J. R. Hersey, C. W. Welsch, G. B. Garner, and M. E. Muhrer. 1962. Gastric concentration of nitrate in rats. *J. Anim. Sci.* 21:1091. Abstract 221.
- Boveri, T. 1914. [The Origin of Malignant Tumors.] Translation by M. Boveri, Williams and Wilkens, Baltimore, Maryland. 64 pp.
- Boyland, E., and S. A. Walker. 1974. Effect of thiocyanate on nitrosation of amines. *Nature* 248:601-602.
- Brash, D. E., and R. W. Hart. 1978. DNA damage and repair in vivo. *J. Environ. Pathol. Toxicol.* 2(1):79-114.
- Braunberg, R. C., and R. E. Dailey. 1973. Formation of nitrosoproline in rats. *Proc. Soc. Exp. Biol. Med.* 142:993-996.
- Brooks, J. B., W. B. Cherry, L. Thacker, and C. C. Alley. 1972. Analysis by gas chromatography of amines and nitrosamines produced in vivo and in vitro by Proteus mirabilis. *J. Infect. Dis.* 126:143-153.
- Brown, L. R., S. Dreizen, S. Handler, and D. A. Johnston. 1975. Effect of radiation-induced xerostomia on human oral microflora. *J. Dent. Res.* 54:740-750.
- Brown-Grant, K. 1961. Extrathyroidal iodide concentrating mechanisms. *Physiol. Rev.* 41:189-213.
- Bryan, B. A. 1981. Physiology and biochemistry of denitrification. Pp. 67-84 in C. C. Delwiche, ed. *Denitrification, Nitrification, and Atmospheric Nitrous Oxide*. John Wiley and Sons, New York.
- Buecheler, J., and P. Kleihues. 1977. Excision of O⁶-methylguanine from DNA of various mouse tissues following a single injection of N-methyl-N-nitrosourea. *Chem. Biol. Interact.* 16:325-333.

- Burdette, W. J. 1955. The significance of mutation in relation to the origin of tumors: A review. *Cancer Res.* 15:201-226.
- Burgen, A. S. V., and N. G. Emmelin. 1961. Physiology of the Salivary Glands; Monographs of the Physiological Society, No. 8. Edward Arnold Ltd., London, United Kingdom. 279 pp.
- Collins-Thompson, D. L., N. P. Sen, B. Aris, and L. Schwinghamer. 1972. Non-enzymic in vitro formation of nitrosamines by bacteria isolated from meat products. *Can. J. Microbiol.* 18:1968-1971.
- Cooper, H. K., and R. F. Itzhaki. 1975. Studies on liver chromatin from rats treated with dimethylnitrosamine. *Biochim. Biophys. Acta* 407:263-272.
- Czygan, P., H. Greim, A. J. Garro, F. Hutterer, F. Schaffner, H. Popper, O. Rosenthal, and D. Y. Cooper. 1973. Microsomal metabolism of dimethylnitrosamine and the cytochrome P-450 dependency of its activation to a mutagen. *Cancer Res.* 33:2983-2986.
- Davenport, H. W. 1943. The secretion of iodide by the gastric mucosa. *Gastroenterology* 1:1055-1061.
- den Engelse, L., M. Gebbink, and P. Emmelot. 1975. Studies on lung tumours. III. Oxidative metabolism of dimethylnitrosamine by rodent and human lung tissue. *Chem. Biol. Interact.* 11:535-544.
- Diem, K., and C. Lentner. 1970. Scientific Tables, 7th ed. Ciba-Geigy Limited, Basel, Switzerland. Distributed by Geigy Pharmaceuticals, Division of Ciba-Geigy Corporation, Ardsley, New York. 810 pp.
- Drasar, B. S., and M. J. Hill. 1974. Human Intestinal Flora. Academic Press, New York, London, and San Francisco. 263 pp.
- Drasar, B. S., M. Shiner, and G. M. McLeod. 1969. Studies on the intestinal flora. I. The bacterial flora of the gastrointestinal tract in healthy and achlorhydric persons. *Gastroenterology* 56:71-79.
- Edwards, D. A. W., K. Fletcher, and E. N. Rowlands. 1954. Antagonism between perchlorate, iodide, thiocyanate, and nitrate for secretion in human saliva: Analogy with the iodide trap of the thyroid. *Lancet* 1:498-499.
- Eisenbrand, G., B. Spiegelhalder, and R. Preussmann. 1981. Analysis of human biological specimens for nitrosamine contents. Pp. 275-283 in W. R. Bruce, P. Correa, M. Lipkin, S. R. Tannenbaum, and T. D. Wilkins, eds. *Gastrointestinal Cancer: Endogenous Factors*, Banbury Report 7. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

- El-Merzabani, M. M., A. A. El-Aaser, and N. I. Zakhary. 1979. A study on the aetiological factors of bilharzial bladder cancer in Egypt--1. Nitrosamines and their precursors in urine. *Eur. J. Cancer* 15:287-291.
- Fine, D. H. 1980. Exposure assessment to preformed environmental N-nitroso compounds from the point of view of our own studies. *Oncology* 37:199-202.
- Fine, D. H., R. Ross, D. P. Rounbehler, A. Silvergleid, and L. Song. 1977. Formation in vivo of volatile N-nitrosamines in man after ingestion of cooked bacon and spinach. *Nature* 265:753-755.
- Fine, D. H., B. C. Challis, P. Hartman, and J. van Ryzin. 1981. Human exposure assessment of nitrosamines from endogenous and exogenous sources: Model calculations and risk assessment. Paper presented at the 7th International Meeting on N-Nitroso Compounds, September 28-October 1, 1981, Tokyo, Japan. Meeting sponsored by the International Agency for Research on Cancer, Lyon, France.
- Fong, L. Y. Y., F. W. T. Wong, and W. C. Chan. 1980. Do chronic urinary tract infections induce cancer in the rat fed nitrate and aminopyrine? Pp. 693-704 in E. A. Walker, M. Castegnaro, L. Gričiute, and M. Bőrzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation, and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Franklin, M. A., and S. C. Skoryna. 1971. Studies on natural gastric flora: Survival of bacteria in fasting human subjects. *Can. Med. Assoc. J.* 105:380-386.
- Frei, J. V. 1976. Some mechanisms operative in carcinogenesis: A review. *Chem. Biol. Interact.* 13:1-25.
- Friedman, M. A., E. J. Greene, and S. S. Epstein. 1972. Rapid gastric absorption of sodium nitrite in mice. *J. Pharm. Sci.* 61:1492-1494.
- Garro, A. J., H. K. Seitz, and C. S. Lieber. 1981. Enhancement of dimethylnitrosamine metabolism and activation to a mutagen following chronic ethanol consumption. *Cancer Res.* 41:120-124.
- Giannella, A., S. A. Broitman, and N. Zamcheck. 1972. Gastric acid barrier to ingested microorganisms in man: Studies in vivo and in vitro. *Gut* 13:251-256.
- Goth, R., and M. F. Rajewsky. 1974a. Molecular and cellular mechanisms associated with pulse-carcinogenesis in the rat nervous system by ethylnitrosourea: Ethylation of nucleic acids and elimination rates of ethylated bases from the DNA of different tissues. *Z. Krebsforsch.* 82:37-64.

- Goth, R., and M. F. Rajewsky. 1974b. Persistence of O^6 -Ethyl-guanine in rat-brain DNA: Correlation with nervous system-specific carcinogenesis by ethylnitrosourea. *Proc. Natl. Acad. Sci. USA* 71:639-643.
- Green, L. C., S. R. Tannenbaum, and P. Goldman. 1981. Nitrate synthesis in the germfree and conventional rat. *Science* 212:56-58.
- Greenblatt, M., and S. S. Mirvish. 1973. Dose-response studies with concurrent administration of piperazine and sodium nitrite to strain A mice. *J. Natl. Cancer Inst.* 50:119-124.
- Greenblatt, M., S. Mirvish, and B. T. So. 1971. Nitrosamine studies: Induction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice. *J. Natl. Cancer Inst.* 46:1029-1034.
- Greene, I., and E. P. Hiatt. 1954. Behavior of the nitrate ion in the dog. *Am. J. Physiol.* 176:463-467.
- Gruener, N., H. I. Shuval, K. Behroozi, S. Cohen, and H. Shechter. 1973. Methemoglobinemia induced by transplacental passage of nitrites in rats. *Bull. Environ. Contam. Toxicol.* 9:44-48.
- Gwatkin, R., and P. J. G. Plummer. 1946. Toxicity of certain salts of sodium and potassium for swine. *Can. J. Comp. Med. Vet. Sci.* 10:183-190.
- Habs, M., D. Schmähel, and M. Wiessler. 1978. Carcinogenicity of acetoxymethyl-methyl-nitrosamine after subcutaneous, intravenous and intrarectal applications in rats. *Z. Krebsforsch.* 91:217-221.
- Halmi, N. S. 1964. The accumulation and recirculation of iodide by the thyroid. Pp. 71-86 in R. Pitt-Rivers and W. R. Trotter, eds. *The Thyroid Gland*, Vol. 1. Butterworths, London, United Kingdom.
- Halmi, N. S., and R. G. Stuelke. 1959. Comparison of thyroidal and gastric iodide pumps in rats. *Endocrinology* 64:103-109.
- Harris, C. C. 1981. Carcinogenesis studies using cultured human epithelial tissues and cells. *J. Supramol. Struct. Cell. Biochem. Suppl.* 5:150. Abstract 407.
- Hartman, P. E. 1981. Nitrates and nitrites: Ingestion, pharmacodynamics and toxicology. Pp. 211-294 in F. J. de Serres and A. Hollaender, eds. *Chemical Mutagens*, Vol. 7. Plenum Press, New York.
- Hashimoto, S., T. Yokokura, Y. Kawai, and M. Mutai. 1976. Dimethyl-nitrosamine formation in the gastro-intestinal tract of rats. *Food Cosmet. Toxicol.* 14:553-556.

- Hawksworth, G. M., and M. J. Hill. 1971. Bacteria and the N-nitrosation of secondary amines. *Br. J. Cancer* 25:520-526.
- Hawksworth, G., and M. J. Hill. 1974. The in vivo formation of N-nitrosamines in the rat bladder and their subsequent absorption. *Br. J. Cancer* 29:353-358.
- Hawksworth, G., M. J. Hill, G. Gordillo, and C. Cuello. 1975. Possible relationship between nitrates, nitrosamines and gastric cancer in south-west Colombia. Pp. 229-234 in P. Bogovski and E. A. Walker, eds. N-Nitroso Compounds in the Environment, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Heath, D. F. 1962. The decomposition and toxicity of dialkylnitrosamines in rats. *Biochem. J.* 85:72-91.
- Hiatt, E. P. 1940. Extreme hypochloremia in dogs induced by nitrate administration. *Am. J. Physiol.* 129:597-609.
- Hicks, R. M., C. L. Walters, I. Elsebai, A.-B. El Aasser, M. El Merzabani, and T. A. Gough. 1977. Demonstration of nitrosamines in human urine: Preliminary observations on a possible etiology for bladder cancer in association with chronic urinary tract infections. *Proc. R. Soc. Med.* 70:413-417.
- Hill, M. J. 1979. In vivo bacterial N-nitrosation and its possible role in human cancer. Pp. 229-240 in E. C. Miller, J. A. Miller, I. Hirono, T. Sugimura, and S. Takayama, eds. Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis. Japan Scientific Societies Press, Tokyo, Japan; University Park Press, Baltimore, Maryland.
- Hill, M. J., G. Hawksworth, and G. Tattersall. 1973. Bacteria, nitrosamines and cancer of the stomach. *Br. J. Cancer* 28:562-567.
- Honour, A. J., N. B. Myant, and E. N. Rowlands. 1952. Secretion of radiiodine in digestive juices and milk in man. *Clin. Sci.* 11:447-462.
- Howell, G. L., and L. Van Middlesworth. 1956. Gastric iodide and chloride clearances in dogs. *Proc. Soc. Exp. Biol. Med.* 93:602-605.
- Hultin, T., E. Arrhenius, H. Löw, and P. N. Magee. 1960. Inhibition by dimethylnitrosamine of incorporation of labelled amino acids into proteins of rat-liver preparation in vitro. *Biochem. J.* 76:109-116.

- Inui, N., Y. Nishi, M. Taketomi, and M. Mori. 1979a. Transplacental action of sodium nitrite on embryonic cells of Syrian golden hamster. *Mutat. Res.* 66:149-158.
- Inui, N., Y. Nishi, M. Taketomi, M. Mori, M. Yamamoto, T. Yamada, and A. Tanimura. 1979b. Transplacental mutagenesis of products formed in the stomach of golden hamsters given sodium nitrite and morpholine. *Int. J. Cancer* 24:365-372.
- Iqbal, Z. M., K. Dahl, and S. S. Epstein. 1980. Role of nitrogen dioxide in the biosynthesis of nitrosamines in mice. *Science* 207:1475-1477.
- Ishiwata, H., P. Boriboon, M. Harada, A. Tanimura, and M. Ishidate. 1975a. Studies on in vivo formation of nitroso compounds (IV): Changes of nitrite and nitrate concentration in incubated human saliva. *J. Food Hyg. Soc.* 16:93-98.
- Ishiwata, H., P. Boriboon, Y. Nakamura, M. Harada, A. Tanimura, and M. Ishidate. 1975b. Studies on in vivo formation of nitroso compounds (II). Changes of nitrite and nitrate concentrations in human saliva after ingestion of vegetables or sodium nitrate. *J. Food Hyg. Soc.* 16:19-24.
- Ishiwata, H., A. Tanimura, and M. Ishidate. 1975c. Studies on in vivo formation of nitroso compounds (III). Nitrite and nitrate concentrations in human saliva collected from salivary ducts. *J. Food Hyg. Soc.* 16:89-92.
- Ishiwata, H., H. Mizushiro, A. Tanimura, A. Takahashi, Y. Omori, and T. Murata. 1977. Metabolic fate of the precursors of N-nitroso compounds (I). Gastro-intestinal absorption of N-nitrosodimethylamine and its precursors in guinea-pigs. *J. Food Hyg. Soc.* 18:524-528.
- Ishiwata, H., H. Mizushiro, A. Tanimura, and T. Murata. 1978. Metabolic fate of the precursors of N-nitroso compounds (III). Urinary excretion of nitrate in man. *J. Food Hyg. Soc.* 19:318-322.
- Johansson, E. B., and H. Tjälve. 1978. The distribution of [¹⁴C]dimethylnitrosamine in mice. Autoradiographic studies in mice with inhibited and noninhibited dimethylnitrosamine metabolism and a comparison with the distribution of [¹⁴C]formaldehyde. *Toxicol. Appl. Pharmacol.* 45:565-575.
- Kakizoe, T., T.-T. Wang, V. W. S. Eng, R. Furrer, P. Dion, and W. R. Bruce. 1979. Volatile N-nitrosamines in the urine of normal donors and of bladder cancer patients. *Cancer Res.* 39:829-832.

- Keith, N. M., M. Whelan, and E. G. Bannick. 1930. The action and excretion of nitrates. *Arch. Intern. Med.* 46:797-832.
- Kleihues, P., and G. P. Margison. 1974. Carcinogenicity of N-methyl-N-nitrosourea: Possible role of excision repair of O⁶-methylguanine from DNA. *J. Natl. Cancer Inst.* 53:1839-1841.
- Kleihues, P., P. L. Lantos, and P. N. Magee. 1976. Chemical carcinogenesis in the nervous system. *Int. Rev. Exp. Pathol.* 15:153-232.
- Kleihues, P., G. Doerjer, L. K. Keefer, J. M. Rice, P. P. Roller, and R. M. Hodgson. 1979. Correlation of DNA methylation by methyl(acetoxymethyl)nitrosamine with organ-specific carcinogenicity in rats. *Cancer Res.* 39:5136-5140.
- Klubes, P., and W. R. Jondorf. 1971. Dimethylnitrosamine formation from sodium nitrite and dimethylamine by bacterial flora of rat intestine. *Res. Commun. Chem. Pathol. Pharmacol.* 2:24-34.
- Klubes, P., I. Cerna, A. D. Rabinowitz, and W. R. Jondorf. 1972. Factors affecting dimethylnitrosamine formation from simple precursors by rat intestinal bacteria. *Food Cosmet. Toxicol.* 10:757-767.
- Knott, F. A. 1927. Addison's (pernicious) anaemia and subacute combined degeneration of the cord: The role of achlorhydria and intestinal infection. *Guys Hosp. Rept.* 77:1-12.
- Kowalski, B., C. T. Miller, and N. P. Sen. 1980. Studies on the in vivo formation of nitrosamines in rats and humans after ingestion of various meals. Pp. 609-617 in E. A. Walker, M. Castegnaro, L. Gričiute, and M. Bőrzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Kraus, F. W., and C. Gaston. 1956. Individual constancy of numbers among the oral flora. *J. Bacteriol.* 71:703-707.
- Kröger, M., and B. Singer. 1979. Ambiguity and transcriptional errors as a result of methylation of N-1 of purines and N-3 of pyrimidines. *Biochemistry* 18:3493-3500.
- Kröger, F. W. 1972. New aspects in metabolism of carcinogenic nitrosamines. Pp. 213-235 in W. Nakahara, T. Takayama, T. Sugimura, and S. Odashima, eds. *Topics in Chemical Carcinogenesis*. University Park Press, Baltimore, London, and Tokyo.

- Kunisaki, N., and M. Hayashi. 1979. Formation of N-nitrosamines from secondary amines and nitrite by resting cells of Escherichia coli B. Appl. Environ. Microbiol. 37:279-282.
- Lake, B. G., M. J. Minski, J. C. Phillips, S. D. Gangolli, and A. G. Lloyd. 1975. Investigations into the hepatic metabolism of dimethylnitrosamine in the rat. Life Sci. 17:1599-1606.
- Lake, B. G., J. C. Phillips, C. E. Heading, and S. D. Gangolli. 1976. Studies on the in vitro metabolism of dimethylnitrosamine by rat liver. Toxicology 5:297-309.
- Lakritz, L., M. L. Simenhoff, S. R. Dunn, and W. Fiddler. 1980. N-Nitrosodimethylamine in human blood. Food Cosmet. Toxicol. 18:77-79.
- Lampé, A. Ed., and H. Strassburger. 1943. [In German.] Zur Bakteriologie des gesunden und des kranken Magens. Arch. Hyg. Bakteriol. 130:150-180.
- Lawley, P. D. 1974. Some chemical aspects of dose-response relationships in alkylation mutagenesis. Mutat. Res. 23:283-295.
- Lawley, P. D. 1976. Methylation of DNA by carcinogens: Some applications of chemical analytical methods. Pp. 181-210 in R. Montesano, H. Bartsch, and L. Tomatis, eds. Screening Tests in Chemical Carcinogenesis, IARC Scientific Publication No. 12. International Agency for Research on Cancer, Lyon, France.
- Lawson, T. A., R. Gingell, D. Nagel, L. A. Hines, and A. Ross. 1981a. Methylation of hamster DNA by the carcinogen N-nitrosobis(2-oxopropyl)amine. Cancer Lett. 11:251-255.
- Lawson, T. A., A. S. Helgeson, C. J. Grandjean, L. Wallcave, and D. Nagel. 1981b. The formation of N-nitrosomethyl(2-oxopropyl)amine from N-nitrosobis(2-oxopropyl)amine in vivo. Carcinogenesis 2:845-849.
- Lijinsky, W., and S. S. Epstein. 1970. Nitrosamines as environmental carcinogens. Nature 225:21-23.
- Lijinsky, W., and M. Greenblatt. 1972. Carcinogen dimethylnitrosamine produced in vivo from nitrite and aminopyrine. Nature New Biol. 236:177-178.
- Lijinsky, W., J. Loo, and A. E. Ross. 1968. Mechanism of alkylation of nucleic acids by nitrosodimethylamine. Nature 218:1174-1175.

- Logothetopoulos, J. H., and N. B. Myant. 1956. Concentration of radio-iodide and ^{35}S -labelled thiocyanate by the stomach of the hamster. *J. Physiol.* 133:213-219.
- Loveless, A., and C. L. Hampton. 1969. Inactivation and mutation of coliphage T_2 by N-methyl- and N-ethyl-N-nitrosourea. *Mutat. Res.* 7:1-12.
- Lowenfels, A. B., A. J. Tuyns, E. A. Walker, and A. Roussel. 1978. Nitrite studies in oesophageal cancer. *Gut* 19:199-201.
- Maekawa, A., M. Takahashi, Y. Kurokawa, T. Kokubo, T. Ogiu, M. Mochizuki, M. Okada, and S. Odashima. 1981. Carcinogenicity of α -oxidized nitrosamines (α -acyloxy, α -hydroperoxy, and α -oxo nitrosamines) in F-344 rats. Paper presented at the 7th International Meeting on Analysis and Formation of N-Nitroso Compounds, September 28-October 1, 1981, Tokyo, Japan.
- Magee, P. N. 1956. Toxic liver injury: The metabolism of dimethylnitrosamine. *Biochem. J.* 64:676-682.
- Magee, P. N. 1980. Metabolism of nitrosamines: An overview. Pp. 1081-1092 in M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette, and P. J. O'Brien, eds. *Microsomes, Drug Oxidations, and Chemical Carcinogenesis*, Vol. 2. Academic Press, New York, London, Toronto, Sydney, and San Francisco.
- Magee, P. N., and J. M. Barnes. 1967. Carcinogenic nitroso compounds. *Adv. Cancer Res.* 10:163-246.
- Magee, P. N., and E. Farber. 1962. Toxic liver injury and carcinogenesis: Methylation of rat-liver nucleic acids by dimethylnitrosamine in vivo. *Biochem. J.* 83:114-124.
- Magee, P. N., R. Montesano, and R. Preussmann. 1976. N-Nitroso compounds and related carcinogens. Pp. 491-625 in C. E. Searle, ed. *Chemical Carcinogens*, A.C.S. Monograph 173. American Chemical Society, Washington, D.C.
- Marriott, W. M., and L. T. Davidson. 1923. The acidity of the gastric contents of infants. *Am. J. Dis. Child.* 26:542-553.
- Mason, E. E., and H. S. Bloch. 1950. Gastric secretion of iodide at low serum iodide levels. *Proc. Soc. Exp. Biol. Med.* 73:488-491.
- Miller, E. C., and J. A. Miller. 1971a. The mutagenicity of chemical carcinogens: Correlations, problems, and interpretations. Pp. 83-119 in A. Hollaender, ed. *Chemical Mutagens: Principles and Methods for Their Detection*. Plenum Press, New York and London.

- Miller, J. A., and E. C. Miller. 1971b. Editorial: Chemical carcinogenesis: Mechanisms and approaches to its control. *J. Natl. Cancer Inst.* 47:V-XIV.
- Milstein, S., and J. B. Guttenplan. 1979. Near quantitative production of molecular nitrogen from metabolism of dimethylnitrosamine. *Biochem. Biophys. Res. Commun.* 87:337-342.
- Mirvish, S. S. 1975. Formation of N-nitroso compounds: Chemistry, kinetics, and in vivo occurrence. *Toxicol. Appl. Pharmacol.* 31:325-351.
- Mirvish, S. S., and C. Chu. 1973. Chemical determination of methylnitrosourea and ethylnitrosourea in stomach contents of rats, after intubation of the alkylureas plus sodium nitrite. *J. Natl. Cancer Inst.* 50:745-750.
- Mirvish, S. S., M. Greenblatt, and V. R. C. Kommineni. 1972. Nitrosamide formation in vivo: Induction of lung adenomas in Swiss mice by concurrent feeding of nitrite and methylurea or ethylurea. *J. Natl. Cancer Inst.* 48:1311-1315.
- Mirvish, S. S., J. Sams, T. Y. Fan, and S. R. Tannenbaum. 1973. Kinetics of nitrosation of the amino acids proline, hydroxyproline, and sarcosine. *J. Natl. Cancer Inst.* 51:1833-1839.
- Mirvish, S. S., K. Patil, P. Ghadirian, and V. R. C. Kommineni. 1975. Disappearance of nitrite from the rat stomach: Contribution of emptying and other factors. *J. Natl. Cancer Inst.* 54:869-875.
- Mirvish, S. S., K. Karlowski, D. F. Birt, and J. P. Sams. 1980. Dietary and other factors affecting nitrosomethylurea (NMU) formation in the rat stomach. Pp. 271-279 in E. A. Walker, M. Castegnaro, L. Gričute, and M. Bőrszőnyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Mirvish, S. S., P. Issenberg, and J. P. Sams. In press. A study of N-nitrosomorpholine synthesis in rodents exposed to nitrogen dioxide and morpholine. In R. A. Scanlan and S. R. Tannenbaum, eds. N-Nitroso Compounds, ACS Symposium Series. American Chemical Society, Washington, D.C.
- Mitchell, H. H., H. A. Shonle, and H. S. Grindley. 1916. The origin of the nitrates in the urine. *J. Biol. Chem.* 24:461-490.
- Mochizuki, M., T. Anjo, and M. Okada. 1980. Isolation and characterization of N-alkyl-N-(hydroxymethyl)nitrosamines from N-alkyl-N-(hydroperoxymethyl)nitrosamines by deoxygenation. *Tetrahedron Lett.* 21:3693-3696.

- Modan, B., F. Lubin, V. Barell, R. A. Greenberg, M. Modan, and S. Graham. 1974. The role of starches in the etiology of gastric cancer. *Cancer* 34:2087-2092.
- Montesano, R., and H. Bartsch. 1976. Mutagenic and carcinogenic N-nitroso compounds: Possible environmental hazards. *Mutat. Res.* 32:179-227.
- Montesano, R., and P. N. Magee. 1971. Evidence of formation of N-methyl-N-nitrosourea in rats given N-methylurea and sodium nitrite. *Int. J. Cancer* 7:249-255.
- Myant, N. B., B. D. Corbett, A. J. Honour, and E. E. Pochin. 1950. Distribution of radioiodide in man. *Clin. Sci.* 9:405-419.
- Naidu, S. R., and P. Venkatrao. 1945. The toxicology of nitrites. *Calcutta Med. J.* 42:79-87.
- Oda, H., H. Tsubone, A. Suzuki, T. Ichinose, and K. Kubota. 1981. Alterations of nitrite and nitrate concentrations in the blood of mice exposed to nitrogen dioxide. *Environ. Res.* 25:294-301.
- Oeff, K., K. Krentz, and M. Kessel. 1955. [In German.] J¹³¹-Clearance der normalen und pathologischen Magenschleimhaut. *Klin. Wochenschr.* 33:59-63.
- Ohshima, H., and H. Bartsch. 1981. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. *Cancer Res.* 41:3658-3662.
- Oliver, T. H., and J. F. Wilkinson. 1933. Critical review: Achlorhydria. *Q. J. Med.* 2:431-462.
- Parks, N. J., K. A. Krohn, C. A. Mathis, J. H. Chasko, K. R. Geiger, M. E. Gregor, and N. F. Peek. 1981. Nitrogen-13-labeled nitrite and nitrate: Distribution and metabolism after intratracheal administration. *Science* 212:58-61.
- Payne, W. J. 1981. The status of nitric oxide and nitrous oxide as intermediates in denitrification. Pp. 85-104 in C. C. Delwiche, ed. *Denitrification, Nitrification, and Atmospheric Nitrous Oxide*. John Wiley and Sons, New York.
- Pegg, A. E. 1977. Formation and metabolism of alkylated nucleosides: Possible role in carcinogenesis by nitroso compounds and alkylating agents. *Adv. Cancer Res.* 25:195-269.
- Pegg, A. E. 1980. Metabolism of N-nitrosodimethylamine. Pp. 3-22 in R. Montesano, H. Bartsch, and L. Tomatis, eds. *Molecular and Cellular Aspects of Carcinogen Screening Tests*, IARC Scientific Publication No. 27. International Agency for Research on Cancer, Lyon, France.

- Pegg, A. E., and B. Balog. 1979. Formation and subsequent excision of O^6 -ethylguanine from DNA of rat liver following administration of diethylnitrosamine. *Cancer Res.* 39:5003-5009.
- Phillips, J. C., B. G. Lake, S. D. Gangolli, P. Grasso, and A. G. Lloyd. 1977. Effects of pyrazole and 3-amino-1,2,4 triazole on the metabolism and toxicity of dimethylnitrosamine in the rat. *J. Natl. Cancer Inst.* 58:629-633.
- Poirier, M. C. 1981. Antibodies to carcinogen-DNA adducts. *J. Natl. Cancer Inst.* 67:515-519.
- Poirier, M. C., and S. H. Yuspa. 1981. Quantitation of carcinogen-DNA modification by immunological techniques. *J. Supramol. Struct. Cell. Biochem. Suppl.* 5:171. Abstract 456.
- Pryor, W. A., and J. W. Lightsey. 1981. Mechanisms of nitrogen dioxide reactions: Initiation of lipid peroxidation and the production of nitrous acid. *Science* 214:435-437.
- Radomski, J. L., D. Greenwald, W. L. Hearn, N. L. Block, and F. M. Woods. 1978. Nitrosamine formation in bladder infections and its role in the etiology of bladder cancer. *J. Urol.* 120:48-50.
- Rao, G. S. 1980. Salivary nitrite and carcinogenic nitrosamine formation--report of research. *Dent. Abstr.* 25:228-231.
- Rath, M., and J. C. Krantz, Jr. 1942. Nitrites: VIII. Blood-nitrite content of man and other species. *J. Pharmacol. Exp. Ther.* 76:27-32.
- Rose, F. L. 1958. Discussion of paper, "Some experimental studies on toxic liver injury," by P. N. Magee. Pp. 116 in A. L. Walpole and A. Spinks, eds. *A Symposium on the Evaluation of Drug Toxicity.* J. and A. Churchill, London; Little, Brown and Company, Boston.
- Ruddell, W. S. J., A. T. R. Axon, J. M. Findlay, B. A. Bartholomew, and M. J. Hill. 1980. Effect of cimetidine on the gastric bacterial flora. *Lancet* 1:672-674.
- Ruegamer, W. R. 1963. The kinetics of I^{131} metabolism in the dog and human. *Arch. Biochem. Biophys.* 47:119-136.
- Sander, J. 1968. [In German; English summary.] Nitrosaminsynthese durch Bakterien. *Hoppe Seylers Z. Physiol. Chem.* 349:429-432.

- Sander, J., and G. Birkle. 1969. [In German; English summary.] [Induction of malignant tumors in rats by simultaneous feeding of nitrite and secondary amines.] *Z. Krebsforsch.* 73:54-66.
- Sander, J., and F. Schweinsberg. 1972. [In German; English summary.] [Interrelationships between nitrate, nitrite, and carcinogenic N-nitroso-compounds. 1. Communication: Nitrate, nitrite and nitrosable amino-compounds in food and drugs, chemistry of N-nitroso compounds.] *Zentralbl. Bakteriol. Parasitenkd. Infektionsk. Hyg. Abt. 1: Orig. Reihe B* 156:299-340.
- Sander, J., and F. Seif. 1969. [In German; English summary.] [Bacterial reduction of nitrate in the human stomach as a cause for nitrosamine formation.] *Arzneim. Forsch.* 19:1091-1093.
- Sander, J., F. Schweinsberg, and H.-P. Menz. 1968. [In German; English summary.] [Studies on the formation of carcinogenic nitrosamines in the stomach.] *Hoppe-Seylers Z. Physiol. Chem.* 349:1691-1697.
- Saul, R. L., S. H. Kabir, Z. Cohen, W. R. Bruce, and M. C. Archer. 1981. Reevaluation of nitrate and nitrite levels in the human intestine. *Cancer Res.* 41:2280-2283.
- Schiff, L., C. D. Stevens, W. E. Molle, H. Steinberg, C. W. Kumpe, and P. Stewart. 1947. Gastric (and salivary) excretion of radiiodine in man (preliminary report). *J. Natl. Cancer Inst.* 7:349-354.
- Schweinsberg, F., J. Sander, E. Schweinsberg, and P. Kollat. 1975. [In German; English summary.] Untersuchungen über die Nitrosierbarkeit von Nicotin und Nornicotin und über die Frage der Bildung von N-Nitrosornicotin im Magen von Rauchern. *Z. Krebsforsch.* 84:81-87.
- Selenka, F. 1970. [In German; English summary.] Entstehung und Abbau von Nitrit in nitrathaltiger Säuglingsnahrung. I. Mitteilung: Durch Bac. subtilis, E. coli, Ps. fluorescens und Staph. albus hervorgerufene Effekte. *Arch. Hyg.* 154:336-348.
- Singer, B. 1979. N-Nitroso alkylating agents: Formation and persistence of alkyl derivatives in mammalian nucleic acids as contributing factors in carcinogenesis. *J. Natl. Cancer Inst.* 62:1329-1339.
- Sleeth, C. K., and E. J. Van Liere. 1941. The effect of sodium nitrite on the emptying time of the normal human stomach. *Arch. Int. Pharmacodyn.* 45:5-8.
- Smith, J. E., and E. Beutler. 1966. Methemoglobin formation and reduction in man and various animal species. *Am. J. Physiol.* 210:347-350.

- Smith, P. L. R., C. L. Walters, and R. Walker. 1978. The transport of nitrite in the blood. *Biochem. Soc. Trans.* 6:665.
- Sollman, T. 1957. *A Manual of Pharmacology and its Applications to Therapeutics and Toxicology*, 8th ed. W. B. Saunders, Philadelphia, Pennsylvania. 1,535 pp.
- Spiegelhalder, B., G. Eisenbrand, and R. Preussmann. 1976. Influence of dietary nitrate on nitrite content of human saliva: Possible relevance to *in vivo* formation of N-nitroso compounds. *Food Cosmet. Toxicol.* 14:545-548.
- Stephany, R. W., and P. L. Schuller. 1980. Daily dietary intakes of nitrate, nitrite and volatile N-nitrosamines in the Netherlands using the duplicate portion sampling technique. *Oncology* 37:203-210.
- Stouthamer, A. H. 1976. Biochemistry and genetics of nitrate reductase in bacteria. *Adv. Microb. Physiol.* 14:315-375.
- Swann, P. F., and P. N. Magee. 1968. Nitrosamine-induced carcinogenesis. The alkylation of nucleic acids of the rat by N-methyl-N-nitroso-urea, dimethylnitrosamine, dimethyl sulphate and methyl methanesulphonate. *Biochem. J.* 110:39-47.
- Tannenbaum, S. R. 1979. Nitrate and nitrite: Origin in humans. *Science* 205:1333-1335.
- Tannenbaum, S. R. 1980. A model for estimation of human exposure to endogenous N-nitrosodimethylamine. *Oncology* 37:232-235.
- Tannenbaum, S. R., A. J. Sinskey, M. Weisman, and W. Bishop. 1974. Nitrite in human saliva. Its possible relationship to nitrosamine formation. *J. Natl. Cancer Inst.* 53:79-84.
- Tannenbaum, S. R., M. Weisman, and D. Fett. 1976. The effect of nitrate intake on nitrite formation in human saliva. *Food Cosmet. Toxicol.* 14:549-552.
- Tannenbaum, S. R., M. C. Archer, J. S. Wishnok, P. Correa, C. Cuello, and W. Haenszel. 1977. Nitrate and the etiology of gastric cancer. Pp. 1609-1625 in H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds. *Origins of Human Cancer, Book C: Human Risk Assessment*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Tannenbaum, S. R., D. Fett, V. R. Young, P. D. Land, and W. R. Bruce. 1978. Nitrite and nitrate are formed by endogenous synthesis in the human intestine. *Science* 200:1487-1489.
- Turek, B., D. Hlavsová, J. Tuček, J. Waldman, and J. Černá. 1980a. [In Czechoslovakian; English summary.] Hygienický význam dusičnanů v životním prostředí. *Cesk. Hyg.* 25:301-305.

- Turek, B., D. Hlavsová, J. Tuček, J. Waldman, and J. Černá. 1980b. The fate of nitrates and nitrites in the organism. Pp. 625-632 in E. A. Walker, M. Castegnaro, L. Gričiute, and M. Bőrzsönyi, eds. *N-Nitroso Compounds: Analysis, Formation and Occurrence*, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Van Stee, E. W., G. A. Boorman, and J. K. Haseman. 1980. Pulmonary adenomas in mice exposed to NO₂ by inhalation and morpholine by ingestion. *Pharmacologist* 22:158. Abstract 13.
- Vanzant, F. R., W. C. Alvarez, G. B. Eusterman, H. L. Dunn, and J. Berkson. 1932. The normal range of gastric acidity from youth to old age: An analysis of 3,746 records. *Arch. Int. Med.* 49:345-359.
- Wallis, R. L. M. 1913. On sulphaemoglobinaemia. *Quart. J. Med.* 7:73-91.
- Wang, T., T. Kakizoe, P. Dion, R. Furrer, A. J. Varghese, and W. R. Bruce. 1978. Volatile nitrosamines in normal human faeces. *Nature* 276:280-281.
- Wang, C. F., R. G. Cassens, and W. G. Hoekstra. 1981. Fate of ingested ¹⁵N-labelled nitrate and nitrite in the rat. *J. Food Sci.* 46:745-748.
- Whong, W.-Z., N. D. Speciner, and G. S. Edwards. 1979. Mutagenicity detection of *in vivo* nitrosation of dimethylamine by nitrite. *Environ. Mutagen.* 1:277-282.
- Wiessler, M., and D. Schmähl. 1976. [In German; English summary.] [On the carcinogenic action of N-nitroso-compounds. 5th Communication: Acetoxymethyl-methyl-nitrosamine.] *Z. Krebsforsch.* 85:47-49.
- Wilson, G. S., and A. A. Miles. 1964. The normal bacterial flora of the human body. Pp. 2461-2482 in Topley and Wilson's *Principles of Bacteriology and Immunity*, Vol. 2, 5th ed. Williams and Wilkins Co., Baltimore, Maryland.
- Witter, J. P., and E. Balish. 1979. Distribution and metabolism of ingested NO₃⁻ and NO₂⁻ in germfree and conventional-flora rats. *Appl. Environ. Microbiol.* 38:861-869.
- Witter, J. P., E. Balish, and S. J. Gatley. 1979a. Distribution of nitrogen-13 from labeled nitrate and nitrite in germfree and conventional-flora rats. *Appl. Environ. Microbiol.* 38:870-878.
- Witter, J. P., S. J. Gatley, and E. Balish. 1979b. Distribution of nitrogen-13 from labeled nitrate (¹³NO₃⁻) in humans and rats. *Science* 204:411-413.

- Witter, J. P., S. J. Gatley, and E. Balish. 1979c. Nitrate and nitrite: Origin in humans. *Science* 205:1335-1337.
- Witter, J. P., S. J. Gatley, and E. Balish. 1981. Evaluation of nitrate synthesis by intestinal microorganisms in vivo. *Science* 213:449-450.
- Yordy, D. M., and K. L. Ruoff. 1981. Dissimilatory nitrate reduction to ammonia. Pp. 171-190 in C. C. Delwiche, ed. *Denitrification, Nitrification, and Atmospheric Nitrous Oxide*. John Wiley and Sons, New York.

CHAPTER 9

ADVERSE EFFECTS OF NITRATE, NITRITE, AND N-NITROSO COMPOUNDS

| | <u>Page</u> |
|--|-------------|
| CARCINOGENICITY..... | 9-3 |
| Studies of Humans and Related Evidence from Laboratory Studies..... | 9-3 |
| Stomach Cancer..... | 9-3 |
| Esophageal Cancer..... | 9-17 |
| Nasopharyngeal Cancer..... | 9-19 |
| Liver Cancer..... | 9-20 |
| Bladder Cancer..... | 9-20 |
| Occupational Studies..... | 9-21 |
| Studies in Animals..... | 9-22 |
| Nitrate..... | 9-22 |
| Nitrite..... | 9-22 |
| N-Nitroso Compounds..... | 9-27 |
| Summary and Discussion: Carcinogenesis..... | 9-41 |
| MUTAGENICITY..... | 9-43 |
| Nitrate and Nitrite..... | 9-43 |
| N-Nitroso Compounds..... | 9-44 |
| Summary and Discussion: Mutagenesis..... | 9-49 |
| OTHER EFFECTS..... | 9-50 |
| Toxic Effects in Humans: Nitrate and Nitrite..... | 9-50 |
| Toxic Effects in Humans: N-Nitroso Compounds..... | 9-54 |
| Toxic Effects in Other Species: Nitrate and Nitrite..... | 9-54 |
| Toxic Effects in Other Species: N-Nitroso Compounds..... | 9-56 |
| Summary: Effects Other Than Carcinogenicity or Mutagenicity..... | 9-58 |
| OVERALL SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS..... | 9-58 |
| REFERENCES..... | 9-61 |

CHAPTER 9

ADVERSE EFFECTS OF NITRATE, NITRITE, AND N-NITROSO COMPOUNDS

Exposure to nitrate, nitrite, and N-nitroso compounds has been implicated in the causation of a variety of diseases in humans and in other species. The first section of this chapter examines studies concerning the role of these compounds in the causation of cancer. This is followed by a discussion of the data on their mutagenicity in bacterial and mammalian systems. The third section reviews reports of other toxic effects following exposure to these agents. After weighing the evidence pertaining to the adverse effects of these compounds, the committee developed specific recommendations, which appear at the end of this chapter.

CARCINOGENICITY

An evaluation of the data from studies of human populations is followed by a review of studies on laboratory animals. Where possible, deficiencies in the data are noted in the discussion.

Studies of Humans and Related Evidence from Laboratory Studies

Research on the role of nitrate, nitrite, and/or N-nitroso compounds in the induction of cancer in humans has focused mainly on hypotheses that cancers of the stomach, esophagus, and nasopharynx are attributable to exposure to these compounds. To provide some perspective on the limited data obtained from epidemiological research, discussions of studies in humans are augmented by descriptions of results obtained in pertinent laboratory studies.

Stomach Cancer

Some investigators have hypothesized that stomach cancer can be attributed to exposure to N-nitroso compounds (see Chapter 8), especially nitrosamides (e.g., nitrosoureas), which are formed endogenously by the reaction of nitrosatable substrates and nitrite (Correa et al., 1975a; Haenszel and Correa, 1975; Mirvish, 1971, 1977; Weisburger, 1979; Weisburger and Raineri, 1975). The committee believes that this hypothesis is plausible for four reasons:

- Nitrosamides can be produced in vitro from nitrite and amides (e.g., methylurea) and other precursors (e.g., methylguanidine) under acidic conditions (Mirvish, 1971, 1975).

- Since nitrosamides are chemically unstable, direct-acting carcinogens, they could lead to cancer in the human stomach if produced there.

- Because of their instability, nitrosamides may not persist in foods, but some of their precursors do persist and could participate in nitrosation reactions following ingestion.

- The feeding of nitrosoureas and nitrosocarbamates to rodents has produced a low incidence of gastric adenocarcinoma, which resembles stomach cancer in humans (Druckrey and Landschütz, 1971; Druckrey *et al.*, 1968, 1970, 1971; Maekawa *et al.*, 1976; Ogiu *et al.*, 1975). Moreover, a related compound, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), is the strongest known carcinogen for stomach cancer in rodents and dogs (Sugimura and Kawachi, 1973, 1978).

Nitrate is converted to nitrite in the mouth and the achlorhydric stomach (see Chapter 8); hence, it could lead to the formation of N-nitroso compounds in the stomach. Laboratory studies have provided support for this mechanism of stomach cancer induction (Franklin and Skoryna, 1971; Ishiwata *et al.*, 1975) and can be compared to attempts to correlate the incidence of stomach cancer with exposure to high levels of nitrate in humans.

Modan *et al.* (1974) reported a correlation between the consumption of high-starch, low-protein diets and a high incidence of stomach cancer in humans. These observations correspond to the findings of Mirvish *et al.* (1980b), who studied the formation of nitrosomethylurea in the stomach of rats fed diets containing methylurea and nitrite. When a low-protein diet was fed to these animals, the concentration of nitrosomethylurea in the stomach was almost 4 times greater than when a high-protein diet was used. The investigators suggested that the protein buffered the stomach contents, thereby causing the observed increase in pH and, hence, inhibiting the nitrosation.

The importance of an elevated gastric pH in nitrosation reactions is not yet clear because nitrosation may be enhanced by two mechanisms--one at low pH and the other at high pH. For example, low gastric pH would favor the production of nitrosamides because chemical nitrosation of amides increases tenfold for each unit drop in pH (Mirvish, 1975; Mirvish *et al.*, 1980b). On the other hand, a high gastric pH (achlorhydria) could also favor the production of N-nitroso compounds because this condition allows the growth of bacteria that reduce nitrate to nitrite, thereby providing an increased level of nitrosating species (Cuello *et al.*, 1976; Ruddell *et al.*, 1976).

Perhaps both views are valid but apply at different stages of gastric carcinogenesis. Thus, an initially low pH may favor the production of nitrosamides, which cause the development of precancerous

lesions. Nitrosation may then continue if the nitrite level is sufficiently high to counterbalance the inhibitory effect of high pH on nitrosation. The resultant nitrosamides may cause the precancerous lesions to develop into cancer. Alternatively, nitrosation might occur when the nitrite is transferred from a part of the stomach with a high pH to one with a low pH; or nitrite might be formed at a high pH at one time of the day, and this could cause nitrosation later when acid is secreted.

Studies with the drug cimetidine may eventually shed some light on the role of pH in nitrosation reactions. Cimetidine is a cyano-guanidine compound that can be nitrosated to give mutagenic N-nitroso compounds, which are similar in structure to MNNG (Bavin et al., 1980; Foster et al., 1980; Ichinotsubo et al., 1981). It is used to reduce gastric acidity in the treatment of peptic ulcers. Apparently, nitrite accumulates in the stomachs of ulcer patients treated with cimetidine (Ruddell, 1981).

The importance of the accumulation of nitrite in the stomach has been investigated. Stemmerman et al. (1980) detected direct-acting mutagens in mucosa removed from the human stomach during surgery. The mutagens were later identified as the N-nitroso derivatives of three drugs (hydroxyzine hydrochloride, diazepam, and cimetidine) that had been administered to the patients (Rice et al., 1981; Stemmerman et al., 1981). Methylation of DNA by nitrosocimetidine has been observed in vivo and in vitro (Gombar et al., 1981; Jensen and Magee, 1980). Additional studies are being conducted to determine whether nitrite can produce large amounts of nitrosocimetidine in the gastric contents (despite the relatively high pH) and whether nitrosocimetidine is carcinogenic in the stomach (Elder et al., 1979; Guslandi, 1979; Hawker et al., 1980; Mullen, 1979; Reed et al., 1979; Ruddell, 1981; Taylor et al., 1979). In one preliminary report, nitrosocimetidine did not cause tumors in laboratory animals (Preussmann, personal communication).

Table 9-1 lists the age-adjusted death rate per 100,000 population for stomach cancer in males and in females and various estimates of ingested nitrate and nitrite ions for the same populations (see footnotes b-e for exceptions). To obtain these ingestion data, investigators used a variety of methods, ranging from assays of prepared meals to estimates based on food consumption tables plus published estimates of the nitrate and nitrite content of foods. Moreover, the assays have been performed as much as a decade apart and by different methodologies in different countries. This table also does not consider the lag period in the induction of cancer and the likelihood that determinants for stomach cancer exert their effect at an early age. Nevertheless, the data indicate that nitrate and nitrite ingestion tend to be higher in countries where gastric cancer is more prevalent. Although suggestive, more precise and complete data for additional countries, especially for populations at very high and very low risk of gastric cancer, are necessary to establish a definite association between ingestion of these ions and induction of gastric cancer.

TABLE 9-1

Relationship between Mortality from Stomach Cancer and Ingestion of Nitrate and Nitrite in 11 Countries

| Country | Stomach Cancer (Age-Adjusted Death Rate per 100,000 Population) ^a | | Estimates of Consumption Ranges and (Averages), mg/Person/Day | | References |
|-----------------------------------|--|--------|--|---|---|
| | Male | Female | Nitrate | Nitrite | |
| Japan | 56.6 | 29.0 | (297) (385) 380 - 490 44 - 864 (218) (388) ^c (271) ^c | (1.5) 0.7 - 10 — — <0.1 - 1.3 — — | Kawabata <i>et al.</i> , 1979 Harada <i>et al.</i> , 1975 Ishidate, 1977, cited in Endo <i>et al.</i> , 1977 Ishiwata <i>et al.</i> , 1978 Maruyama <i>et al.</i> , 1979 Maruyama <i>et al.</i> , 1979 |
| Czechoslovakia | 33.3 | 16.6 | (142) | — | Turek <i>et al.</i> , 1980 |
| Federal Republic of Germany | 27.1 | 14.1 | (75) ^d (49) ^e | (3.3) (1.7) ^e | Selenka and Brand-Griem, 1976 Selenka and Brand-Griem, 1976 |
| Yugoslavia | 24.0 | 11.5 | (156) ^f | (6.5) ^f | Adsmovic, 1979 |
| Netherlands | 21.4 | 9.7 | (110) | (2.8) | Stephany and Schuller, 1980 |
| United Kingdom | 19.7 | 9.0 | (58) ^g (115) ^h | — — | Ashton, 1970 Walker, 1975 |
| Switzerland | 18.1 | 10.2 | (91) | — | Tremp, 1980 |
| Norway | 17.4 | 9.8 | (32) ⁱ (48) | (1.1) ^h (0.11) | Gislason and Dahle, 1980 Høyem, 1974 |
| Sweden | 16.3 | 8.3 | (150) 24 - 68 (42) 110 - 190 | 0.5 - 5 0.9 - 7.3 (3.7) 2 - 10 | Sandberg, 1978, cited in Slorach, 1981 Jägerstad and Nilsson, 1976, Jägerstad <i>et al.</i> , 1976 Boström and Tammelin, 1981 |
| Denmark | 14.8 | 8.1 | (54) | (0.74) | Statens Lægemiddelinstitut |
| United States | 7.2 | 3.7 | (75) | (0.78) | Tables 5-20 and 5-21 |

^aFrom American Cancer Society, 1980.^bResidents of Tokyo.^cResidents of Nagano Prefecture.^dIn an area with nitrate-free drinking water.^eCalculated from food consumption statistics.^fResidents of Serbia.^gIncludes only meat products, water, and vegetables (excluding potatoes).^hExcludes contributions from cereals, fruits, and dairy produce, and assumes maximal estimates for nitrate contents of meat products and water.ⁱIncomplete tabulation; vegetables are listed as the only significant source of nitrate and cured meats and potatoes as the sources of nitrite.

The following paragraphs describe in greater detail the epidemiological studies of stomach cancer conducted in various countries throughout the world.

Japan. Japan has the highest reported incidence of stomach cancer in the world. This high rate has been associated with the consumption of salted dried fish products known to contain high levels of certain secondary amines (Singer and Lijinsky, 1976) and other precursors of N-nitroso compounds (see below). Salt has been suggested as a promoting factor for this disease (Haenszel and Correa, 1975; Joosens and Geboers, 1981; Sato et al., 1959) and has been demonstrated to be a cocarcinogen in test animals (Capoferro and Torgersen, 1974; Kinoshita, 1969; Tatematsu et al., 1976). Japanese who immigrate to Hawaii continue to be at high risk for stomach cancer, but the risk is substantially lower for the next generation (Nisei) (Haenszel et al., 1972).

Epidemiological studies of these immigrants indicate an association between a high incidence of stomach cancer and the consumption of salted dried fish and salt-pickled vegetables (Haenszel et al., 1973). In Japan, this association was not confirmed, but investigators reported an elevated risk for stomach cancer among Japanese farmers who consume both types of food more frequently than do other population groups (Haenszel et al., 1976b). Other important sources of N-nitroso compound precursors were not ruled out. For example, Japanese farmers, more often than other occupational groups in that country, drink well water that could contain elevated levels of nitrate. They also consume fewer vitamin-C-containing fruits and vegetables. Vitamin C intake has been hypothesized to be inversely correlated with the incidence of gastric cancer because of its inhibitory effect on nitrosation reactions (Chapters 4 and 8). Kolonel et al. (1979) have also reported a negative association between fresh fruit consumption and gastric cancer in a prospective study conducted in Hawaii, which included Japanese immigrants.

In contrast to the studies linking stomach cancer to exposure to nitrate or nitrite, there is no evidence linking large bowel cancer to such exposures (Haenszel et al., 1973). Table 9-2 summarizes the major results and conclusions of epidemiological studies of gastrointestinal cancer among the Japanese.

Laboratory studies lend some support to the suggestion that salted dried fish plays a role in the etiology of gastric cancer. For example, treatment of a Japanese dried bonito fish product with excess nitrite and then with acid ("nitrosation-dinitrosation") produced 25 mg of methylurea per kilogram. The carcinogen nitrosomethylurea was formed after treatment with excess nitrite alone (Mirvish et al., 1980a). The methylurea precursor was identified as creatinine (Mirvish and Cairnes, 1981), which is derived from creatine when the fish is dried (Kawabata et al., 1979). A Japanese mackerel fish

TABLE 9-2

Gastrointestinal Cancer Among Japanese

| <u>Subject of Study</u> | <u>Methods</u> | <u>Results and Interpretation</u> | <u>References</u> |
|---|--|--|--------------------------------|
| Stomach cancer among Japanese in Hawaii | Interviews of 220 Japanese stomach cancer patients and 440 matched controls | Immigrants (Issei) continued to be at high risk, but risks declined in their children (Nisei). Association with pickled vegetables and dried salted fish; inverse association with vegetables, especially tomatoes, celery, and corn. Dried fish said to contain high concentrations of secondary amines. | Haenszel <u>et al.</u> , 1973 |
| Pathology of gastric carcinoma in the Japanese population; comparisons between Miyagi prefecture, Japan, and Hawaii | Pathology review of 407 cases from Japan and 256 from Hawaii | Incidence of diffuse carcinoma was similar; incidence of intestinal, mixed, and other types was lower in Japan. | Correa <u>et al.</u> , 1973 |
| Large-bowel cancer in Hawaiian Japanese | Interviews of 179 Japanese colorectal cancer patients and 357 matched hospital controls | Unlike stomach cancer, results were similar for immigrants and their children. Bowel cancer patients ate meat, legumes, and starches more frequently. Relative risk was as high or higher for meats containing little or no nitrate, e.g., preserved pork products, and there was no association with dried and salted fish rich in nitrate and nitrite. | Haenszel <u>et al.</u> , 1973 |
| Stomach cancer in Japan | Interviews of 783 stomach cancer patients and 1,566 hospital controls from Hiroshima and Miyagi prefectures in Japan | Elevated risk among farmers. Hawaiian Japanese finding of increased salted dried fish and salt-pickled vegetable consumption not reproduced, but these foods were more widely used by farmers. Farmers also used well water and ate less lettuce and celery. | Haenszel <u>et al.</u> , 1976b |

product treated with excess nitrite at pH 3 was found to contain a direct-acting mutagen, which was not nitrosomethylurea (Marquardt et al., 1977a; Mower and Weisburger, 1978). The mutagen has not yet been identified. When the same product was fed to 12 rats, it produced glandular stomach tumors in five of the animals (Weisburger et al., 1980).

Mutagens are also produced in similarly treated beans and beets, which are eaten in Colombia and Chile. Both of these countries also have high rates of gastric cancer (see below) (Marquardt et al., 1977a,b).

Colombia. Colombia has one of the highest rates of mortality from stomach cancer, especially among persons living at high altitudes in certain rural areas (Correa et al., 1975a). Cuello et al. (1976) reported a fourfold geographic variation in the incidence of gastric cancer within this country. Correa et al. (1970, 1975a, 1976) have shown geographic correlations of stomach cancer incidence not only with nitrate content of well water but also with the prevalence of atrophic gastritis and, to some extent, with the nitrate in urine and saliva. In carefully conducted studies involving gastroscopy of volunteers, they found three-fourths of the high-risk population to have superficial gastritis or other dysplasias. In contrast, a much lower frequency was found in persons from the lower risk areas of Colombia.

Correa and his colleagues have suggested that superficial gastritis, atrophic gastritis, and intestinal metaplasia are precursor lesions for gastric cancer. They described in detail the histopathology of the gastric lesions, beginning with the normal mucosa and progressing through various degrees of dysplasia to stomach cancer. Analyses of gastric fluid of individuals with atrophic gastritis indicated that nitrite levels were elevated in patients whose gastric pH was above 5 (Correa et al., 1979). Since nitrosation reactions could occur more readily at higher nitrite concentrations, the formation of N-nitroso compounds may be a key factor in the development of gastric cancer. Data from case-control studies of persons with dysplasia indicate that a history of a high corn diet is associated with dysplasia but ingestion of lettuce, which contains ascorbic acid (vitamin C), is inversely associated. Additional data supporting the role of N-nitroso compounds in gastric cancer were reported by Montes et al. (1979), who detected a direct-acting mutagen, possibly a nitrosamide, in the gastric juice of fasting subjects from an area with a high incidence of gastric cancer, and by Hawksworth et al. (1975), who observed concentrations of nitrate as high as 146 mg/liter in water supplies serving three areas of Colombia with high rates of stomach cancer.

Table 9-3 summarizes the major results and conclusions of epidemiological studies of gastric cancer conducted in Colombia.

Chile. Chile has natural deposits of nitrate, and for decades has used large amounts of these compounds in fertilizers. Gastric cancer mortality in 1960, 1962, and 1964 was statistically correlated with the use of nitrate fertilizers from 1960 to 1964 for each Chilean province (Zaldívar, 1977; Zaldívar and Robinson, 1973; Zaldívar and Wetterstrand, 1975). An association was found between mortality from stomach cancer and the proportion of farmers using nitrate-containing fertilizers in each province; in contrast, there was no association with miners. This association remained significant when data from the industrialized provinces were excluded in order to eliminate the interference of occupational or industrial factors.

A more detailed study was conducted by Armijo and Coulson (1975) from 1957 to 1972. These investigators found no association between stomach cancer and exposure to nitrate in drinking water, but they noted that the levels of nitrate throughout Chile were well below 100 mg/liter -- the 1972 maximum acceptable level established by the World Health Organization (Armijo and Coulson, 1975). However, they did report a high correlation between the gastric cancer mortality in each province and the estimated per capita exposure to nitrogen from fertilizers.

Armijo et al. (1981b) interviewed 389 gastric cancer patients at the gastroenterology clinics of seven Santiago hospitals and 845 controls. They found that the patients had resided in high-risk areas during their early lives for longer periods than had the controls. Gastric cancer was also found to be associated with previous occupation in agriculture.

Table 9-4 summarizes the major results and conclusions of the epidemiological studies of gastric cancer in Chile. The committee, however, has some reservations about these data, particularly because inadequate attention was directed toward the latent period in cancer induction and, in one study (Cuello et al., 1976), the validity of the sampling methods used to measure urinary nitrate is doubtful.

England. In an epidemiological study conducted in England, Hill et al. (1973) correlated differences in stomach cancer mortality rates with the nitrate content of drinking water in two towns. They found that the town with the higher level of nitrate (average, 90 mg/liter) had the higher mortality from this cancer. The ratios of observed to expected gastric cancer cases were 1.3 for males and 1.9 for females. Hill and coworkers, however, did not consider socioeconomic status, which is known to be inversely correlated with the risk for developing gastric cancer (Haenszel, 1967). Davies (1980) suggested that the higher stomach cancer mortality was not related to nitrate intake from drinking water but, rather, to the lower socioeconomic status of the inhabitants of the town, which was a mining community.

TABLE 9-3

Gastric Cancer in Colombia

| <u>Subject of Study</u> | <u>Methods</u> | <u>Results and Interpretation</u> | <u>References</u> |
|--|--|--|------------------------------|
| Histological types of gastric carcinoma in high- and low-risk areas | Study of 322 cases from Colombia and Mexico City. | Two histological types: "intestinal" (glandular) and "diffuse" (undifferentiated). Intestinal more common in high risk areas (52% vs 36% in lower risk areas) and may follow precancerous lesions. | Munõz <u>et al.</u> , 1968 |
| Carcinoma and intestinal metaplasia of the stomach in Colombian migrants | Cali registry incidence rates were correlated with intestinal metaplasia in natives vs immigrants. | Intestinal metaplasia correlated with gastric cancer incidence. Authors hypothesized that premalignant change in young predispose to later cancer. | Correa <u>et al.</u> , 1970 |
| A model for gastric cancer epidemiology | Hypothesis based on other studies. | Hypothesis that gastric atrophy and intestinal metaplasia may result from abrasives or irritants in diet (e.g., hard grains, salt, surfactants). Increased pH allows bacterial growth, which increases reduction of nitrate to nitrite. Nitroso compounds formed are mutagenic-carcinogenic. Water supplies in high risk towns reported to contain high concentrations of nitrate. | Correa <u>et al.</u> , 1975b |
| Developments in the epidemiology of stomach cancer over the past decade | Review. | Early life exposure critical for stomach cancer. Reference to two histological types, precursor lesions, and nitroso compounds as candidate carcinogens. Epidemiological features summarized. | Haenszel and Correa, 1975 |

TABLE 9-3 continued

| Subject of Study | Methods | Results and Interpretation | References |
|---|--|---|--------------------------------|
| The cancer registry of Cali, Colombia--epidemiological studies of gastric cancer | Description of registry. Study (in progress) uses questionnaire, chemical tests, gastroscopy, and gastric biopsy of charity hospital patients, and correlations with nitrate in drinking water. | Colombia has a high stomach cancer mortality rate. Immigrants from rural, high-altitude areas of Colombia have highest rates. Intestinal metaplasia and atrophic gastritis are also common in this group. Water in areas with highest stomach cancer rates has high nitrate content. | Correa <u>et al.</u> , 1975a |
| Gastric cancer in Colombia. I. Cancer risk and suspect environmental agents | Case-control study in Narino, Colombia, including 276 stomach cancer patients and 276 matched controls from four hospitals. Also, 463 gastroscopies of healthy persons, and 173 water sources were tested for nitrate. Urine and saliva were tested for nitrate and nitrite. | Fourfold geographic variation in stomach cancer shown by case-control studies. Geographic correlation with chronic atrophic gastritis and intestinal metaplasia, nitrate content of well water, and nitrate in urine and saliva. Nitrate consumption by stomach cancer patients and controls not studied. | Cuello <u>et al.</u> , 1976 |
| Gastric cancer in Colombia. II. Case-control epidemiological study of precursor lesions | Epidemiological questionnaire sent to 463 volunteers who had gastroscopy. | Atrophic gastritis and intestinal metaplasia were associated with history of corn ingestion and were inversely associated with lettuce. | Haenszel <u>et al.</u> , 1976a |
| Gastric cancer in Colombia. III. Natural history of precursor lesions | Subjects included 586 volunteers from areas of high and low risk for stomach cancer who had gastroscopy. A mathematical model related transition of "precursor lesions" to risk level of population. | Seventy-five percent of high-risk population has superficial gastritis or more superficial gastritis or more advanced "precursor" by age 25 years, in contrast to low-risk population. | Correa <u>et al.</u> , 1976 |

TABLE 9-3 continued

| <u>Subject of Study</u> | <u>Methods</u> | <u>Results and Interpretation</u> | <u>References</u> |
|---|--|---|---------------------------------|
| Spermidine nitrosation and gastric cancer | Mutagenesis of nitrite plus spermidine tested. | Spermidine nitrosation yields mutagen most active at pH 3.5-6.0, the pH present with hypochlorhydria from "precursor" lesions. Ascorbic acid inhibited mutagenesis. | Correa <u>et al.</u> , 1978 |
| Gastric cancer in Colombia. IV. Nitrite and other ions in gastric contents of residents from a high-risk region | Gastric fluid of persons with "precursor lesions" tested for pH, nitrite, nitrate, thiocyanate, and chlorides. | Above pH 5, nitrite was correlated with nitrate. This is compatible with bacterial reduction of nitrate in persons with "precursor" lesions. | Tannenbaum <u>et al.</u> , 1979 |
| The gastric micro-environment in populations at high risk of stomach cancer | Similar to those for study described immediately above. | Possible role of N-nitroso compounds discussed. | Correa <u>et al.</u> , 1979 |
| Histopathology of gastric dysplasia correlated with gastric juice chemistry | Pathology review of biopsy materials. | Gastric dysplasias described. Correlations with gastric juice nitrite noted. | Cuello <u>et al.</u> , 1979 |
| Nitrate and etiology of gastric cancer | Review and discussion. | Two gastric cancer models involving nitrite discussed--one in normal acidic stomach, the other in achlorhydria. | Tannenbaum <u>et al.</u> , 1977 |

TABLE 9-4

Gastric Cancer in Chile

| <u>Subject of Study</u> | <u>Methods</u> | <u>Results and Interpretation</u> | <u>Reference</u> |
|---|--|--|---------------------------------|
| Epidemiological investigation of stomach cancer mortality in Chileans and its association with nitrate fertilizer | Stepwise multiple regression analysis of gastric cancer mortality in 1960 in association with tons of fertilizer used per province, rainfall, and latitude during 1960-1961. | Mortality significantly associated with fertilizer use. Stomach cancer deaths had borderline association with proportion of farmers in each province but no association with proportion of miners. | Zaldívar and Robinson, 1973 |
| Further evidence of a positive correlation between exposure to sodium and potassium nitrate fertilizers and gastric cancer death rates attributed to nitrite and nitrosamines | Same as above. Repeated using 1960, 1962, and 1964 deaths. | Further association of gastric cancer deaths and use of nitrate fertilizers by province. | Zaldívar and Wetterstrand, 1975 |
| Nitrate fertilizers as environmental pollutants; positive correlation between sodium and potassium nitrate used per unit area and stomach cancer mortality rate | Same as above. Repeated using 1960-1964 nitrate use by province; reanalysis excluding industrialized provinces. | Association still significant. | Zaldívar, 1977 |

TABLE 9-4 continued

| Subject of Study | Methods | Results and Interpretation | References |
|---|---|--|------------------------------|
| Epidemiology of stomach cancer in Chile: The role of nitrogen-containing fertilizers | Statistical correlations of gastric cancer mortality in 1957-1972 in association with nitrate in drinking water and nitrogen fertilizers used per province. | No association with nitrate in drinking water, which contains much less than WHO maximum acceptable level. High correlation between estimated per capita nitrogen exposure and gastric cancer. | Armijo and Coulson, 1975 |
| Epidemiology of gastric cancer in Chile. I. Case-control study | Study of 389 gastric cancer cases from gastroenterology clinics of seven Santiago hospitals and 845 controls interviewed, 1977-1978. | Cases had long-term residence in high-risk areas in early life; controls had long-term residence in low-risk areas. Association found between stomach cancer and previous occupation in agriculture. | Armijo <u>et al.</u> , 1981b |
| Epidemiology of gastric cancer in Chile. II. Nitrate exposures and stomach cancer frequency | Nitrate levels in urine and nitrite in saliva determined in 231 11- to 13-year-old school-children in two areas with high and two with low stomach cancer mortality in Chile. Levels of nitrate and nitrite in selected vegetables were also tested in high and low rate areas. | Children in low mortality areas had higher urinary nitrate. Salivary nitrite was similar in high and low mortality areas. No overall correlation was seen between nitrate levels in vegetables and mortality rates for stomach cancer. | Armijo <u>et al.</u> , 1981a |

United States. In the United States, gastric cancer was the commonest form of cancer in males during the 1920's and 1930's. Although the incidence rate of this form of cancer has dropped by two-thirds since then, there are still many cases reported each year. The American Cancer Society (1980) has estimated that 23,000 new cases can be expected in 1981. Weisburger and Raineri (1975) speculated that the decline in the incidence of stomach cancer in the United States is due to the introduction of refrigeration, which slows down bacterial contamination of food and, hence, inhibits the production of nitrite from nitrate. Other possible contributing factors could include the increase in the year-round consumption of fruits and vegetables (which contain protective factors such as antioxidants), and the decreased use of varying levels of nitrite to preserve products in the home in favor of better regulated, industry-prepared products. These suggestions, although interesting and supportive of the hypothesis that nitrate, nitrite and N-nitroso compounds may be causative agents in gastric cancer, have not been investigated to determine their validity. One possible exception is the finding of an inverse correlation between consumption of vitamin C-containing fruits and vegetables and the risk of developing gastric cancer (Bjelke, 1978).

Geleperin et al. (1976) compared cancer mortality rates in three Illinois communities whose water supplies contained different levels of nitrate and found no significant correlations. However, few details about the cancer patients or the communities were provided in this report. Higginson (1966) compared diets of 93 stomach cancer patients to controls. Stomach cancer patients had a history of a slightly higher consumption of pork, ham, sausage, and bacon and a lower consumption of dairy products and fresh fruits. However, the differences between the patients and the controls were not statistically significant. Bjelke (1978) also reported a negative association between consumption of fresh fruits containing vitamin C and gastric cancer patients in Norway and the north-central region of the United States.

The Netherlands. Meinsma (1964) interviewed 340 stomach cancer patients and a comparable group of other hospital patients in the Netherlands. The stomach cancer patients of both sexes ate bacon more frequently than did their controls, but the bacon consumption of female patients was lower than that of the male controls. Meinsma also noted that the intake of citrus fruits by cases was lower than that of controls.

In a laboratory study to investigate the possibility that stomach cancer is associated with bacon consumption, Mirvish et al. (1980a) observed that methylurea was produced when fried (but not raw) bacon had been treated with nitrite and then exposed to strong acid. The methylurea probably arose from creatinine, which occurs at high levels in fried bacon (Mirvish and Cairnes, 1981).

China. Stomach cancer is also common in various regions of China; however, few epidemiological results have been reported. Armstrong (1980) reported that people in regions of China where stomach cancer is common tend to eat small amounts of fresh fruits and vegetables, meat, or eggs, but do consume large amounts of salted and pickled vegetables and sweet potatoes.

Esophageal Cancer

Esophageal cancer is especially common in parts of Iran, China, the USSR, and South Africa. Epidemiological studies conducted in several of these high incidence areas are described below.

Iran. The high incidence of esophageal cancer in the Caspian Littoral of Iran has been studied by a Joint Iran-International Agency for Research on Cancer Study Group (1977). Villages with different rates of esophageal cancer were surveyed for dietary, work, and personal habits. Their main foodstuffs were analyzed for volatile nitrosamines, nitrate, nitrite, and other compounds, and their drinking water was tested for nitrate and nitrite.

The average daily intake of nitrate and nitrite was not significantly different for high- and low-incidence areas. For example, a comparison of the nitrate and nitrite content of water showed no elevation in high-risk areas. In contrast to these findings concerning nitrate and nitrite in water, Eisenbrand *et al.* (1980) stated that there were intermittently high levels of nitrite in the saliva of children in the high-incidence area of Iran, especially on hot days when water intake may not have been sufficient.

The study group also found that in the regions where esophageal cancer was most common, the diet was limited largely to bread and tea, and was low in vitamin C, vitamin A, and riboflavin. In a subsequent case-control study in this region, Cook-Mozaffari *et al.* (1979) also found lower intakes of uncooked vegetables (as well as fruits) in cases compared with controls. However, no association was found between the intake of preformed volatile nitrosamines and high-risk of esophageal cancer (Joint Iran-International Agency for Research on Cancer Study Group, 1977). The results of this investigation did not implicate nitrate, nitrite, or N-nitroso compounds, but indicated that the high esophageal cancer risk in the Caspian Littoral of Iran "arises from the severely limited and probably irritant nature of the diet, in conjunction with exposure to a carcinogenic agent derived either from the opium tars or from wheat contaminants."

Table 9-5 summarizes the major results and conclusions of the epidemiological studies of esophageal cancer in Iran.

TABLE 9-5

Esophageal Cancer in Iran

| <u>Subject of Study</u> | <u>Methods</u> | <u>Results and Interpretations</u> | <u>References</u> |
|--|---|---|--|
| Esophageal cancer studies in the Caspian Littoral of Iran; results of population studies | Villages with varying esophageal cancer incidence were sampled. A census was taken; households surveyed about diet, work, and personal habits; medical exams given to a subsample; main foodstuffs analyzed for volatile nitrosamines, nitrate, nitrite, and other chemicals; and water tested for nitrite and nitrate. | Average daily intakes of nitrate and nitrite were similar for high- and low-incidence areas. Preformed volatile nitrosamines did not appear to be important. The diet in the highest incidence area was markedly restricted to bread and tea and was low in vitamin C, vitamin A, and riboflavin. The investigators tentatively concluded that the high esophageal cancer risk "arises from the severely limited and probably irritant nature of the diet, in conjunction with exposure to a carcinogenic agent derived either from the opium tars or from wheat contaminants." | Joint Iran-International Agency for Research on Cancer Study Group, 1977 |
| Esophageal cancer studies in the Caspian Littoral of Iran; results of a case-control study | Epidemiological interviews of 344 cases (of 638 registered) and matched controls. | Strong association with low socioeconomic status and low intake of fruits and vegetables. | Cook-Mozaffari <u>et al.</u> , 1979 |

China. Chinese scientists reported a multifaceted study of esophageal cancer in their country (Li *et al.*, 1980; Yang, 1980), and suggested that nitrosamines are likely to be the causative agents. The incidence of esophageal cancer is highest in Lin-Xien county in Henan province, where there is also a high prevalence of esophageal dysplasia. The occurrence of squamous cell carcinoma in the gullet (esophagus) of domestic chickens in the same area is intriguing. The diet and gastric juice of noncancer patients have been found to contain secondary amines, nitrate, nitrite, and nitrosamines. In laboratory experiments, treatment of a fungus-infected corn bread (eaten in a high-incidence area) with nitrite produced nitrosamines, which may be esophageal carcinogens in rats and possibly in humans (Chuan *et al.*, in press; Li *et al.*, 1980). The diet of this population includes nitrite-rich pickled vegetables and is low in riboflavin and ascorbic acid. The Chinese have begun a preventive effort, discouraging the use of pickled vegetables and fungus-infected corn bread and encouraging the consumption of foods containing ascorbic acid.

The diets of populations in areas with a high incidence of esophageal cancer mortality are similar but not identical to those with high mortality rates from stomach cancer (see section on stomach cancer, above).

United States. The American Cancer Society (1980) has estimated that there will be 8,800 new cases of esophageal cancer in the United States during 1981. There has been no significant change in the rate of this type of cancer in recent years. However, the incidence is rising among the black population.

Esophageal cancer in the United States has been associated with the use of tobacco, which can contain tobacco-specific nitrosamines in concentrations as high as 88 mg/kg. Cancer at this site could be attributable to exposure to carcinogenic tobacco-specific nitrosamines, especially nitrosonornicotine (NNN), which induces esophageal cancer in rats when administered in the drinking water (Hoffmann *et al.*, in press). Cigarette smoke contains other carcinogens that could be involved as well. Furthermore, Wynder and Bross (1961) have suggested that alcohol may promote the carcinogenic effect of tobacco at this site. Additional evidence for the role of nitrosamines in esophageal cancer has been reported by Mettlin *et al.* (1980), who computed indices for the intake of vitamins A and C in a study of male esophageal cancer patients and controls and found an inverse correlation between intake of these vitamins, especially vitamin C, and cancer risk.

Nasopharyngeal Cancer

Nasopharyngeal cancer has been associated with exposure to N-nitroso compounds or their precursors. Some nitrosamines

[e.g., nitrosodimethylamine (NDMA) and nitrosodiethylamine (NDEA) at low doses (Druckrey *et al.*, 1967) and nitrosomethylamylamine (Bulay and Mirvish, 1979) at higher doses] induce tumors of the nasal cavity in rats.

Nasopharyngeal cancer is rare in the United States, but is common among the Chinese in parts of Southeast Asia, including Hong Kong and Singapore. It may be relevant that NDMA has been detected in salted fish from Hong Kong, although the levels were only 1-35 $\mu\text{g}/\text{kg}$ (Huang *et al.*, 1978a). When tested in animals, the same fish product induced tumors of the nasal cavity in 4 of 20 rats (Huang *et al.*, 1978b). Other factors in addition to N-nitroso compounds may also be important in the etiology of this disease in humans, including infection with the Epstein-Barr virus (EBV) (Zur Hausen, 1976).

Liver Cancer

In China, the geographic pattern of liver cancer incidence is different from that for cancers of the stomach or esophagus. High levels of nitrate and nitrite in the soil reportedly correlate with increased liver cancer mortality, and nitrosamines have been detected in salted vegetables, which are commonly eaten in areas with high rates of liver cancer (Armstrong, 1980). However, other agents such as aflatoxin and hepatitis B virus have also been implicated as etiologic agents in this disease (Armstrong, 1980).

Bladder Cancer

Some investigators have reported that volatile nitrosamines are present in the urine of healthy volunteers (El-Merzabani *et al.*, 1979; Hicks *et al.*, 1977; Kakizoe *et al.*, 1979); however, when all the necessary controls for artifact formation are implemented, e.g., the use of marker nitrosamines, excess vitamin C, etc. (see Chapter 7), volatile nitrosamines are not found in analytically significant amounts in the urine of healthy volunteers (Archer, personal communication; Eisenbrand *et al.*, 1981; Tannenbaum, 1981).

Dimethylamine is the principal secondary amine in urine and can be present at levels of approximately 0.5 mM (Asatoor and Simenhoff, 1965). Brooks *et al.* (1972) and Radomski *et al.* (1978) have reported that high levels of NDMA are present in the urine of patients with urinary tract infections. Although the analytical methods used by both groups of investigators did not include appropriate detection procedures or controls for artifacts, the reported concentration of NDMA was so high that artifact formation may not have been especially relevant. In addition, certain nitrosamines such as nitrosodi-N-butylamine are bladder carcinogens in rats and mice (Druckrey *et al.*, 1964). Thus, the speculation that bladder cancer might be associated with urinary tract infection is plausible, but needs to be studied by using more precise analytical methods.

In an epidemiological study by Wynder et al. (1963), 6% of a group of men with bladder cancer had a history of cystitis -- a significantly higher percentage than that of the controls. However, in the general population, bladder cancer predominates in men, whereas urinary tract infections are more common in women. Howe et al. (1980) reported an increased risk of bladder and kidney cancer in persons with a history of bladder infections and kidney stones. Although it is plausible that the formation of N-nitroso compounds in the infected bladder is related to the occurrence of cancer, further epidemiological studies are needed to determine the validity of this hypothesis.

Occupational Studies

In certain occupations, workers are exposed to airborne N-nitroso compounds. The highest exposures have been reported for leather tanners, who are exposed to NDMA (Fine, 1980a,b; Rounbehler et al., 1979), and rubber-industry workers, who are exposed to N-nitrosomorpholine (NMOR) and NDMA (Fajen et al., 1979; Preussmann et al., 1980). Several epidemiological studies have been conducted to examine the incidence of cancer among rubber-industry workers.

Monson and Nakano (1976) reported increases in certain cancers among rubber-industry workers in specific jobs: cancer of the stomach and large intestine in processers, lung cancer in tire curers, brain tumors and lymphatic cancer in tire builders, and bladder cancer and leukemia in all workers. In other studies of these workers, Mancuso et al. (1968) reported an increase in brain tumors, while McMichael et al. (1974) found high mortality ratios for leukemia and cancer of the stomach and prostate, and low ratios for cancer of the bladder, lung, brain, and pancreas. The data from these epidemiological studies are not very consistent, and the exposure to N-nitroso compounds was not specifically examined. Moreover, comparisons of exposures may be misleading because the purity of chemicals used in different factories within the same country and in different countries varies greatly.

Some other occupational groups that might be exposed to nitrosamines include foundry workers (Egan et al., 1979), agricultural workers who use pesticides (Bontoyan et al., 1979), workers in chemical plants manufacturing amines, and, perhaps, brewery workers (Walker et al., 1979). Although no studies of nitrate and/or nitrite exposures of workers have been reported, butchers and meat cutters have been shown to have a high incidence of prostate cancers (Registrar General's for England and Wales, 1978).

Studies in Animals

This section reviews the results of studies conducted in animals to investigate the carcinogenicity of nitrate, nitrite, or N-nitroso compounds.

Nitrate

The few experiments conducted in animals have provided no evidence that nitrate is carcinogenic. Greenblatt and Mirvish (1973) gave sodium nitrate in distilled water (12,300 mg/liter) to 40 Strain A mice as a substitute for drinking water. An equal number of mice served as controls for this and other experiments. The animals were treated for 25 weeks, and the experiment was terminated 13 weeks later. The number of lung tumors in treated and control mice was similar. Lijinsky et al. (1973) administered 40 mg of sodium nitrate daily in drinking water to each of 15 male and 15 female MRC-derived rats for 84 weeks and terminated the experiment 20 weeks later. An increase in pituitary adenomas was observed in treated female rats, but this was not statistically significant. There were no other tumors that could be associated with the treatment. Sugiyama et al. (1979) reported another experiment in which there was no significant difference in the incidence of tumors among the controls and ICR mice fed 50,000 and 25,000 mg of sodium nitrate per kilogram of diet over a lifetime.

Nitrite

There have been very few adequate studies to test the carcinogenicity of nitrite in animals and, until recently, most of the information came from data on tumor incidence in control animals administered nitrite alone in experiments that were designed to study the carcinogenic effects resulting from the simultaneous administration of nitrite and an amine.

In one recent study, which was designed to test the carcinogenic effect of nitrite, Mirvish et al. (1980a) reported that papillomas of the forestomach developed in 8 of 45 MRC Wistar rats (of both sexes) given a 3,000 mg/liter solution of sodium nitrite in distilled drinking water for 5 days/week for life (more than 100 weeks). Two of 91 untreated rats developed these tumors. This significant increase in the yield of benign tumors was produced by the maximum tolerated dose of nitrite. The 2-day-per-week rest period enabled the rats to recover from gross methemoglobinemia.

Aoyagi et al. (1980) induced benign liver tumors in two groups of 24 male Wistar rats given 800 mg and 1,600 mg sodium nitrite per kilogram of food for 646 days (92 weeks). An equal number of animals

was given a basal diet without sodium nitrite. Although none of the untreated rats developed liver tumors, one rat fed the lower dose developed a benign liver tumor and five rats receiving the higher dose developed three benign and two malignant liver tumors. Both the benign and malignant liver tumors were derived either from parenchymal or hemangioendothelial cells. However, because NDMA and nitrosopyrrolidine were found in the diet containing sodium nitrite, the authors concluded that these N-nitroso compounds were probably the principal cause of the liver tumors in this experiment.

Inai et al. (1979) fed sodium nitrite at 5,000, 2,500, and 1,000 mg/liter in drinking water to groups of 50 male and 50 female ICR mice for 18 months. No tumors attributable to nitrite treatment were observed. In a cocarcinogenesis assay of morpholine plus sodium nitrite, Shank and Newberne (1976) reported that control Sprague-Dawley rats fed sodium nitrite at a level of 1,000 mg/kg in the diet led to a 27% incidence of tumors of the lymphoreticular system compared to approximately 6% in untreated control rats.

In another, larger lifetime study conducted for the Food and Drug Administration (FDA), Newberne (1978, 1979) administered sodium nitrite to groups of approximately 68 male and 68 female Sprague-Dawley rats under a variety of conditions. Groups 1 to 5 received 0, 250, 500, 1,000, or 2,000 mg/kg sodium nitrite in the diet, and groups 6 and 7 received 1,000 or 2,000 mg/liter in drinking water. For these groups, an agar-based semisynthetic diet was used. For groups 9 to 11, a commercial chow diet was substituted, and sodium nitrite concentrations of 0, 1,000, or 2,000 mg/kg diet were fed to the animals. Groups 13 and 14 were given a refined casein diet containing nitrite at 0 or 1,000 mg/kg, while another two groups, 15 and 16, were fed the original semisynthetic diet containing nitrite at 0 or 1,000 mg/kg. Each of the latter two groups contained only 34 animals -- the dams that supplied the pups for groups 1 and 4. Groups 17 and 18 were also fed the semisynthetic diet containing nitrite at 0 or 1,000 mg/kg. Groups 1 through 16 were exposed prenatally, while groups 17 and 18 were exposed at 21 days. Groups 8 and 12 served as positive controls and received urethane (2,000 mg/liter) in drinking water or the semisynthetic diet, respectively. The rats survived the sodium nitrite regimens well, the only adverse effects being a loss of weight in groups receiving 2,000 mg/kg in their diet and, to a lesser extent, in groups receiving 2,000 mg/liter in drinking water.

Newberne's histopathologic assessment of the tissues indicated that by considering all the groups receiving sodium nitrite together, there was a statistically significant excess of lymphoid tumors ($p < 0.01$, based on chi-square analysis). This was reflected especially in the groups receiving sodium nitrite in drinking water, where the excess of lymphoid tumors was statistically significant, but the results were not significant in the other groups treated with sodium nitrite.

In addition to malignant tumors of the lymphatic system, Newberne (1979) observed an alteration referred to as immunoblastic cell proliferation in the spleen and, occasionally, in the lymph nodes of some members of all groups except the positive controls (groups 8 and 12). The incidence of this abnormality in nitrite-treated animals, however, was greater (11.2%) than in the untreated animals (7%). The disease in humans, which is histologically similar to that observed in rats, is considered by some to develop into lymphoma; others consider it not to be preneoplastic.

Newberne interpreted these results to indicate that nitrite is an enhancer or promoter of carcinogenesis in rats.

After Newberne's report was submitted, a Government Interagency Working Group on Nitrite Research reviewed a sample of histological slides from that study and decided that there was sufficient difference of opinion in the diagnoses to warrant a further evaluation of the histopathological findings. The Universities Associated for Research and Education in Pathology (UAREP), a nonprofit consortium of 15 universities organized to carry out educational and research activities in pathology, was selected by the FDA to review the slides (Food and Drug Administration, 1980a). A Joint Committee of Experts, which was established by the UAREP to perform this review, diagnosed fewer lymphomas than Newberne had reported (Food and Drug Administration, 1980a). The disparity between the two series of diagnoses involved the differentiation of lymphomas from extramedullary hematopoiesis, plasmacytosis, or histiocytic sarcoma. Furthermore, the committee was unable to confirm Newberne's diagnosis of immunoblastic hyperplasia.

In its final report to the FDA, the Government Interagency Working Group summarized its assessment of the UAREP committee's findings as follows:

"The major result of the histopathology review was that most of the lymphoma diagnoses originally reported were not confirmed. A relatively high incidence of lymphomas had been reported by Dr. Newberne, with a significant increased incidence in the total combined treated groups as compared to combined controls. The UAREP pathologists, on the other hand, diagnosed very few lesions as lymphoma, with a resulting reduction of incidence to approximately 1 percent among treated and control groups. This rate of lymphoma incidence is similar to that usually seen spontaneously in Sprague-Dawley rats.

"UAREP pathologists did report a greater than 1% incidence of other types of tumors, including histiocytic sarcomas, angiosarcomas, liver neoplasms, ear duct tumors, pancreatic tumors, pituitary tumors, and mammary tumors. However, after statistical analysis and careful review by the IAWG, no demonstration could be found that the increased incidences of these tumors were induced by the ingestion of sodium nitrite." (Food and Drug Administration, 1980b, pp. 28-29)

The committee has reviewed the data in 21 additional studies identified by Birdsall (1981) in which the carcinogenicity of nitrite could be examined. In the committee's view, three of the 21 reports were too brief for an adequate evaluation (Olsen and Meyer, 1977; Pearson et al., 1980; Procter and Rona, 1977). Of the remaining 18 studies, nine were conducted in rats, eight in mice, and one in guinea pigs. The experimental designs of these studies varied greatly, and the end points for carcinogenicity in some of them were specific lesions. For example, the incidence of pulmonary adenomas was the carcinogenic "end point" in four studies.

In two relatively short-term studies (7.5 to 12 months), nitrite was administered to pregnant Swiss mice intragastrically (10 mg/kg body weight) each day during the last week of pregnancy (Börzsönyi et al., 1978) or in drinking water (500 mg/liter during the entire pregnancy (Börzsönyi et al., 1976). In a third study, nitrite was given to rats in drinking water (100 mg/kg body weight) for three generations (Druckrey et al., 1962b). In a fourth study, nitrite was injected subcutaneously (12 mg per mouse over 90 days), and the experiment was concluded at 11 months (Nakahara and Fukuoka, 1959).

In another four experiments, nitrite was fed in doses of 1,600 or 2,000 mg/kg, and the experiments were terminated at 16 (Matsukura et al., 1977), 29 (Lijinsky and Reuber, 1980; Van Logten et al., 1972), or 32 (Taylor and Lijinsky, 1975) months. In 10 studies, nitrite was added to the drinking water for periods varying from 7.5 (Greenblatt and Mirvish, 1973) to 30 months (Greenblatt et al., 1973; Sen et al., 1975). In five of these, the observation period was longer than 24 months (Garcia and Lijinsky, 1973; Greenblatt et al., 1973; Lijinsky et al., 1973, 1980; Sen et al., 1975). None of these studies indicated that nitrite was carcinogenic; however, many of them were not designed to test nitrite, and some of them do not meet accepted standards for the adequate assessment of the carcinogenicity of chemicals (Interagency Regulatory Liaison Group, 1979).

The absence of evidence that nitrite is a direct carcinogen does not diminish the importance of the possibility that nitrite may interact with specific dietary components or endogenous metabolites to produce N-nitroso compounds that induce cancer. The initial reports on this subject suggested that ingested nitrite could react with secondary amines in the acidic conditions of the stomach to produce nitrosamines that induce tumors (Sander, 1970; Sander and Bürkle, 1969). Three main lines of research have developed since these first studies were reported. First, many experiments in animals were performed to confirm and amplify this novel observation (Greenblatt and Mirvish, 1973; Greenblatt *et al.*, 1971; Mirvish *et al.*, 1972a; Shank and Newberne, 1976). Second, the kinetics of nitrosation were intensively studied to determine the rate of carcinogen formation for different reactants. Third, attempts were made to measure the amounts of N-nitroso compounds formed under defined conditions (Mirvish, 1975; Mirvish and Chu, 1973; Mirvish *et al.*, 1973, 1980b).

The second and third areas of research are particularly important since many entities are able to react with nitrite: some of them react readily, while others react very slowly (Chapter 4), and only some of these reactions will lead to the formation of carcinogenic N-nitroso compounds and the subsequent development of tumors. In one study, for example, Greenblatt and Mirvish (1973) reported that the kinetics of *in vivo* nitrosation of piperazine, as evidenced by tumor formation, closely resembled those occurring *in vitro*. They reported that the incidence of lung tumors in Strain A mice exposed for 5 to 6 months was directly related to the concentration of piperazine and to the square of the nitrite concentration.

Because the tumorigenic effect is very dependent on the dose of nitrite and nitrosatable substrate, caution must be exercised when using nitrosation data from laboratory experiments to predict the response of humans exposed to nitrite and nitrosatable agents. Nitrosation experiments designed to produce a measurable incidence of tumors are generally carried out with very high levels of nitrite and nitrosatable agents. With the possible exception of exposure to nitrosatable drugs, humans are generally exposed to much lower levels of these agents. Since the concentrations of both nitrite and nitrosatable substances are related to the final concentration of the N-nitroso compound formed, extrapolation of this information probably means that the carcinogenic response in the human population is likely to be less than that observed in experiments on laboratory animals.

For example, according to chemical kinetic equations, lowering the nitrite concentration 100-fold, from 10,000 mg/liter to 100 mg/liter, and lowering concentrations of the nitrosatable agent by an equal factor will lower the concentration of the N-nitroso compound a millionfold for the uncatalyzed nitrosation of secondary amines and ten-thousandfold for the catalyzed reaction for the formation of nitrosamides.

In light of the above considerations, the potential for tumor induction by nitrite is probably best evaluated by considering the carcinogenicity of N-nitroso compounds that may be formed in the environment (Chapter 7) or endogenously (Chapter 8) from the reaction of nitrite or nitrogen oxides with nitrosatable substances.

N-Nitroso Compounds

Chronic Exposure Experiments. Shortly after NDMA was introduced as a novel industrial solvent, severe and, on one occasion, fatal liver damage occurred in humans exposed to the compound. A laboratory investigation of this agent (Barnes and Magee, 1954) confirmed the clinical observations. The investigators found that subchronic treatment with NDMA led to changes in the liver of rats similar to those produced by known liver carcinogens. Carcinogenicity tests on NDMA in rats (Magee and Barnes, 1956, 1959) demonstrated that chronic administration of the chemical for 42 or 102-104 weeks at low levels (10, 20, 50 mg/kg diet) led to liver cancer, whereas shorter exposures (1 to 4 weeks) at higher doses (100-200 mg/kg diet) induced kidney tumors.

Following this initial demonstration, numerous carcinogenicity studies on variety of N-nitroso compounds have been reported. Druckrey and his colleagues (1967) exposed rats to 65 N-nitroso compounds, most of which were potent carcinogens. Lijinsky and Reuber (1981) examined many other N-nitroso compounds for their carcinogenic potential, mainly in rats. Of the approximately 300 N-nitroso compounds tested to date, 85% of the 209 nitrosamines and 92% of the 86 nitrosamides have been shown to induce cancer in laboratory animals (Preussmann and Stewart, personal communication, 1981). Schmähl et al. (1978) reported in a review that NDEA was carcinogenic in 20 species of animals. Among the species in which N-nitroso compounds have been shown to be carcinogenic are rats, mice, guinea pigs, rabbits, dogs, monkeys, grass parakeets, and pigs (Schmähl and Osswald, 1967), hamsters (Dontenwill and Mohr, 1961), hedgehogs (Graw et al., 1974), mink (Koppang and Rimeslätten, 1976), and trout (Halver et al., 1962). N-Nitroso compounds have induced tumors in all species tested to date.

Some of the N-nitroso compounds shown to be carcinogenic in animals have been found in various environments to which humans are exposed (Chapter 7). In addition to NDMA and NDEA, these compounds include: nitrosodiphenylamine (NDPhA) (Cardy et al., 1979), NMOR (Bannasch and Müller, 1964), nitrosodiethanolamine (NDELA) (Druckrey et al., 1967; Lijinsky et al., 1980; Preussmann et al., in press), nitrosopyrrolidine (NPYR) (Druckrey et al., 1967; Greenblatt and Lijinsky, 1972b), nitrosodi-n-propylamine (NDPA) (Druckrey et al., 1967), nitrosodi-n-butylamine (NDBA) (Druckrey et al., 1962a, 1967; Mohr et al., 1970; Takayama and Imaizumi, 1969), NNN (Hoffmann et al., 1975; Singer and Taylor, 1976), 4-(N-methyl-N-nitrosamine-1-(3-pyridyl)-1-butanone (NNK) (Hoffmann et al., in press), nitroso-n-dodecylmethylamine (NDOMA) (Althoff and Lijinsky, 1977; Lijinsky and

Taylor, 1975), and nitrosopiperidine (NPIP) (Druckrey et al., 1967; Takayama, 1969). (References given for each compound provide carcinogenicity data; See Chapter 7 for discussion of the distribution and concentrations of these nitrosamines in various environmental media.)

N-Nitroso compounds that are not carcinogenic include the nitroso derivatives of some amino acids (Druckrey et al., 1967; Greenblatt and Lijinsky, 1972a; Mirvish et al., 1980a) and certain nitrosamines that do not contain one or more alpha hydrogen atoms on the carbon next to the N-NO group (Druckrey et al., 1967).

One of the important conclusions reached in the many studies of carcinogenic N-nitroso compounds is that different compounds have the ability to induce tumors of specific tissues. Under certain conditions, NDMA and NDEA were carcinogenic in the liver of rats. NDEA, even in low doses, was also carcinogenic in the lungs and/or esophagus of rats and hamsters. NDBA and certain metabolic derivatives were bladder carcinogens in rats and mice (Druckrey et al., 1964; Wood et al., 1970). Unsymmetrical nitrosodialkylamines, such as nitrosomethylamylamine, induced esophageal cancer in rats (Bulay and Mirvish, 1979; Druckrey et al., 1967; Mirvish et al., 1978), whereas MNNG in rats provides a standard animal model for the induction of gastric cancer (Saito and Sugimura, 1973). Nitrosoethylurea, especially when administered transplacentally to rats, led to tumors of the brain and spinal cord (Ivankovic and Preussmann, 1970), whereas peripheral nervous system tumors resulted in hamsters (Rustia and Shubik, 1974). More recently, a group of nitrosamines analogous to, or converted metabolically to, nitrosobis(2-oxopropyl)amine (BOP) or nitrosomethyl-2-oxopropylamine (MOP) were shown to produce ductular cell carcinomas of the pancreas in hamsters (Pour et al., 1974, 1978); however, in other species, such as rats, guinea pigs, or mice, tumors only occurred at sites other than the pancreas.

Thus far, determinations of structure-activity relationships have applied to partial structures that may lead to cancer at selected sites. In fact, because most N-nitroso compounds are carcinogenic, the amount of useful information on structure-activity relationships that may be obtained is limited unless these relationships are derived from quantitative measures of carcinogenicity.

Dose-Response Studies and Carcinogenic Potency. Druckrey (1967) determined the relationship of time to death and tumor incidence as a function of dose for NDEA in BD II rats given a series of daily doses ranging from 0.075 to 14.2 mg/kg body weight (Table 9-6). At each of seven dose levels, ranging from 0.3 to 14.2 mg/kg body weight/day, liver carcinomas were induced in every surviving rat, but the time to death ("induction time") decreased from 457 to 68 days with increased dose. At the two lowest doses (0.15 and 0.075 mg/kg body

TABLE 9-6

Average Total Dose and Average Induction Time Dependent on Daily Dosage in NDEA-Induced Carcinogenesis in BD II Rats^a

| <u>Daily Dosage, mg/kg Body Weight</u> | <u>Yield of Carcinomas/ No. of Survivors</u> | <u>Average Total Dose, mg/kg Body Weight</u> | <u>Average Induction Time, Days</u> |
|--|--|--|---|
| 14.2 | 5/5 | 1,000 | 68 |
| 9.6 | 25/25 | 963 | 101 |
| 4.8 | 25/25 | 660 | 137 |
| 2.4 | 34/34 | 460 | 192 |
| 1.2 | 36/36 | 285 | 238 |
| 0.6 | 49/49 | 213 | 355 |
| 0.3 | 67/67 | 137 | 457 |
| 0.15 | 27/30 | 91 | 609 |
| 0.075 | 5/7 | 64 | 840 |

^aData adapted from Druckrey, 1967, and Preussmann, 1978.

weight/day), the tumor yield was less than 100% and the induction times were longer. The investigators suggested that these data fit the expression:

$$dt^n = \text{constant},$$

where d is the daily dose, t is the average time to tumor, and n is a constant, which was 2.3 for NDEA and varied between 1.2 and 4.7 for other carcinogens.

Several factors limit the ability to draw definite conclusions from these data. The lowest tumor yield was 71% (five rats with tumors of seven that survived long enough to develop tumors), and there can be no certainty that extrapolation from tumor incidence at high doses to lower doses will be meaningful. Time-to-death rather than time-to-tumor is used, implying either that the time for the induced liver tumors to kill the rats is short or is a relatively constant fraction of the induction time. Such assumptions may or may not be valid, and can only be assessed by experiments involving serial sacrifices. The number of surviving rats, especially those in the highest and lowest dose groups (five and seven, respectively), is insufficient. The importance of this study is that at a particular dose of the tumorigen, time-to-tumor occurrence, as well as the number of rats with tumors, are clearly shown to be of critical importance in quantifying the effects of a carcinogen.

It is unlikely that all of this information will be experimentally determined in animal models for every N-nitroso compound. Hence, there is a need for a simple expression of carcinogenic potency that can be derived from the simplest protocol used in a carcinogenicity study.

Clayson (in press) has described an expression that may give an approximate indication of the potency of an N-nitroso compound:

$$\text{Potency} = 7 - \log d_{E50},$$

where d_{E50} is that dose in $\mu\text{mol/kg}$ body weight per week that will give a 50% tumor yield in a lifetime study, and 7 is an arbitrary constant that ensures all values are positive. Using this expression and appropriate approximations, it is possible to obtain an indication of the potency of any chemical that has been demonstrated to be carcinogenic below the 100% tumor incidence level (Table 9-7). These methods have been used to determine potency values for the N-nitroso compounds shown in Table 9-8. To develop these values, the committee used data reported in the literature in which the incidence of tumors was as close to 50% as possible in order to avoid unnecessary extrapolation. Where necessary, the following assumptions were made:

• Tumor yield and survival. In most carcinogenicity tests, the survival time is less than the normal lifespan of the test animal and the tumor probability differs from 0.50. To correct for this, we assume $d_{E50} = d_x \cdot \frac{0.50}{P_x} \cdot \frac{S_x}{S_L}$, where d_{E50} has the same meaning as in the potency expression, P_x is the probability of obtaining a tumor in an animal at dose rate d_x , S_x is the survival at dose rate d_x , and S_L is the expected lifespan of the animal. These assumptions are approximations that should be acceptable if (1) tumor probability and survival do not differ too greatly from 0.50 and S_L , respectively, and (2) potency is calculated in log units. For example, Druckrey (1967) suggested that for a carcinogen, dt^n is a constant, where d = dose rate and t is time to tumor occurrence. It is assumed here that $n = 1$, although Druckrey *et al.* (1963) indicated that it may be higher for specific chemicals.

• Dose rate. When a dose of carcinogen was administered for 60% or more of the survival period of the animals, it was assumed that this represented the actual dose rate for the entire experiment.

The approximate potency values shown in Table 9-8 demonstrate that N-nitroso compounds have a wide range of carcinogenic activity differing by more than three orders of magnitude -- from NDEA (6.4) (Druckrey *et al.*, 1963) to NDPhA (3.2) (Cardy *et al.*, 1979) and nitrosobis(2-ethoxyethyl)amine (2.7) (Lijinsky and Taylor, 1978).

TABLE 9-7

The Potency of a Range of Chemicals that are Carcinogenic in Rats or Mice Following Continuous Feeding^a

| <u>Chemical</u> | <u>Species (Tumor Site)</u> | <u>Potency (Log Units)</u> |
|----------------------|-----------------------------|----------------------------|
| Aflatoxin | Rat (liver) | 9.2 |
| NDEA | Rat (liver) | 6.5 |
| Michler's Ketone | Rat (liver) | 4.6 |
| Carbon tetrachloride | Rat (liver) | 3.9 |
| 2-Aminoanthraquinone | Rat (liver) | 4.4 |
| Trichloroethylene | Mouse (liver) | 2.1 |
| Saccharin | Rat (bladder) | 1.9 |

^aAdapted from a table in Clayson (in press), based on data from selected experiments reported in U.S. Public Health Service, 1951-1978.

TABLE 9-8

Approximate Potency Value for Specific N-Nitroso Compounds^a

| <u>Agent</u> | <u>Species</u> | <u>Route</u> | <u>Target Tissue</u> | <u>Potency (Log Units)</u> |
|----------------------------------|----------------|--------------|----------------------|----------------------------|
| NDEA | Rat | Oral | Liver | 6.4 |
| NDMA | Rat | Oral | Liver | 5.7 |
| Nitroso-1,1-diethyl-3-methylurea | Rat | Oral | Nervous System | 4.4 |
| Nitrosotriethylurea | Rat | Oral | Nervous System | 4.1 |
| Dimethylnitramine | Rat | Oral | Liver | 3.2 |
| NDPhA | Rat | Oral | Bladder | 3.2 |
| Nitrosobis(2-ethoxyethyl)amine | Rat | Oral | Liver | 2.7 |

^aPotency was calculated by the committee, based on data from U.S. Public Health Service, 1951-1978.

Table 9-9 shows the variation in the potency of one nitrosamine, NDEA, in six different species. The range is approximately a thousandfold.

The potency values presented in Tables 9-7, 9-8, and 9-9 for a comparison of relative carcinogenicity are crude measures and depend only on one point, the 50th percentile, on the dose-response curve. Other measures of potency depending on lower percentiles or the shape and slope of the dose-response curve may prove to be more relevant if carcinogenicity experiments that have more extensive dose-response curves in the low-dose range are performed. However, the measures for potency given in these tables are useful to illustrate the wide variability in the potency of various carcinogens, especially the N-nitroso compounds.

Potency values are useful in assessing the relative carcinogenicity of various N-nitroso compounds; however, when considering the in vivo formation of N-nitroso compounds, the ease of nitrosation and concentration of the amino substrate will also be important factors. For example, the kinetic rate constants may vary by up to five orders of magnitude for

TABLE 9-9

Carcinogenic Potency of NDEA in Several Species After Continuous Administration^a

| <u>Species</u> | <u>Stock</u> | <u>Route</u> | <u>Estimated Lifespan, Months</u> | <u>Tissue of Origin of Tumors</u> | <u>Potency Log Units</u> |
|---|---------------|-------------------|-----------------------------------|-----------------------------------|--------------------------|
| Rat (<u>Rattus norvegicus</u>) | BD II | Oral | 24 | Liver | 6.5 |
| White-tailed rat (<u>Mystromys albicaudatus</u>) | -- | Oral | 72 | Liver | 6.3 |
| Chicken (<u>Gallus domesticus</u>) | White Leghorn | i.m. ^b | 100 | Liver | 5.3 |
| Guinea pig (<u>Cavia procellus</u>) | Hybrid | Oral | 84 | Liver | 4.2 |
| Mouse (<u>Mus musculus</u>) | NMRI | Oral | 18 | Liver | 4.0 |
| Syrian golden hamster (<u>Mesocricetus auratus</u>) | RF | Oral | 18 | Liver | 3.9 |
| | -- | Oral | 18 | Trachea | 3.7 |

^aPotency was calculated by the committee based on data from U.S. Public Health Service, 1951-1978.

^bi.m. - intramuscular.

different N-nitroso compounds (Chapter 4; Mirvish, 1975). This difference, combined with a three orders of magnitude range of potency for the carcinogenicity of these compounds, suggests that certain agents that are readily nitrosated to form more potent carcinogenic N-nitroso compounds (amines or amides) may be of much greater importance in the production of cancer by nitrite than are other agents. It also offers the possibility of determining which nitrosatable agents may be most important in the induction of cancer, even when they are present in small quantities. In addition, information on the relative potency of N-nitroso compounds may aid in studies of chemical structure and carcinogenic activity.

Single Dose Exposure. A single application of certain N-nitroso compounds to adult animals may induce cancer. For example, nitrosomethylurea administered to rats in a single oral dose of 90 mg/kg body weight induced cancers in the kidney, stomach (squamous cell carcinoma), small and large intestine, skin, and jaw (Leaver *et al.*, 1969); NP1P and NMOR led to tracheal and laryngeal cancer in Syrian golden hamsters after a single subcutaneous injection (Althoff *et al.*, 1974); and NDEA resulted in adenomas and carcinomas in the kidneys of rats after a single intravenous injection (Mohr and Hilfrich, 1972).

Induction of kidney tumors by single doses of NDMA has been studied intensively by Hard and his colleagues (Hard, 1979; Hard and Butler, 1970). Magee and Barnes (1962) clearly showed that limited exposure of rats to NDMA led to a high incidence of renal adenomas and carcinomas. The amount of carcinogen that could be applied was limited by liver toxicity, which led to the early death of the rats. However, if the rats were fed a very low ("zero") protein diet for up to 10 days before the single intraperitoneal injection of carcinogen was administered, toxicity in the liver was reduced and higher doses of NDMA could be administered. Under these conditions, a 100% incidence of renal neoplasms was consistently observed (McLean and Magee, 1970; McLean and Verschuuren, 1969; Swann and McLean, 1971).

There is a relative paucity of dose-response data on the effects of single doses of N-nitroso compounds. Mohr and Hilfrich (1972) reported that single doses of NDEA given to female rats at 1.25 mg/kg body weight induced renal adenomas in 10% of the animals. Syrian golden hamsters given a single dose of NDEA in a study by Ii *et al.* (1979) showed a linear dose-response between 10 and 1.25 mg/kg body weight. Pour *et al.* (1980) noted that a single injection of nitrosobis(2-oxypropyl)amine at 2.5 mg/kg body weight induced a marked incidence of pancreatic carcinoma in Syrian golden hamsters.

Vesselinovitch *et al.* (1979) reported that a single dose of NDEA at 1.5, 5, and 10 mg/kg body weight injected into 1- or 15-day-old (C57BL/6J X C3HeB/FeJ) F_1 mice induced metastasizing liver tumors. They observed a dose-dependent tumor response to this compound. The highest dose administered to 1- and 15-day-old mice resulted in a 96% and 100% tumor incidence, respectively. Five mg/kg resulted in a 64% and 74% incidence, and 1.5 mg/kg in a 25% and 54% incidence, respectively.

Transplacental Carcinogenesis. The developing organism, i.e., the fetus and neonate, differs from the adult in many ways, several of which are important in the chemical induction of cancer. These differences include metabolic capability, cell proliferation and cell types in certain tissues, hormonal balance, and immunological capacity. The metabolic capability of fetal tissues is generally lower, and often different, than tissues in the adult. In rats, for example, the liver changes from a hematopoietic tissue to its more normal function in the third to the fifth day of extrauterine life. Exposure to trace levels of toxicants such as tetrachlorodibenzo-dioxin (TCDD) during fetal life appears to affect certain metabolizing enzymes for long periods after exposure (Hook *et al.*, 1975; Lucier *et al.*, 1977). At certain stages of development, the nervous system, the liver, and the bladder undergo rapid cell division in contrast to the absence, or very low levels, of cell division later in life. Also, because the immune system does not fully develop until after birth, the fetus and the neonate depend on maternal antibodies for immunologic protection.

The differences in metabolic capability are especially important when considering transplacental carcinogenesis of nitrosamines. Nitrosamides, nitrosoureas, nitrosocarbamates, and nitrosoguanidines generally decompose spontaneously to their ultimate carcinogenic form (Miller and Miller, 1976). Nitrosamines require metabolism, which, it is believed, consists of hydroxylation to an α -hydroxynitrosamine (Chapter 8). The product then decomposes to a carcinogenic electrophile.

The pattern of response of the developing organism to chemical carcinogens is therefore different from that of adults (Tomatis, 1979). The first observation supporting this conclusion was made by Druckrey *et al.* (1966), who administered nitrosoethylurea at 80 mg/kg body weight to three female BD IX rats on day 15 of pregnancy. Five rats from one litter survived for 160 or more days and developed, or showed signs of, intracranial tumors. In a second experiment, Ivankovic and Druckrey (1968) demonstrated that nitrosoethylurea administered to pregnant Sprague-Dawley rats at concentrations of 1 to 50 mg/kg body weight led to the development of brain and spinal cord tumors in 16% to 100% of the offspring. These tumors were also induced in adults by nitrosoethylurea, but, on a dose/body weight basis, adults required 50 times the dose used in utero.

The transplacental effect of nitrosoethylurea has been investigated in other species. In SWR and AKR mice, lung tumors and leukemia developed in less than 10 weeks (Diwan and Meier, 1974). In rabbits given nitrosoethylurea at 60 mg/kg body weight, 14 of 15 animals developed kidney tumors after a mean latency period of 3.5 months, a very short time to tumor occurrence in this species (Fox *et al.*, 1975). In Syrian golden hamsters treated with ethylurea and sodium nitrite (which react in the acidic conditions in the stomach to give nitrosoethylurea), Rustia and Shubik (1974) observed tumors of the peripheral nervous system instead of the tumors of the central nervous system induced in rats. These were more prevalent and had a shorter latency period in females than in males, thereby providing a useful model for neurofibromatosis (von Recklinghausen's disease) in humans.

Other nitrosamides and nitrosooureas given transplacentally to rats or mice have proved to be carcinogenic. These include nitroso-methylurea (Napalkov, 1973; Tomatis *et al.*, 1975), nitrosoethylbiuret (Druckrey and Landschütz, 1971), and nitrosomethylurethane (Tanaka, 1973). Each of these well-designed experiments demonstrated that these compounds have considerable carcinogenic activity.

Transplacental administration of nitrosamines provided much less adequate evidence for carcinogenicity. The best comparative studies in this area were reported by Althoff and Grandjean (1979), who studied the carcinogenesis of 10 nitrosamines in Syrian golden hamsters. In each case, the dams that were treated during pregnancy were maintained so that there could be a direct comparison between the effects of a single dose in the mothers and those in the offspring. The investigators ascertained that the nitrosamine reached the fetal circulation. The chemicals investigated were NDMA, NDPA, NDBA, NPiP, nitrosohexamethyleneamine, nitroso-2-hydroxypropylpropylamine, nitroso-2-oxopropylpropylamine, nitroso-di(2-hydroxypropyl)amine, nitroso-4-hydroxybutylbutylamine, and nitrosomethylpropylamine. Overall, the low tumor yields were remarkably similar in the dams and the female offspring. The major overall difference was a greater sensitivity of the dams to respiratory tract tumors in response to 7 of the 10 chemicals investigated. This experiment clearly demonstrated that the offspring are not exquisitely sensitive to the carcinogenic effects of nitrosamines. The probable explanation of this observation is that nitrosamines require metabolic activation and the fetus does not necessarily possess the requisite enzymes for this purpose.

Although the fetus is not highly sensitive to the carcinogenicity of nitrosamines, neonates, in some instances, are extremely sensitive to their carcinogenic effects. For example, Rao and Vesselinovitch (1973) found that the rate at which hepatomas developed in mice injected

with NDEA at 48 days of age lagged far behind the development of tumors in mice injected with the same dose at 15 days of age. This finding and others reviewed by Craddock (1976) suggest that cell proliferation may play an important role in carcinogenesis mediated by N-nitroso compounds.

Inhibitors or Enhancers of Carcinogenesis. A variety of agents either enhance or inhibit the carcinogenicity of N-nitroso compounds. Such agents may influence the amounts of N-nitroso compounds formed by in vivo nitrosation or may either act on the process of carcinogenesis induction by preformed N-nitroso compounds. These two aspects must be considered separately.

Inhibition of the carcinogenicity of several N-nitroso compounds has been observed in systems where the formation of N-nitroso compounds has been inhibited. Following the initial demonstration by Mirvish et al. (1972b) that ascorbic acid (vitamin C) prevented oxytetracycline from producing NDMA in the presence of nitrite, many experiments were conducted in a variety of animal species with different nitrosatable agents. These studies have clearly demonstrated the inhibition of in vivo nitrosation reactions by ascorbic acid (Akin and Wasserman, 1975; Archer et al., 1975; Fong and Chan, 1976; Greenblatt, 1973; Ivankovic et al., 1975; Mirvish et al., 1975; Rustia, 1975). The principle behind nitrosation inhibition is that ascorbic acid and a variety of other agents compete with the nitrosatable agent for the available nitrite in the acid conditions of the stomach, thereby inhibiting the formation of N-nitroso compounds. A number of other agents that interact readily with nitrite have also been shown to inhibit nitrosation. Among these are other isomers of ascorbic acid, some phenols, and α -tocopherol. Most of these interactions have been observed at the chemical rather than at the biological level. These interactions are described in Chapters 4 and 6.

The formation of N-nitroso compounds can also be enhanced by a variety of ions, especially thiocyanate and iodide, which may catalyze the nitrosation reaction in the stomach (Boyland and Walker, 1975; Boyland et al., 1971; Lathia and Rütten, 1979; Mirvish et al., 1975). Since these ions are present in foodstuffs, their catalytic action could be of some importance in assessing the risk of nitrosation in vivo. The majority of the information on this mechanism has been obtained in vitro using chemically defined conditions, although a recent report by Pignatelli et al. (1981) showed that certain phenolic catalysts enhanced in vivo formation of N-nitroso compounds.

For nitrosamines, metabolic activation is required for carcinogenic effect. Thus, agents that alter such activation influence carcinogenicity. The effect of a temporary, low ("zero") protein diet on tumorigenesis by NDMA was discussed above. The "zero" protein

diet reduces the capacity of the liver, but not the kidney, for metabolic activation of NDMA, thereby reducing acute toxic effects in the liver and raising the dose that kills 50% of the animals (LD_{50}). The higher doses that may be given with this protein-deficient diet lead to a 100% incidence of induced kidney tumors. In studies in rats, Rogers *et al.* (1974) showed that diets deficient in lipotropic agents such as choline and methionine enhanced hepatocarcinogenesis by NDEA and NDBA, but not that induced by NDMA. They also enhanced the induction of esophageal tumors by NDBA.

Overall, however, the effort directed toward the study of factors that may affect the metabolism of nitrosamines appears to have been much less extensive than that directed toward other classes of carcinogens, perhaps because most metabolic studies have been conducted on compounds with relatively simple structures, such as NDMA or NDEA (e.g., Phillips *et al.*, 1975). Thus, there have been few bioassays for carcinogenicity using combinations of agents that may influence metabolic activation in combination with carcinogenic nitrosamines. For example, 3-methylcholanthrene inhibits liver tumorigenesis when administered with 3-methyl-4-dimethylaminoazobenzene, a carcinogenic aminoazo dye (Richardson *et al.*, 1952), but does not have a major effect on the induction of liver tumors by NDMA or NDEA (Hoch-Liget *et al.*, 1968; Makiura *et al.*, 1973). The former, but not the latter, authors reported that cofeeding 3-methylcholanthrene and NDMA marginally increased the yield of subcutaneous sarcomas induced by 3-methylcholanthrene alone. Schmähl and von Stackelberg (1968) failed to observe any effect of lactoflavin, nicotinamide, or dipyridamole on rat liver carcinogenesis induced by NDEA.

Enhancement of carcinogenicity by N-nitroso compounds can also occur following metabolic activation, at the stages of tumor initiation or promotion. The concept of multistage carcinogenesis has been well established by clinical observations and animal experimentation (Farber and Cameron, 1980). It appears that many, if not all, neoplasms arise from precursor lesions through a series of steps, during which they acquire increasing degrees of autonomy (Foulds, 1958; Medina, 1975). This process is called tumor progression. The two general stages in the progression of tumors are initiation and promotion.

Tumor initiation refers to the earliest irreversible effect of exposure to a carcinogen. This effect might be a consequence of somatic mutation, and it may not be associated with any recognizable phenotypic changes. One of the most important factors altering the magnitude of tumor initiation is the rate of cell replication in tissues that are at risk of carcinogenesis. This is probably best exemplified by the increased incidence of hepatic neoplasms in newborn and suckling animals (in comparison to adults) that have been treated with single doses or a few doses of N-nitroso compounds

(Craddock, 1976). The amplifying effect of cell proliferation on the hepatocarcinogenesis of N-nitroso compounds was also observed in a number of instances when carcinogens were administered in single doses during the phase of most rapid DNA synthesis within one day after partial hepatectomy (Craddock, 1976). Thus, enhancement at the initiation stage requires that the carcinogen be administered after the cells have been stimulated to multiply.

Promotion, on the other hand, refers to a process that enhances or fosters further development of neoplasms from the initiated state through a series of potentially reversible but recognizable stages to a stage of self-sustained neoplasia (Berenblum and Shubik, 1947; Boutwell, 1974). In this case, then, the carcinogen is administered first, followed by the promoting agent. Pure tumor promoters are agents that by themselves have little or no carcinogenic effect, but that greatly increase the incidence of neoplasms when administered after an initiator or low dose of a complete carcinogen. Tumor promotion has been observed in at least nine different organ systems in rats, mice, and dogs (Pitot and Sirica, 1980). Phenobarbital has been found to be a promoter of NDMA-induced carcinogenesis in the rat liver (Pitot *et al.*, 1978), and lithocholic acid was reported to be a promoter of carcinogenesis induced by MNNG in the rat colon (Lipkin, 1975; Reddy and Watanabe, 1979).

Probably the best documented modification of the carcinogenicity of N-nitroso compounds is the enhancement of NDMA- or NDEA-induced liver carcinogenesis in rats and mice by carbon tetrachloride (Pound *et al.*, 1973a; Schmähl *et al.*, 1965; Taylor *et al.*, 1974). Pound *et al.* (1973a) gave the animals a single necrotizing dose of carbon tetrachloride, followed by a single dose of NDMA 42 or 60 hours later. When administered separately, carbon tetrachloride did not induce liver tumors under these conditions, and NDMA (20 mg/kg body weight) induced two hepatocellular tumors in 27 animals (7%). But the combination induced hepatocellular tumors in 3 of 27 (11%) rats at 42 hours and in 7 of 34 (21%) rats at 60 hours after the carbon tetrachloride was administered. Pound *et al.* (1973b) observed that the single dose of carbon tetrachloride induced a considerable increase in the synthesis of rat liver DNA, which reached its maximum 60 hours after treatment.

Other investigators have studied the effects of carbon tetrachloride on nitrosamine-induced liver tumors. Schmähl *et al.* (1965) fed NDMA and carbon tetrachloride concurrently to rats. A high incidence (75%) of liver tumors was obtained in animals receiving the nitrosamine with or without carbon tetrachloride, but tumor latency was somewhat reduced by the combined treatment. Taylor *et al.* (1974) reported a similar experiment in which aminopyrine and sodium nitrite led to a higher incidence of hepatocellular tumors in

the presence of carbon tetrachloride than in its absence (6 of 18 rats exposed to carbon tetrachloride developed these tumors, whereas 0 of 15 did so in the absence of this chemical). The implication of these experiments with carbon tetrachloride is that the liver, normally having a very low rate of cell proliferation, is made more sensitive to carcinogenesis induced by N-nitroso compounds in the presence of agents that stimulate proliferation.

In a more recent study, carbon tetrachloride was found to enhance NDEA-induced hepatocarcinogenesis when it was administered before and/or after the N-nitroso compound (Pound and McGuire, 1978). A control group of the random-bred mice used in the study did not develop hepatocellular neoplasms during the 1-year course of the experiment. Furthermore, administration of carbon tetrachloride alone did not induce neoplasms. The investigators found that a single intraperitoneal injection of NDEA at 80 mg/kg body weight induced 26 hepatocellular tumors in 14 of 29 mice after 1 year. When seven doses of carbon tetrachloride were administered after NDEA, the number of tumors was nearly doubled (46 tumors in 21 of 26 mice). Administration of a single dose of carbon tetrachloride 24 hours prior to NDEA tripled the number of neoplasms (79 tumors induced in 20 of 26 mice). Finally, when carbon tetrachloride was administered before and after the NDEA, 172 tumors were induced in 28 of 28 mice -- a synergistic, sixfold enhancing effect. The results suggest that carbon tetrachloride enhances NDEA-induced hepatocarcinogenesis at both initiation and promotion stages of tumor development, probably by inducing hepatic parenchymal cell proliferation in response to cellular necrosis.

The carcinogenic effects of N-nitroso compounds can also be enhanced if administered with other carcinogens having a similar organotropy (Schmährl, 1980). In one study, during which equally carcinogenic doses of NDEA and 4-dimethylaminoazobenzene were fed to rats (Schmährl *et al.*, 1963), the induction time for liver tumors greatly shortened when the carcinogens were fed together (153 days) in comparison to the time required when NDEA or 4-dimethylaminoazobenzene was given (233 and 235 days, respectively). In a later experiment, four liver carcinogens (NDMA, NDEA, NMOR, and *p*-dimethylaminoazobenzene) were administered in doses that did not cause liver cancer when they were applied separately, i.e., in subthreshold doses (Schmährl, 1970). The investigator speculated that if the effects were additive, some rats would develop carcinomas of the liver after 600 or 700 days. The results of the study supported the speculation, since 50% of the rats developed carcinomas by 700 days.

Short-Term, In Vivo Bioassays. As indicated earlier, most spontaneous neoplasms develop from initiated cells through a series of stages. Since some of these intermediate lesions may be reversible, the precursor lesions should be distinguished from purely reactive lesions arising in response to cellular injury.

The characterization and quantitation of premalignant lesions may have important implications for development of reliable short-term in vivo bioassays for N-nitroso carcinogens. The committee believes that the development of these tests is important in view of the shortage of trained personnel and facilities needed for lifetime rodent studies. The use of short-term in vivo bioassays for carcinogenesis is being actively evaluated in a number of different organs including the skin and lungs of mice and the liver of rats, but none of them are considered to be adequate substitutes for lifetime studies. Recent studies of NDEA-induced hepatocarcinogenesis suggest that it might be useful to develop short-term tests for agents that are carcinogenic for the liver.

There is an increasing body of direct and indirect evidence that hepatocellular carcinomas in rats develop from enzyme-altered hyperplastic foci or islands (Goldfarb and Pugh, in press). Scherer and Emmelot (1976) induced ATPase-deficient hepatocellular foci by administering NDEA to adult rats that had been partially hepatectomized 24 hours earlier. They reported a progressive increase in the size of foci over time. Furthermore, they documented a linear response in the incidence of islands over a broad range of doses of the carcinogen. This observation suggested to the authors that islands arose from normal cells after a single carcinogen "hit" and that the further progression to trabecular carcinomas required additional "hits." In another study, Kunz et al. (1978) plotted the time of NDEA treatment against decreasing daily intake of the carcinogen on a log-log scale. They observed that the relationship for the induction of GGTase (γ -glutamyltranspeptidase) positive cells in 1% of the liver section area and for 50% mortality due to liver tumors (Druckrey, 1967) was described by straight lines with similar slopes. Thus, the relationship:

$$K = \text{daily dose of carcinogen} \times \text{time}^{2.3},$$

which was originally described by Druckrey (1967) for the induction of malignant neoplasms, also applies to the development of enzyme-altered islands. Of practical importance for devising a short-term in vivo bioassay was the observation that when island number, average area of islands, and total island areas were determined on tissue slides, the total island area was statistically most sensitive for quantifying the hepatocarcinogenic effect.

Recent studies in mice suggest that this species may also be useful in short-term bioassays for assessing the hepatocarcinogenic potency of N-nitroso compounds. Ribonucleic acid-rich hepatocellular foci with high nuclear-to-cytoplasmic ratios and a deficiency of glucose-6-phosphatase activity were found to increase in number and size following a single injection of NDEA in 15-day-old mice (Goldfarb et al., 1981). Since the foci were first noted 10 weeks following injection of the carcinogen (but 20% of them were found to invade terminal hepatic veins by 20 weeks) and since they acquired increasing

degrees of anaplasia during the course of the experiment, they were considered to be the precursors of trabecular hepatocellular carcinomas. The progressive, irreversible nature of the lesions was also supported by their almost spherical shape and high thymidine-labeling indices (10- to 80-fold times greater than "background" hepatocytes). However, NDEA is known to be a potent carcinogen. No studies of hepatocellular foci and less potent N-nitroso hepatocarcinogens have been reported.

Summary and Discussion: Carcinogenesis

Data on Humans. Perhaps the greatest limitation in the epidemiological studies described in this chapter is their lack of sufficient data on the history of exposure to nitrate, nitrite, and N-nitroso compounds for the individuals who develop cancer. One of the most thorough attempts to accumulate such data has been made by Correa and his colleagues (Correa *et al.*, 1976, 1979; Cuello *et al.*, 1976) in their studies of gastric cancer in Colombia. Despite the difficulties of ascertaining dietary intake and the inadequate medical diagnoses in poorly developed regions, these investigators were able to document the high frequency of both stomach cancer and dysplasia in certain areas of that country. It is likely that dysplasia is a true precursor, but this has not been proven. That nitrate, nitrite, or N-nitroso compounds play a role in the etiology of dysplasia and the subsequent development of stomach cancer in Colombia is plausible, but this, too, has not been proven. The finding of a negative association between consumption of vegetables containing the nitrosation inhibitor vitamin C lends further indirect support to this hypothesis.

Carefully conducted studies of populations in Chile, Japan, Iran, and China do not demonstrate a clear causal link between exposure to nitrate and nitrite and the risk of gastric and esophageal cancer in humans, nor do they rule out alternative causes. One study conducted in Chile showed an inverse correlation between stomach cancer and urinary nitrate levels. Studies of Japanese in Hawaii and in Japan were rigorous, but did not focus on N-nitroso compounds. In Iran, nitrate and nitrite intakes were similar in areas with a high and low incidence of esophageal cancer. The Chinese have recommended dietary changes to discourage the use of pickled vegetables and encourage the consumption of foods containing ascorbic acid. Further study is needed to determine if these recommended changes affect the incidence of esophageal cancer in that country.

The cancer risks for humans from consumption of processed meats has been studied by Meinsma (1964) in the Netherlands, where a correlation between stomach cancer mortality and bacon consumption was reported, and by Higginson (1966) in the United States, where a difference in consumption of bacon and other cured meats between

controls and stomach cancer patients was detected, but was not statistically significant. The negative association between consumption of fresh fruits and vegetables and gastric cancer incidence reported in these two studies also suggests a possible causative role for nitrate, nitrite, and/or N-nitroso compounds.

Despite the valuable research opportunities, studies in certain occupational groups, such as rubber-industry workers, have not examined the exposure to nitrate, nitrite, or N-nitroso compounds in those who have developed cancer.

In summary, epidemiological studies have failed to provide convincing evidence that exposure is associated with cancer. Future studies to examine this association will require clearly defined populations, well-documented conditions of exposure for an adequate length of time, and documentation that the exposed individuals and not the controls are getting the cancers. In addition, alternative causations such as the presence of other, possibly carcinogenic substances in the environment must also be considered.

Data on Animals. The data on the carcinogenicity of nitrate and nitrite in animals are not definitive. However, there is convincing evidence that nitrite can react with nitrosatable agents in the acidic conditions of the stomach to produce N-nitroso compounds, most of which, when adequately tested in animals, have been proven to be carcinogenic. The impact of in vivo formation of N-nitroso compounds on cancer induction in humans cannot be determined with precision because the amounts of N-nitroso compounds formed following administration of nitrite and amines or amides at the low doses to which humans are normally exposed are not yet known. A further complication is the wide range (three orders of magnitude) in the carcinogenic potency of the various nitrosamines.

In estimating the extent to which humans are exposed as a result of such nitrosation reactions, it is important to use peak concentrations of nitrite and nitrosatable agents such as those that may occur after a meal, rather than the average daily intake, if meaningful results are to be obtained. It may also be helpful to focus on the importance of N-nitroso compound formation when higher levels of nitrosatable agents, such as certain drugs, are deliberately administered.

The original observations of Mirvish and his colleagues (1972b) that the formation of nitrosamines is inhibited by ascorbic acid have been confirmed, and a variety of nitrosation inhibitors (e.g., ascorbic acid) and accelerators (e.g., certain phenols) have been discovered. Again, there is a need for a more quantitative assessment of the availability of inhibitors and accelerators in the gastric contents of humans and animals, or in normal and specialized diets, to permit an adequate assessment of the hazards of in vivo nitrosation (see Chapter 6).

There is no doubt that most N-nitroso compounds are carcinogenic. Of the approximately 300 N-nitroso compounds tested, nearly 90% have been found to be carcinogenic. Moreover, the induction of tumors by single doses of N-nitroso compounds testifies to the potency of this group of chemicals.

Nitrosamides that do not require metabolic activation may be more potent carcinogens in rapidly proliferating fetal tissues than in adult tissues. For example, nitrosoureas have a strong propensity to induce tumors in the nervous systems of rats when administered transplacentally. Similarly, the activity of agents that increase cell proliferation in the liver can enhance carcinogenicity of N-nitroso compounds. The formation of carcinogenic N-nitroso compounds can also be enhanced or inhibited by the presence of certain chemical anions or reducing agents.

Despite the difficulties in extrapolating carcinogenicity data from animals to humans, data indicate that N-nitroso compounds will probably be found to be carcinogenic in humans. Two important facts support this conclusion: (1) These compounds are carcinogenic in every species tested thus far. (2) Humans are apparently capable of converting nitrosamines to the metabolic intermediates that alkylate the DNA. These intermediates are presumably the ultimate carcinogens (Montesano and Magee, 1970).

MUTAGENICITY

Assays for mutagenicity and other forms of genotoxicity may be valuable as indicators of mutagenic potential of a chemical in vivo and predictors of whether a chemical may be a carcinogen. In this section, results of mutagenicity assays for nitrate, nitrite, and N-nitroso compounds are described and areas requiring further research are identified.

Nitrate and Nitrite

The only published information on the mutagenicity of nitrate appeared in an abstract prepared by Konetzka (1974), who studied the mutagenicity in Escherichia coli under aerobic and anaerobic conditions. He observed a hundredfold increase in mutant colonies in the presence of nitrate under anaerobic conditions, but no increase under aerobic conditions. In contrast, Salmonella typhimurium was not susceptible to mutagenesis by nitrate, even under anaerobic conditions. The author postulated that the observed mutations were due to the reduction of nitrate to nitrite -- not directly to the nitrate itself.

Nitrite, as nitrous acid, may lead to mutations by one of three mechanisms (Zimmermann, 1977):

- Nitrous acid may deaminate DNA bases. This has been demonstrated in viruses containing single-stranded DNA (Mundry and Gierer, 1958; Schuster and Schramm, 1958; Tessman, 1959). However, spontaneous deaminations are frequent, even in the absence of nitrous acid, and DNA repair systems that correct such lesions are present in bacteria and probably in all healthy mammalian cells (Hartman, 1980; Lindahl, 1979).

- In organisms with double-stranded DNA, mutagenesis by nitrous acid may also proceed by other mechanisms (Frankel *et al.*, 1980; Hayakawa *et al.*, 1978; Kotaka and Baldwin, 1964; Litman, 1961; Oeda *et al.*, 1978). It may proceed the creation of intra- or interstrand cross-links between purine residues, which lead to helix distortion (Dubelman and Shapiro, 1977). The induction of helix-distorting lesions by nitrous acid appears to be enhanced by the presence of molecules proximate to DNA, such as polyamines, glycols, alcohols, or phenols (Murphey-Corb *et al.*, 1980; Thomas *et al.*, 1979b).

- In a third mechanism, nitrite reacts with nitrosatable substrates to produce N-nitroso compounds that are known carcinogens and mutagens. It may also contribute to the formation of C-nitroso, aryl-nitroso, and S-nitroso compounds, some of which are carcinogenic and mutagenic (Gilbert *et al.*, 1980; Nataka *et al.*, 1979). C-Nitroso compounds such as nitrosophenols are generally regarded as nongenotoxic. Specific aryl-nitroso compounds are carcinogenic, but they result from the metabolism of aromatic amines rather than from direct nitrosation. S-Nitroso compounds have not been investigated because of their instability.

The few *in vivo* studies that have been conducted have provided little useful information on the mutagenicity of nitrite. In a host-mediated assay, nitrite was without activity (Couch and Friedman, 1975); however, the nitrite may never have reached the site where the test organisms were located. *In vitro* assays of cultured mouse cells demonstrated nitrite-induced mutagenicity only when substrate concentrations higher than 1 mM were obtained (Kodama *et al.*, 1976).

Despite the unequivocal demonstration of nitrite-induced mutagenicity in *in vitro* systems in which single-stranded and double-stranded DNA are the targets, no evidence that this occurs in intact mammalian organisms has been provided either directly or by host-mediated assays.

N-Nitroso Compounds

Many N-nitroso compounds are shown to be mutagenic when assayed under certain conditions. Bacterial mutagenicity assays of nitrosamines require supplementation with animal-derived enzymes to detect mutagenicity because the bacteria are not capable of activating these compounds.

Nitrosamides, which decompose to the active form in bacteriological media, do not require enzyme activation. More than 40 N-nitroso compounds have been shown to be mutagenic in microbial systems (McCann et al., 1975; Montesano and Bartsch, 1976).

There have been several reviews of the data on the mutagenicity of N-nitroso compounds (Montesano and Bartsch, 1976). The Ames Salmonella typhimurium microsome test (Ames, 1979; Ames et al., 1973a,b) is the system applied most often to these compounds. The original Ames method was not very responsive to N-nitroso compounds, but by using a different technical approach, i.e., preincubating the nitrosamine in liquid suspension with the bacteria, investigators were able to obtain highly satisfactory results (Yahagi et al., 1977). Many such tests have been performed using Salmonella tester strains TA1535, TA1537, TA1538, TA98, and TA100. Mutations are produced in one or more of these strains, depending on the nature of the test chemical (Andrews et al., 1980). S. typhimurium is by no means the only monocellular organism to be used in this way. Other microbes, such as E. coli, and yeasts, such as Saccharomyces cerevisiae, are also suitable (Elespuru and Lijinsky, 1976; Larimer et al., 1978). Test systems have been reviewed by Hollstein et al. (1979) and Brusick (1980).

Microbial tests have also been used to demonstrate the nitrosation of amines by nitrite (Andrews et al., 1980; Couch and Friedman, 1975) and the ability of ascorbate to inhibit the mutagenicity of nitrosamines (Guttenplan, 1978).

Many investigators have used the microbial mutation assay to examine food as well as fluids or excreta from the human body for traces of mutagens (Bruce et al., 1977; Lin et al., 1979; Scheutwinkel-Reich et al., 1980). Studies of this nature need very careful evaluation for two reasons: artifacts may form during mutagen extraction (Iwaoka et al., 1981) and mutagenicity may be due to agents other than N-nitroso compounds.

N-Nitroso compounds have been used in numerous other test systems as well (Hollstein et al., 1979; Montesano and Bartsch, 1976; Neale, 1976). Drosophila melanogaster was the first organism shown to develop germ-line mutations as a result of treatment with N-nitroso compounds (Coulston and Olajos, 1980; Rapoport, 1948; Vogel and Sobels, 1976). Russell et al. (1979, 1980) reported that nitrosoethylurea is a potent mutagen for germ-line mutations in the mammal, in contrast to nitrosomethylurea, which is only weakly mutagenic for mammalian germ cells (Ehling et al., 1968). Taken together, these results provide substantial evidence of the mutagenic potential of the N-nitroso compounds; however, for a class of compounds containing mostly established carcinogens, the qualitative prediction for carcinogenicity is less valuable than quantitative determinations that may predict the relative carcinogenic potency of the various compounds.

Correlations of Mutagenic and Carcinogenic Potency. The test methods used to assess mutagenic potency of N-nitroso compounds provide results that correspond to carcinogenic potency in varying degrees. Langenbach *et al.* (1980) determined the mutagenic potency of four compounds known to be carcinogenic in the hamster pancreas. The agents, in descending order of carcinogenic potency, were nitrosomethyl-2-oxopropylamine (MOP), nitrosobis(2-oxopropyl)amine (BOP), nitroso-2-oxopropyl-2-hydroxypropylamine (HPOP), and nitrosobis(2-hydroxypropyl)amine (BHP). These investigators reported that the use of liver S-9 fraction placed the four carcinogens in an order of mutagenic potency in the Ames test with *S. typhimurium* TA1535 that was almost the inverse of carcinogenic potency (Figure 9-1). However, when hamster lung V79 cells were used and resistance to ouabain was the method of selecting mutants, cocultivation of uninduced primary explants of hamster liver cells with the mutable V79 cells placed the four nitroso compounds in the correct order of potency.

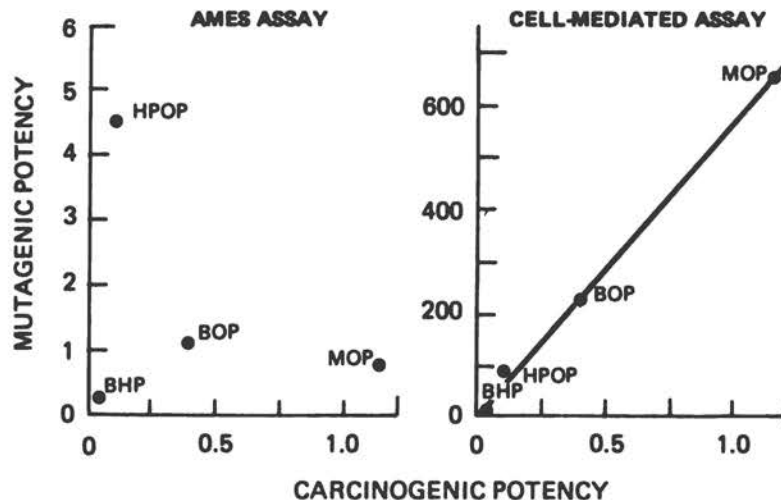


FIGURE 9-1. Relationship of carcinogenicity and mutagenicity in the Ames *Salmonella* and cell-mediated assays. Carcinogenic potency is expressed as the reciprocal of the lowest weekly dose that produced a tumor incidence of at least 60% to 70%. From Langenbach *et al.*, 1980.

The reason that the mammalian cell assay provides much more convincing results than the microbial test probably lies in the use of whole liver cells that are better able to mimic metabolic activation in vivo than the crude S-9 fraction. Jones et al. (in press) have now examined 27 N-nitroso compounds by the hepatocyte-mediated V79 mammalian cell method and obtained a high degree of correlation between carcinogenic and mutagenic potency. In their study, they defined mutagenic potency as the concentration of nitrosamine that yields a frequency of mutation 10 times higher than the frequency of spontaneous mutation. The index for carcinogenicity was defined as a function of the nitrosamine dose and time to death resulting from tumors in 50% of the exposed animals. Using these indices, the authors established a linear relationship between the degrees of carcinogenicity and the degree of mutagenicity for the nitrosamines with a p-value of 0.0001.

In contrast, the correlation of mutagenic potencies for N-nitroso compounds tested in the bacterial systems does not correlate very strongly with potency determinations from carcinogenicity studies (Ames, 1979; Meselson and Russell, 1977). However, for certain N-nitroso compounds with closely related structures (Andrews et al., 1980; Preussmann et al., 1979; Rao et al., 1977, 1979a,b; Wakabayashi et al., 1981), which are only minimally affected by use of the S-9 fraction for metabolic activation, some rough correlations have been obtained.

Variation in Mutagenicity Data. Variation in the results from assays stems from three major considerations. First, potentially mutagenic events take place at multiple sites on DNA (Kröger and Singer, 1979; Singer, 1979). The position of these events (i.e., alkylation of DNA) and, thus, the resulting mutagenic effectiveness of different N-nitroso compounds depend on the nature of the alkylation (McMahon et al., 1979). For example, nitrosomethylurea is more potent than nitrosoethylurea in the Ames test (Lee et al., 1977), in the host-mediated assay (Couch and Friedman, 1975), in cytotoxicity tests, and in tests for HGPRT (hypoxanthine guanine phosphoribosyltransferase) locus mutations in Chinese hamster ovary (CHO) cells (Couch and Hsie, 1978). In contrast, E. coli strains carrying the trpA58 mutation (Hince and Neale, 1974) and strain WP-2 derivatives (Garner et al., 1979) exhibit a greater mutagenic responsiveness to nitrosoethylurea than to nitrosomethylurea. Lawley (1980) reported that the ratio of O⁴-thymine and O⁶-guanine alkylation is approximately 0.05 for nitrosomethylurea and 0.3 for nitrosoethylurea. This suggests that nitrosomethylurea would favor G/C to A/T mutations, whereas nitrosoethylurea would favor A/T to G/C mutations. Thus, nitrosomethylurea would be more active in the Ames tester bacteria that carry the histidine G46 missense mutational triplet (GGG/GCC) since this triplet can only revert to wild type by a G/C to A/T transition (Ames, in press), whereas nitrosoethylurea would

be expected to be more active in *E. coli* W-2 strains that carry a TAA/ATT ochre or nonsense-coding triplet and revert by an A/T to G/C shift. Similarly, the trpA58 mutation (GAC) could form true wild-type revertants only by reversion to GGC -- an A/T to G/C base-pair change (Nicholas and Yanofsky, 1979; Yanofsky, 1967).

Differences in the results of mutagenicity assays may also be due to a nonlinear relationship between dose and mutagenic response, which may be a consequence of the necessity for metabolic activation (Guttenplan, 1979; Guttenplan et al., 1976; Malling, 1971; McCann et al., 1975). However, even for the direct-acting nitrosoureas, the dose-response curve at the lowest doses shows an area of minimum response, followed at increasing doses by a strong and increasing response (Brundrett et al., 1979; Franza et al., 1980; Guttenplan, 1979; Lee et al., 1977). The slope of the biphasic dose-response curve is dependent on alkyl chain length and the test protocol (Brundrett et al., 1979). Other direct-acting alkylating mutagens, i.e., linear aryl-monoalkyl triazines, show similar biphasic dose-response curves (Endo et al., 1980; Thomas et al., 1979a).

The biphasic nature of the dose-response curve for the mutagenic response to a dose of alkylating agent may be partly explained by an adaptive response in repair proficiency in the repair of O⁶-guanine lesions at low levels of the alkylating agent and the saturation or inactivation of this system at higher levels (Cairns, 1980; Jeggo et al., 1977; Karran et al., 1979; Robins and Cairns, 1979; Samson and Cairns, 1977). Similar attributes have been demonstrated for DNA repair at O⁶-guanine *in vivo* in mammals (Buckley et al., 1979; Montesano et al., 1979; Pegg, 1978).

Finally, differences in results from mutational testing may be due to the lack of standardization among the many laboratories conducting these studies.

Correlation Between In Vitro and In Vivo Mutagenicity Assays.
In vitro mutagenicity tests are valuable because they can be applied to many more chemicals than can in vivo tests for mutagenicity or carcinogenicity, which are limited by their requirement for large numbers of animals.

Unfortunately, there is little information on chemically induced mutation in whole animals for comparison with the results of in vitro tests. However, it is clear that the distribution of the chemical in the whole animal and its pharmacodynamic fate provide one source of difference because such considerations do not arise in in vitro tests. Furthermore, there is relatively little knowledge about whether the germ cells or embryo are protected from the effects of electrophiles, such as nucleophile-containing molecules. Finally, it is not known whether germ cells or the structures proximate to them have the capability for metabolic conversion of procarcinogens to their active

forms. Thus, predictions of mutagenicity in whole animals based on in vitro results must be regarded with reservations. This is especially true for nitrosamines that require metabolic activation than for nitrosamides that do not, primarily because, unlike the normal intact liver, the S-9 fraction (prepared from livers of rats treated with phenobarbital or Aroclor) may not activate or detoxify carcinogens (Clayson, 1980; Wright, 1980).

Another major difference between in vitro assays and in vivo tests can be observed in DNA repair mechanisms. Chemically induced lesions in DNA are often removed by one or more DNA repair systems. Tester cells in both microbial and mammalian cell mutation assays are usually more sensitive to chemical effects when their DNA repair capability is deficient, which leads to another area of discordance between in vitro and in vivo tests. There would still be discrepancies even if cells with a normal DNA repair capacity were used, since the repair capacity of cells from humans is markedly different from that of cells from rodents. Finally, the tumor progenitor cell in which the test chemical has induced one or more genetic errors must be developed within the constraints of the body systems to a frank clinical tumor; no such constraints affect the in vitro system. Despite these limitations, however, most N-nitroso compounds that are carcinogenic in animals are mutagenic when tested under appropriate conditions, although exceptions do occur (Rao et al., 1979).

Summary and Discussion: Mutagenesis

Nitrate does not appear to be directly mutagenic. In microbial systems, nitrite may be mutagenic by three different mechanisms: deamination of DNA bases in single-stranded DNA; formation of intra-strand or interstrand lesions leading to helix distortions in double-stranded DNA; and formation of mutagenic N-nitroso compounds. In mammalian systems, however, there is no evidence that nitrite is mutagenic (except for one study, in which a high dose of nitrite was used).

Despite several limitations of the in vitro mutational assays stemming from differences in metabolic activation and DNA repair mechanisms among species, appropriate mutagenicity tests on N-nitroso compounds have usually provided a high level of correlation with whole animal studies, although exceptions do occur. The majority of N-nitroso compounds considered for testing are usually positive for carcinogenicity and, under appropriate conditions, for mutagenicity. This leads to a good degree of correlation at the qualitative level, and quantitative correlations have been observed in mammalian mutagenesis systems.

OTHER EFFECTS

Exposure to high doses of nitrate, nitrite, and N-nitroso compounds has been associated with a variety of adverse health effects in humans and other animal species. This section contains a review of data pertaining to the role of these compounds in the induction of disease in humans and descriptions of those diseases. Data on diseases in animals associated with exposure to nitrate and nitrite are also reviewed along with data from experiments on toxicity and teratogenicity of N-nitroso compounds in animals.

Toxic Effects in Humans: Nitrate and Nitrite

The toxic effects of nitrate and nitrite have been extensively reviewed (Archer, in press; Corré and Breimer, 1979; Green *et al.*, in press; National Academy of Sciences, 1978; World Health Organization, 1978). The committee has summarized this information and described the most prevalent toxic effects. Nitrate and nitrite are discussed together since most toxic reactions are due to nitrite derived from bacterial reduction of nitrate, either prior to ingestion or within the host (Chapter 8).

Corré and Breimer (1979) and Burden (1961) have summarized the literature documenting the toxic and lethal levels of nitrate and nitrite. Different studies have reported that the lethal level of nitrate for a 60-kg adult ranges from 4 to 50 g, whereas for nitrite, it ranges from 1.6 to 9.5 g (Corré and Breimer, 1979). Although the criteria for toxicity vary, most authors accept as a criterion for toxicity a single dose that will induce methemoglobinemia. In four studies, the listed toxic dose for nitrate ranged between 2 and 4 g, whereas for nitrite it ranged from 60 to 500 mg (Corré and Breimer, 1979).

Methemoglobinemia. Methemoglobinemia is the most prevalent and potentially the most serious known complication of nontherapeutic, excessive intake of nitrate and nitrite. The condition, characterized clinically by cyanosis and anoxia, is due to defective transport of oxygen by high levels of circulating methemoglobin. Methemoglobin is an oxidation product of hemoglobin in which the ferrous iron of hemoglobin is oxidized to the ferric form (Jaffé, 1981). The mechanisms by which nitrite causes methemoglobinemia are complex, apparently involving the formation of intermediate complexes between hemoglobin and nitrite redox products (Kiese, 1974).

After formation of methemoglobin, oxygen can no longer reversibly bind to red blood cells. If the oxidation damage proceeds far enough, the hemoglobin may be irreversibly damaged, leading to the formation of hemochromes. Finally, the hemoglobin may be denatured and precipitated as Heinz bodies, leading ultimately to the death of the

red blood cells. The hypoxic effect of methemoglobin results not only from an inability of the oxidized hemoglobin to transport oxygen, but also from interference by methemoglobin with normal delivery of oxygen transported by oxyhemoglobin. The latter effect was originally suggested by Darling and Roughton (1942) and has been confirmed in more recent years (Brewer, 1972; Enoki et al., 1969). Approximately 1% of the hemoglobin circulates as methemoglobin in the normal adult; in children, it is usually less than 2%. Clinical cyanosis usually appears when approximately 10% of the hemoglobin is converted to methemoglobin; symptoms of cerebral anoxia supervene at approximately 20%; and stupor, coma, and death usually result when conversion levels reach 60% or more.

Patients with mild methemoglobinemia may be treated with oral doses of ascorbic acid administered 3 times daily. When patients are in extremis, immediate intravenous injection of methylene blue, which rapidly reverses the methemoglobinemia, is usually life-saving.

There are no proven cases of chronic sequelae of nitrite- or nitrate-induced methemoglobinemia in humans. Petukhov et al. (1972) reported slightly delayed reaction time in children drinking water containing high levels of nitrate (average, 105 mg/liter); however, this effect, if confirmed, may not be specifically related to methemoglobinemia. A delayed reaction time was also observed in mice that were given drinking water containing up to 2,000 mg of sodium nitrite per liter and enough ascorbic acid to inhibit the formation of methemoglobin (Shuval and Gruener, 1972).

The true incidence of methemoglobinemia in the United States is not known since there are no regulations requiring that cases be reported. However, approximately 2,000 cases were documented in North America and Europe between 1945 and 1971 (Shuval and Gruener, 1971). In the United States, from 1939 to 1950, there were reports of approximately 320 cases of methemoglobinemia in infants who ingested nitrate-rich well water (Walton, 1951). In the Federal Republic of Germany, where some of the best data are available (Simon et al., 1964), 745 cases between 1956 and 1964 were attributed to water containing high concentrations of nitrate. Private wells had supplied the water for 97.3% of these cases; public water supplies had been used by the remaining 2.7%. Eighty-four percent were related to water supplies containing more than 100 mg of nitrate per liter. Nitrite was detectable in samples of water used by only 10% of these cases.

Infants are at greatest risk of developing methemoglobinemia from excessive intake of nitrate. This increased susceptibility is related to at least four factors (Anonymous, 1966; Lee, 1970; Phillips, 1971). First, fetal hemoglobin is much more readily oxidized than hemoglobin in adults. At birth, between 60% and 80% of the hemoglobin remains in the immature form of the molecule,

but this decreases to approximately 30% by the third month of life (Künzer and Schneider, 1953; Pisciotto *et al.*, 1959). Second, the infant has a transitory physiologic deficiency of methemoglobin reductase or its cofactor NADH (reduced nicotinamide adenine dinucleotide), which are necessary to maintain iron in its reduced state (Ross and Desforges, 1959). Third, on a weight basis, infants consume approximately 10 times more water (the most important source of nitrate in the etiology of methemoglobinemia) than do adults (Burden, 1961). Finally, relative achlorhydria in the very young probably favors overgrowth of nitrate-reducing organisms in the upper gastrointestinal tract (Walton, 1951; see also Chapter 8). Ewing and Mayon-White (1951) reported that only infants with a gastric pH above 4.0 developed methemoglobinemia after ingesting water containing high levels of nitrate. Similarly, infantile epidemic gastroenteritis may favor the development of methemoglobinemia when the upper gastrointestinal tract becomes overgrown with bacteria (Fandre *et al.*, 1962).

Several other categories of individuals with altered physiological states or with either hereditary or acquired disease may also be predisposed to the development of nitrite- or nitrate-induced methemoglobinemia. These include pregnant women (Metcalf, 1961), individuals with glucose-6-phosphate dehydrogenase deficiency (Kohl, 1973), adults with reduced gastric acidity (including those being treated for peptic ulcer or individuals with chronic gastritis or pernicious anemia), and a rare group with a hereditary lack of NADH or methemoglobin reductase activity in their red blood cells (Scott, 1960). This hereditary enzyme deficiency seems also to account for the somewhat increased incidence of methemoglobinemia among Alaskan Eskimos and Indians (Scott and Griffith, 1959; Scott and Hoskins, 1958). Individuals with hereditary structural abnormalities in hemoglobin, referred to as hemoglobin Ms, are probably also at increased risk from dietary nitrate or nitrite. In these unusual hemoglobinopathies (two types have been described), substituted amino acids in the globin moiety create new, very strong bonds with the heme iron, maintaining it in the ferric state (Jaffé and Heller, 1964).

Nitrate in drinking water is the factor most commonly associated with the genesis of methemoglobinemia. In the most comprehensive study of its kind, the cases of water-induced methemoglobinemia were classified according to the concentration of nitrate-nitrogen in water used in the preparation of infant formulae (Walton, 1951). Although there were shortcomings in both clinical diagnosis and water analysis, a very obvious finding was the absence of methemoglobinemia in populations whose drinking water contained nitrate-nitrogen concentrations less than 10 mg/liter. In addition, only 2.3% of the cases were infants who drank water containing 10 to 20 mg of nitrate-nitrogen per liter. These figures seem to validate the current standards, which limit nitrate-nitrogen in drinking water to 10 mg/liter.

Although high levels of nitrate (not nitrite) in water have been implicated in the development of methemoglobinemia, ingestion of nitrite has been implicated in all of the other, exceedingly rare cases of secondary methemoglobinemia. An unusual case of methemoglobinemia was traced to the use of nitrate-rich water in a home dialysis unit (Carlson and Shapiro, 1970). Vegetables containing high levels of nitrate, such as spinach, pose a potential threat when their processing or refrigeration is delayed or inadequate. However, less than two dozen cases of methemoglobinemia associated with spinach have been documented, and most of these occurred in the Federal Republic of Germany (Anonymous, 1966) where it has been customary to prepare pureed vegetables by retaining the nitrate-enriched water (Sinios and Wodsak, 1965). For each case of infantile methemoglobinemia traced to this source, toxic levels of nitrite were held responsible. The conversion of nitrate to nitrite in spinach is probably due to the activation of nitrate reductase, which is present in high concentrations in the vegetable (Paneque *et al.*, 1965) (see Chapter 5) or to a similar enzymatic action by bacterial contamination (see Chapter 8).

Ingestion of improperly processed sausage (Bakshi *et al.*, 1967; Orgeron *et al.*, 1957) or fish (Singley, 1962) has also been associated with the development of methemoglobinemia. In one outbreak, sodium nitrite was inadvertently substituted for table salt in a restaurant (Greenberg *et al.*, 1945). In all cases attributed to food processing, the levels of sodium nitrite in the food were far in excess of the permitted maximum residual of 200 mg/kg (U.S. Department of Agriculture, 1970).

Miscellaneous Effects. Excess intake of nitrate esters, which are vasodilators used to treat angina pectoris, may induce headache, facial flushing, and, in severe cases, even syncope and hypotension (Opie, 1980). A remarkable consequence of chronic exposure to relatively high levels of nitrate esters is the development of tolerance to the vascular effect. This has been observed in workers in explosives factories who frequently suffer from severe headache, dizziness, and postural weakness during the first few days of employment in factories manufacturing nitroglycerin. These workers soon develop tolerance to the compound. To maintain this short-lived tolerance while away from work, the workers have learned to rub the nitrate esters into their skin, thereby preventing recurrence of symptoms upon their return to the workplace.

A potentially serious effect of chronic exposure to nitrate esters is the now well-documented development of dependence on organic nitrate derivatives (Nickerson, 1975). This dependence was first observed in some explosives workers who were free of clinical atherosclerosis; however, after several days away from work, they developed severe myocardial ischemia and even myocardial infarction. Although

the precise mechanism of this dependence is not known, the affected individuals recover rapidly after treatment with nitroglycerin.

Toxic Effects in Humans: N-Nitroso Compounds

The literature contains only a few reports of fatalities resulting from exposure to nitrosamines. In two of the earliest reports, occupational exposure to NDMA resulted in acute liver necrosis, which later developed into cirrhosis. In one case, death resulted from the exposure (Barnes and Magee, 1954; Freund, 1937). In a more recent report, criminal poisoning was proven (Fussgaenger and Ditschuneit, 1980). The victim had apparently been fed repeated doses of NDMA over many months and ultimately died of hepatic decompensation and cirrhosis. An autopsy of the liver showed fibrosis and hyperplastic nodules (cirrhosis). In another incident where NDMA poisoning was suspected as a cause of death, the liver from the victim was subjected to DNA analysis (Herron and Shank, 1980). Very high levels of methylated DNA adducts (O⁶-methylguanine and 7-methylguanine) known to result from exposure to NDMA were found in the hepatic DNA from this person, but were absent from the liver and kidneys of the patients who had died from other causes. Based on the known rate of in vitro metabolism of nitrosamines by the human liver, the authors speculated that the victim had been exposed to 20 mg or more of NDMA per kilogram of body weight.

Toxic Effects in Other Species: Nitrate and Nitrite

Turner and Kienholz (1972) and Emerick (1974) have reviewed nitrate-nitrite toxicity in livestock and other animals. Although the toxic effect of most agents is similar in various species, including humans, there appear to be large differences in the effective dose of the agent required to produce these effects among species. In general, ruminants are much more susceptible to the toxic effect of nitrate than are nonruminants, probably because of the longer retention and greater opportunity for bacterial reduction in the rumen. For example, the lethal dose of nitrate in pigs is 300 mg/kg body weight, a level that is approximately 4 times greater than the lethal dose in ruminants (Gwatkin and Plummer, 1946).

Tolerance to nitrate has been induced experimentally in animals. In one extreme example, lambs weighing 32 kg were adapted to increasing levels of potassium nitrate and were able to consume rations containing 1.28% of the compound without showing any obvious ill effects (Sokolowski et al., 1960).

The "no-effect" level for the chronic feeding of nitrate has been determined for rats and dogs (World Health Organization, 1962). Rats were found to tolerate 10,000 mg/kg sodium nitrate in the diet for 2 years with no adverse reaction, and 50,000 mg/kg in the diet of sodium nitrate produced only mild retardation of growth. Dogs were not adversely affected when they were fed a diet containing 20,000 mg/kg sodium nitrate for 105-125 days.

As expected, orally administered sodium nitrite was 10 times more toxic in ruminants than in nonruminants (Emerick, 1974). Intravenous injections of approximately 6 mg of nitrite-nitrogen per kilogram of body weight have produced consistent moderate to severe methemoglobinemia in pigs (Emerick et al., 1965), dogs (Jensen and Anderson, 1941), and ruminants (Emerick et al., 1965). Some reports indicate that rats are relatively resistant to the long-term toxic effects of nitrite. The feeding of sodium nitrite to two generations of rats at 240 to 460 mg/kg in the diet was without effect on litter size, infant mortality, growth rate, or longevity (Shank and Newberne, 1976). However, 2,000 to 3,000 mg of sodium nitrite per liter of drinking water given to rats for 2 years induced heart and lung damage. After exposure for only 2 weeks to levels between 100 and 200 mg of sodium nitrite per liter of drinking water, rats had abnormal electroencephalogram patterns, which persisted after discontinuation of the treatment (Shuval and Gruener, 1972).

The effect of nitrate and nitrite on the reproductive capacity of farm animals continues to be of concern, especially since Thorp (1938) suggested that there was an increased incidence of abortion after animals ingested hay containing high levels of nitrate. However, more recent studies indicate that nitrate exerts no significant abortifacient effect on heifers and ewes at levels approaching those that induce fatal methemoglobinemia (Davison et al., 1964, 1965; Simon et al., 1958).

Emerick (1974) reported that chronic nitrate and nitrite intoxication induces a deficiency of vitamin A in a number of animals, including poultry, pigs, turkeys, and sheep. Most of the evidence indicates that nitrite, but not nitrate, depletes vitamin A in nonruminants by destroying it in the gut lumen under acidic conditions (Roberts and Sell, 1963; Sell and Roberts, 1963). In view of the generally low level of nitrate in feeds, grains, and water, Emerick (1974) believes that the effect of nitrate and nitrite on vitamin A utilization or storage is of no great significance, at least in poultry and swine.

Toxic Effects in Other Species: N-Nitroso Compounds

Acute Toxicity. Barnes and Magee (1954) observed that NDMA-induced acute and chronic toxicity in rodents was most striking in the liver, probably because that is the site of major metabolic activation. Other studies indicate that the hepatotoxicity of nitrosamines follows a marked impairment of protein synthesis. This is probably a consequence of defective RNA processing (Emmelot, 1964; Mager *et al.*, 1965; Mizrahi and de Vries, 1965), which is believed to be responsible for a secondary defect in peptide chain initiation (Pegg, 1977). DNA synthesis is also acutely impaired after administration of NDMA, possibly because of decreased DNA polymerase activity (Salisbury and O'Connor, 1976) or the induction of lesions in the DNA template. In the rat, single toxic doses of NDMA (20 mg/kg body weight or greater) and NDEA (200 mg/kg body weight) result in striking central zonal necrosis of the liver (Barnes and Magee, 1954; Solt *et al.*, 1977), which, for NDMA, begins within 6 hours. Milder hepatotoxicity, also accompanied by central zonal localization, resulted from administration of the heterocyclic nitrosamine NPYR (Hendy and Grasso, 1977).

Studies of nitrosamine-induced acute toxicity in organs other than the liver are relatively uncommon. Renal toxicity is of particular interest since it appears to correlate with the late appearance of neoplasia of the kidney in a manner somewhat similar to that in the liver. Administration of single, very high doses of NDMA to young rats resulted in a decrease in DNA synthesis in the kidney after only 24 hours. Focal periglomerular collections of replicating mesenchymal cells, which were observed on the second day, continued to enlarge, ultimately resulting in mesenchymal neoplasms. Since the replicating cells were located in precisely the same place as the toxically injured cells and since the degree of toxic injury and the tumor incidence were both dose dependent, these findings indicate a relationship between acute cellular damage and ultimate tumor development (Hard and Butler, 1970, 1971).

Greenblatt and Rijhsinghani (1969) reported that susceptibility for necrosis of the olfactory epithelium 48 hours after administration of a nitrosamine seemed to correlate with carcinogenic response in Syrian golden hamsters. The acute toxicity of nitrosoalkylamines generally decreased with increasing chain length, and at the LD₅₀ level, NDEA was a far more effective nasal toxin and carcinogen than was NDMA. The LD₅₀'s of many of these agents have been determined. The most potent is considered to be nitrosomethylbenzylamine, which has an LD₅₀ of 18 mg/kg body weight in rats (Druckrey *et al.*, 1969; Shank, 1975). When measuring the LD₅₀'s of several carcinogens, such as NP1P, that are not carcinogenic in the liver, Mirvish (personal communication, 1981) observed that death occurs almost immediately after ingestion because of toxic effects on the nervous system characterized by convulsions.

De Munter *et al.* (1979) reported that an early "rebound" replication in response to nitrosamine-induced injury also appeared to reflect susceptibility to the carcinogen. During the first week following a single injection of NDMA, the tritiated-thymidine-labeling indices of Type 2 pneumocytes, the putative precursor cell population of lung adenomas, were 2 times higher in GRS/A mice than in C3H/A mice. In contrast, labeling of hepatocytes was 2 times higher in C3H/A mice than in the livers of GRS/A mice during that same period. Since GRS/A mice develop lung adenomas, but C3H/A mice develop hepatocellular carcinomas, the early replicative difference may provide a measure of the organ's susceptibility to the carcinogen.

Teratogenic Effects. When administered to rats, Syrian golden hamsters, or minipigs during the first half of pregnancy, high doses of nitrosomethylurea or nitrosoethylurea induced brain and bone malformations (Ivankovic, 1979). Apparently, the route of administration was not important and high molecular weight homologues of the agents were less teratogenic than those with a lower molecular weight. A teratogenic dose-response effect was observed in BD rats that had been treated with single intravenous injections of nitrosoethylurea. The median teratogenic dose was 46 mg/kg body weight, corresponding to about one-fifth of the LD₅₀. If a linear extrapolation is valid, these limited data suggest that the maximum dose of nitrosoethylurea producing no teratogenic effect is approximately 20 mg/kg body weight. Administration of ethylurea with nitrite to rats during the first trimester of pregnancy resulted in *in vivo* formation of nitrosoethylurea, resulting in hydrocephalus in the offspring. However, when administered to the rats during the second half of pregnancy, the progeny developed neurogenic tumors. In a related study, Ivankovic *et al.* (1973) found that coadministration of ascorbate with the nitrosating mixture effectively blocked the induction of hydrocephalus.

Druckrey (1973) conducted a systematic investigation of the transplacental teratogenic and carcinogenic effects of a number of N-nitroso compounds in rats. He observed that chemical structure, manner of metabolic activation, and stage of fetal development all had a similar effect on teratogenesis and carcinogenesis. Malformations of the central and peripheral nervous system were evident in many instances and, similar to the studies by Ivankovic and his colleagues (1973), the administration of the agents early in embryogenesis caused brain malformations in the absence of neoplasms, whereas treatment with these compounds at later stages of pregnancy was associated with the development of a variety of neoplasms. Clearly, then, the site of action for the teratogenic effects is different from that of the carcinogenic effect.

Summary: Effects Other Than Carcinogenicity or Mutagenicity

Nitrate- and nitrite-induced methemoglobinemia in humans occurs mainly in infants and other predisposed individuals following ingestion of high levels of nitrate in drinking water or nitrite in prepared vegetables. The disease is characterized by cyanosis and anoxia, and is largely reversible by administering ascorbic acid or methylene blue, depending on the severity of the disease. N-Nitroso compounds can be toxic at very high levels of exposure, and death has resulted from irreversible liver damage in several instances.

In animals, nitrate and nitrite can be toxic, especially in ruminants. N-Nitroso compounds may cause fetal death and/or teratogenesis in several species that have been tested. This preliminary evidence of teratogenicity of N-nitroso compounds merits further research, especially because N-nitroso compounds are also mutagenic and carcinogenic in other test systems. The finding that nitrosamides are teratogenic is consistent with the findings that these compounds cause tumors transplacentally and that they are directly mutagenic (i.e., they do not require metabolic activation). It may be that nitrosamines are less active than nitrosamides transplacentally because they require metabolic activation and the necessary enzymes may not be produced in sufficient quantities in the developing fetus.

OVERALL SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

An excessive intake of nitrate or nitrite leads to the development of methemoglobinemia. The effect has been well-documented in humans, and a similar effect has been observed in animals exposed to high doses of these two chemicals. The disease appears to be induced by a complex mechanism in which nitrite oxidizes hemoglobin to methemoglobin -- a type of hemoglobin that is incapable of reversibly binding oxygen.

Evidence implicating nitrate, nitrite, and N-nitroso compounds in the development of cancer in humans is circumstantial. Several epidemiological studies of certain geographical/nationality groups have provided data that are consistent with the hypothesis that exposure of humans to high levels of nitrate and nitrite may be associated with an increased incidence of cancers of the stomach and esophagus. However, in none of these studies was there a direct attempt to investigate actual exposures of nitrate, nitrite, or N-nitroso compounds in individuals who developed cancer. In most of the studies, several other plausible causative agents were also identified. In addition, the few studies conducted on worker populations that may be exposed to nitrate, nitrite, or N-nitroso compounds have also not considered the level, duration, and time of

exposure to these compounds in relation to cancer incidence, nor have they ruled out exposure to other known or suspected carcinogens.

There is no definitive evidence to suggest that either nitrate or nitrite is carcinogenic. In animals, nitrate has not been shown to be directly carcinogenic or mutagenic. The limited data on nitrite indicate that it may not act directly as a carcinogen but that it is mutagenic in microbial systems.

In contrast, the N-nitroso compounds are clearly carcinogenic in every species of animals in which they have been tested. Positive results have been obtained for approximately 90% of the 300 N-nitroso compounds tested for carcinogenicity in one or more species. Most of these compounds are also mutagenic. Several nitrosamides have been shown to be teratogenic in animals. Thus, tests in animals provide strong evidence that N-nitroso compounds are likely to be carcinogenic in humans. These tests have also revealed the importance of enhancers and inhibitors of carcinogenicity. For example, agents that promote cell proliferation in the liver enhance carcinogenicity. In contrast, ascorbic acid, α -tocopherol, and other antioxidants can inhibit carcinogenicity by blocking the formation of N-nitroso compounds from the reaction of nitrite and nitrosatable substrates. These findings indicate that humans exposed to a chemical or virus that stimulates proliferation of liver cells may be predisposed to carcinogenesis induced by N-nitroso compounds. Moreover, they suggest that diets low in vitamin C content may create conditions favorable for the in vivo formation of N-nitroso compounds in humans.

The following recommendations of the committee are based on the data reviewed in this chapter.

1. The committee recommends that future epidemiological studies focus on correlating actual exposures to nitrate, nitrite, and N-nitroso compounds to incidence of cancer. Also, emphasis should be placed on determining other factors, such as the presence of precursor lesions affecting susceptibility. Where possible, exposure should be correlated with actual levels of nitrate, nitrite, and N-nitroso compounds in biological fluids such as blood, saliva, gastric juice, and urine.

2. Results of limited experiments suggest that nitrate is not carcinogenic or mutagenic. The committee recommends that future tests of the carcinogenicity of nitrate be correlated with the test species' ability to reduce nitrate to nitrite in the saliva, which may be of an important mechanism in humans.

3. Current evidence indicates that nitrite may not act directly as a carcinogen in animals. However, because of its mutagenicity in microbial systems and its possible role in the induction of stomach and esophageal cancer in humans, further testing in animals may be

warranted. If such tests provide evidence of a carcinogenic effect, attempts should be made to determine if nitrite acts as a complete carcinogen, a cocarcinogen, or a promoter.

4. Many N-nitroso compounds are clearly carcinogenic in laboratory animals and mutagenic in microbial and mammalian test systems; some are teratogenic in hamsters and rats. However, the value of these tests in the prediction of risk to humans is unknown. The committee recommends that future carcinogenicity assays emphasize quantitative assessment of potency and dose-response relationships as well as the qualitative outcome. It also recommends continued development of mammalian cell mutation assays with emphasis on the use of whole cells of liver and other tissues to provide a better model for the metabolism of the test agent in vivo. These tests should be extended to the use of human cells to learn more about the potency of the N-nitroso compounds in humans. Metabolic studies with human tissues may also help in this regard.

5. Premalignant lesions induced by N-nitroso compounds in humans and laboratory animals should be characterized and short-term in vivo bioassays should be developed to determine the carcinogenicity of N-nitroso compounds based on accurate quantitation of these experimental lesions.

Histopathological diagnoses play a crucial role in the interpretation of bioassays. Although the committee realizes that this subject falls outside the scope of its immediate charge, it believes that special efforts should be made to validate results from such diagnoses before the findings are used as a basis for decisions affecting public health.

6. Many N-nitroso compounds present in the environment or those formed from nitrate or nitrite in vivo have been shown to be carcinogenic in experimental studies. At certain dose levels, they are also acutely toxic, inducing, for example, liver damage. It is reasonable to consider that they are probably carcinogenic in humans. Therefore, the committee recommends that exposure to the precursors of N-nitroso compounds -- especially nitrate and nitrite -- and to preformed N-nitroso compounds be reduced.

REFERENCES

- Adamovic, V. M. 1979. [In Serbo-Croatian.] Occurrence of N-nitrosamines and possible health hazards. Pp. 153-162 in Nitriti Nitratu Predelavi Mesa, Simp. SOZD hp, Ljubljana, Yugoslavia. Chem. Abstr. 95:40901n.
- Akin, F. J., and A. E. Wasserman. 1975. Effect on guinea-pigs of feeding nitrosomorpholine and its precursors in combination with ascorbic acid. Food Cosmet. Toxicol. 13:239-242.
- Althoff, J., and C. Grandjean. 1979. In vivo studies in Syrian golden hamsters: A transplacental bioassay of ten nitrosamines. Natl. Cancer Inst. Monogr. 51:251-255.
- Althoff, J., and W. Lijinsky. 1977. Urinary bladder neoplasms in Syrian hamsters after administration of N-nitroso-N-methyl-N-dodecylamine. Z. Krebsforsch. 90:227-231.
- Althoff, J., R. Wilson, A. Cardesa, and P. Pour. 1974. Comparative studies of neoplastic response to a single dose of nitroso compounds. Z. Krebsforsch. 81:251-259.
- American Cancer Society. 1980. Cancer Facts and Figures: 1981. American Cancer Society, New York. 31 pp.
- Ames, B. N. 1979. Identifying environmental chemicals causing mutations and cancer. Science 204:587-593.
- Ames, B. N. In press. Lipid peroxidation and oxidative damage to DNA. In K. Yagi, ed. Lipid Peroxides in Biology and Medicine. Academic Press, New York.
- Ames, B. N., W. E. Durston, E. Yamasaki, and F. D. Lee. 1973a. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Natl. Acad. Sci. USA 70:2281-2285.
- Ames, B. N., F. D. Lee, and W. E. Durston. 1973b. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Natl. Acad. Sci. USA 70:782-786.
- Andrews, A. W., J. A. Fornwald, and W. Lijinsky. 1980. Nitrosation and mutagenicity of some amine drugs. Toxicol. Appl. Pharmacol. 52:237-244.
- Anonymous. 1966. Spinach - A risk to babies. Brit. Med. J. 1:250-251.

- Aoyagi, M., N. Matsukura, E. Uchida, T. Kawachi, T. Sugimura, S. Takayama, and M. Matsui. 1980. Induction of liver tumors in Wistar rats by sodium nitrite given in pellet diet. *J. Natl. Cancer Inst.* 65:411-414.
- Archer, M. C. In press. Hazards of nitrate, nitrite, and N-nitroso compounds in human nutrition. In J. N. Hatchcock, ed. *Nutritional Toxicology*, Vol. I. Academic Press, New York.
- Archer, M. C., S. R. Tannenbaum, T.-Y. Fan, and M. Weisman. 1975. Reaction of nitrite with ascorbate and its relation to nitrosamine formation. *J. Natl. Cancer Inst.* 54:1203-1205.
- Armijo, R., and A. H. Coulson. 1975. Epidemiology of stomach cancer in Chile -- The role of nitrogen fertilizers. *Int. J. Epidemiol.* 4:301-309.
- Armijo, R., A. Gonzalez, M. Orellana, A. H. Coulson, J. W. Sayre, and R. Detels. 1981a. Epidemiology of gastric cancer in Chile: II. Nitrate exposures and stomach cancer frequency. *Int. J. Epidemiol.* 10:57-62.
- Armijo, R., M. Orellana, E. Medina, A. H. Coulson, J. W. Sayre, and R. Detels. 1981b. Epidemiology of gastric cancer in Chile: I. Case-control study. *Int. J. Epidemiol.* 10:53-56.
- Armstrong, B. 1980. The epidemiology of cancer in the Peoples Republic of China. *Int. J. Epidemiol.* 9:305-315.
- Asatoor, A. M., and M. L. Simenhoff. 1965. The origin of urinary dimethylamine. *Biochim. Biophys. Acta* III:384-392.
- Ashton, M. R. 1970. The Occurrence of Nitrates and Nitrites in Foods; Literature Survey No. 7. British Food Manufacturing Industries Research Association, Leatherhead, Surrey, United Kingdom. 33 pp.
- Bakshi, S. P., J. L. Fahey, and L. E. Pierce. 1967. Sausage cyanosis--Acquired methemoglobinemic nitrite poisoning. *N. Engl. J. Med.* 277:1072.
- Bannasch, P., and H.-A. Müller. 1964. [In German.] Lichtmikroskopische Untersuchungen über die Wirkung von N-Nitrosomorpholin auf die Leber von Ratte und Maus. *Arzneimit. Forsch.* 14:805-814.
- Barnes, J. M., and P. N. Magee. 1954. Some toxic properties of dimethylnitrosamine. *Br. J. Ind. Med.* 11:167-174.
- Bavin, P. M. G., G. J. Durant, P. D. Miles, R. C. Mitchell, and E. S. Pepper. 1980. Nitrosation of cimetidine [N"-cyano-N-methyl-N'-(2-[(5-methylimidazol-4-yl)methylthio]ethyl)guanidine]. *J. Chem. Res. Synop.* 1980:212-213.

- Berenblum, I., and P. Shubik. 1947. A new, quantitative, approach to the study of the stages of chemical carcinogenesis in the mouse's skin. *Br. J. Cancer* 1:383-391.
- Birdsall, J. J. 1981. Human dietary nitrite intake; and, results of animal feeding studies of nitrite. Paper presented at the Public Meeting of the Committee on Nitrite and Alternative Curing Agents in Food, January 22, 1981, National Research Council, National Academy of Sciences, Washington, D.C. [16] pp.
- Bjelke, E. 1973. Epidemiologic Studies of Cancer of the Stomach, Colon, and Rectum; with Special Emphasis on the Role of Diet, Vol. 1-4. Ph.D. thesis from the University of Minnesota, Minneapolis, Minn. Abstract available in *Diss. Abstr. Int.* 34(8).
- Bontoyan, W. R., M. W. Law, and D. P. Wright, Jr. 1979. Nitrosamines in agricultural and home-use pesticides. *J. Agric. Food Chem.* 27:631-634.
- Börzsönyi, M., A. Pintér, A. Surján, and I. Farkas. 1976. Transplacental induction of lymphomas in Swiss mice by carbendazim and sodium nitrite. *Int. J. Cancer* 17:742-747.
- Börzsönyi, M., A. Pintér, A. Surján, and A. Török. 1978. Carcinogenic effect of a guanidine pesticide administered with sodium nitrite on adult mice and on the offspring after prenatal exposure. *Cancer Lett.* 5:107-113.
- Boström, C.-E., and L.-E. Tammelin. 1981. Occurrence of precursors and N-alkyl-N-nitroso compounds. *Var Foda* 33 supp. 2:143-146.
- Boutwell, R. K. 1974. The function and mechanism of promoters of carcinogenesis. *Crit. Rev. Toxicol.* 2:419-443.
- Boyland, E., and S. A. Walker. 1975. Thiocyanate catalysis of nitrosamine formation and some dietary implications. Pp. 132-136 in P. Bogovski and E. A. Walker, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publications No. 9. International Agency for Research on Cancer, Lyon, France.
- Boyland, E., E. Nice, and K. Williams. 1971. The catalysis of nitrosation by thiocyanate from saliva. *Food Cosmet. Toxicol.* 9:639-643.
- Brewer, G. J. 1972. Clinical implications of variation in erythrocyte oxygen affinity: A. Blood storage and B. Arteriosclerosis. Pp. 629-645 in M. Rørth and P. Astrup, eds. *Oxygen Affinity of Hemoglobin and Red Cell Acid Base Status; Proceedings of the Alfred Benzon Symposium IV*. Munksgaard, Copenhagen, Denmark; and Academic Press, New York.

- Brooks, J. B., W. B. Cherry, L. Thacker, and C. C. Alley. 1972. Analysis by gas chromatography of amines and nitrosamines produced in vivo and in vitro by Proteus mirabilis. J. Infect. Dis. 126:143-153.
- Bruce, W. R., A. J. Varghese, R. Furrer, and P. C. Land. 1977. A mutagen in the feces of normal humans. Pp. 1641-1646 in H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds. Origins of Human Cancer: Book C. Human Risk Assessment. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Brundrett, R. B., M. Colvin, E. H. White, J. McKee, P. E. Hartman, and D. L. Brown. 1979. Comparison of mutagenicity, antitumor activity, and chemical properties of selected nitrosoarenes and nitrosoamides. Cancer Res. 39:1328-1333.
- Brusick, D. 1980. Principles of Genetic Toxicology. Plenum Press, New York. 279 pp.
- Buckley, J. D., P. J. O'Connor, and A. W. Craig. 1979. Pretreatment with acetylaminofluorene enhances the repair of O⁶-methylguanine in DNA. Nature 281:403-404.
- Bulay, O., and S. S. Mirvish. 1979. Carcinogenesis in rat esophagus by intraperitoneal injection of different doses of methyl-n-amylnitrosamine. Cancer Res. 39:3644-3646.
- Burden, E. H. W. J. 1961. The toxicology of nitrates and nitrites with particular reference to the potability of water supplies. Analyst 86:429-433.
- Cairns, J. 1980. Efficiency of the adaptive response of Escherichia coli to alkylating agents. Nature 286:176-178.
- Capoferro, R., and O. Torgersen. 1974. The effect of hypertonic saline on the uptake of tritiated 7,12-methylbenz(a)anthracene by the gastric mucosa. Scand. J. Gastroenterol. 9:343-349.
- Cardy, R. H., W. Lijinsky, and P. K. Hildebrandt. 1979. Neoplastic and nonneoplastic urinary bladder lesions induced in Fischer 344 rats and B6C3F₁ hybrid mice by N-nitrosodiphenylamine. Ecotoxicol. Environ. Saf. 3:29-35.
- Carlson, D. J., and F. L. Shapiro. 1970. Methemoglobinemia from well water nitrates: A complication of home dialysis. Ann. Intern. Med. 73:757-759.
- Chun, J., G. M. Singer, W. Lijinsky, and L. M. Xin. 1981. Factors Determining Formation of Nitroso Compounds in Moldy Corn Bread. A report prepared for the National Cancer Institute under Contract No. N01-CO-75380. National Cancer Institute, Bethesda, Maryland. 15 pp. + tables and figures.

- Clayson, D. B. 1980. Comparison between in vitro and in vivo tests for carcinogenicity. *Mutat. Res.* 75:205-213.
- Clayson, D. B. In press. Carcinogens and carcinogenesis enhancers. *Mutat. Res.*
- Cook-Mozaffari, P. J., F. Azordegan, N. E. Day, A. Rassicaud, C. Sabai, and B. Aramesh. 1979. Oesophageal cancer studies in the Caspian Littoral of Iran: Results of a case-control study. *Br. J. Cancer* 39:293-309.
- Corre, W. J., and T. Breimer. 1979. Nitrate and Nitrite in Vegetables. Literature Survey No. 39. Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands. 85 pp.
- Correa, P., C. Cuello, and E. Duque. 1970. Carcinoma and intestinal metaplasia of the stomach in Colombian migrants. *J. Natl. Cancer Inst.* 44:297-306.
- Correa, P., N. Sasano, G. N. Stemmermann, and W. Haenszel. 1973. Pathology of gastric carcinoma in Japanese populations: Comparisons between Miyagi Prefecture, Japan, and Hawaii. *J. Natl. Cancer Inst.* 51:1449-1459.
- Correa, P., O. Bolaños, F. T. Garcia, G. Gordillo, E. Duque, and C. Cuello. 1975a. The cancer registry of Cali, Colombia--Epidemiologic studies of gastric cancer. Recent results. *Cancer Res.* 50:155-169.
- Correa, P., W. Haenszel, C. Cuello, S. Tannenbaum, and M. Archer. 1975b. A model for gastric cancer epidemiology. *Lancet* 2:58-60.
- Correa, P., C. Cuello, E. Duque, L. C. Burbano, F. T. Garcia, O. Bolanos, G. Brown, and W. Haenszel. 1976. Gastric cancer in Colombia. III. Natural history of precursor lesions. *J. Natl. Cancer Inst.* 57:1027-1035.
- Correa, P., M. G. Kokatnur, and M. L. Murray. 1978. Letter to the Editor: Spermidine nitrosation and gastric cancer. *Lancet* 1:324.
- Correa, P., C. Cuello, G. Gordillo, G. Zarama, J. Lopez, W. Haenszel, and S. Tannenbaum. 1979. The gastric microenvironment in populations at high risk to stomach cancer. *Natl. Cancer Inst. Monogr.* 53:167-170.
- Couch, D. B., and M. A. Friedman. 1975. Interactive mutagenicity of sodium nitrite, dimethylamine, methylurea and ethylurea. *Mutat. Res.* 31:109-114.
- Couch, D. B., and A. W. Hsie. 1978. Mutagenicity and cytotoxicity of congeners of two classes of nitroso compounds in Chinese hamster ovary cells. *Mutat. Res.* 57:209-216.

- Coulston, F., and E. J. Olajos. 1980. Comparative toxicology of N-nitroso compounds and their carcinogenic potential to man. Pp. 25-86 in F. Coulston and J. F. Dunne, eds. The Potential Carcinogenicity of Nitrosatable Drugs, WHO Symposium, Geneva, Switzerland, June 1978. Ablex Publishing Corp., Norwood, New Jersey.
- Craddock, V. M. 1976. Cell proliferation and experimental liver cancer. Pp. 153-201 in H. M. Cameron, D. A. Linsell, and G. P. Warwick, eds. Liver Cell Cancer. Elsevier/North Holland Biomedical Press, Publishers, New York and Amsterdam.
- Cuello, C., P. Correa, W. Haenszel, G. Gordillo, C. Brown, M. Archer, and S. Tannenbaum. 1976. Gastric cancer in Colombia. I. Cancer risk and suspect environmental agents. J. Natl. Cancer Inst. 57:1015-1020.
- Cuello, C., P. Correa, G. Zarama, J. Lopez, J. Murray, and G. Gordillo. 1979. Histopathology of gastric dysplasias: Correlations with gastric juice chemistry. Am. J. Surg. Pathol. 3:491-500.
- Darling, R. C., and F. J. W. Roughton. 1942. The effect of methemoglobin of the equilibrium between oxygen and hemoglobin. Am. J. Physiol. 137:56-68.
- Davies, J. M. 1980. Stomach cancer mortality in workshop and other Nottinghamshire mining towns. Br. J. Cancer 41:438-445.
- Davison, K. L., W. Hansel, L. Krook, K. McEntee, and M. J. Wright. 1964. Nitrate toxicity in dairy heifers. I. Effects on reproduction, growth, lactation, and vitamin A nutrition. J. Dairy Sci. 47:1065-1073.
- Davison, K. L., K. McEntee, and M. J. Wright. 1965. Responses in pregnant ewes fed forages containing various levels of nitrate. J. Dairy Sci. 48:968-977.
- De Munter, H. K., L. den Engelse, and P. Emmelot. 1979. Studies on lung tumours. IV. Correlation between [³H] thymidine labelling of lung and liver cells and tumour formation in GRS/A and C3Hf/A male mice following administration of dimethylnitrosamine. Chem. Biol. Interact. 24:299-316.
- Diwan, B. A., and H. Meier. 1974. Strain- and age-dependent transplacental carcinogenesis by 1-ethyl-1-nitrosourea in inbred strains of mice. Cancer Res. 34:764-770.
- Dontenwill, W. and U. Mohr. 1961. [In German.] [Carcinoma of the respiratory tract following treatment of the golden hamster with diethylnitrosamine.] Z. Krebsforsch. 64:305-312.

- Druckrey, H. 1967. Quantitative aspects in chemical carcinogenesis. Pp. 60-78 in R. Truhaut, ed. Potential Carcinogenic Hazards from Drugs: Evaluation of Risks, UICC Monograph Series, Vol. 7, Springer-Verlag, Berlin, Heidelberg, New York.
- Druckrey, H. 1973. Specific carcinogenic and teratogenic effects of 'indirect' alkylating methyl and ethyl compounds, and their dependency on stages of ontogenic developments. *Xenobiotica* 3:271-303.
- Druckrey, H., and Ch. Landschütz. 1971. [In German; English summary.] [Transplacental and neonatal carcinogenesis by ethylnitrosobiuret (ENBU) in BD IX rats.] *Z. Krebsforsch.* 76:45-48.
- Druckrey, H., R. Preussmann, D. Schmähl, and M. Müller. 1962a. [In German.] Erzeugung von Blasenkrebs an Ratten mit N,N-Dibutyl-nitrosamin. *Naturwissenschaften* 49:19.
- Druckrey, H., D. Steinhoff, H. Beuthner, H. Schneider, and P. Klärner. 1962b. [In German; English summary.] Screening of nitrite for chronic toxicity in rats.] *Arzneim. Forsch.* 13:320-323.
- Druckrey, H., R. Preussmann, S. Ivankovic, C. H. Schmidt, H. D. Mennel, and K. W. Stahl. 1964. [In German; English summary.] [The selective induction of bladder cancer in rats using di-butyl- and N-butyl-N-butanol(4)-nitrosamine] *Z. Krebsforsch.* 66:280-290.
- Druckrey, H., S. Ivankovic, and R. Preussmann. 1966. Teratogenic and carcinogenic effects in the offspring after single injection of ethylnitrosourea to pregnant rats. *Nature* 210:1378-1379.
- Druckrey, H., R. Preussmann, S. Ivankovic, D. Schmähl, J. Afkham, G. Blum, H. D. Mennel, M. Müller, P. Petropoulos, and H. Schneider. 1967. [In German; English summary.] [Organotropic carcinogenic effects of 65 different N-nitroso-compounds on BD-rats.] *Z. Krebsforsch.* 69:103-201.
- Druckrey, H., S. Ivankovic, J. Bücheler, R. Preussmann, and C. Thomas. 1968. [In German.] Erzeugung von Magen- und Pankreas-Krebs beim Meerschweinchen durch Methylnitroso-harnstoff und -urethan. *Z. Krebsforsch.* 71:167-182.
- Druckrey, H., R. Preussmann, and S. Ivankovic. 1969. N-Nitroso compounds in organotropic and transplacental carcinogenesis. *Ann. N.Y. Acad. Sci.* 163:676-696.
- Druckrey, H., S. Ivankovic, and R. Preussmann. 1970. [In German.] Selektive Erzeugung von Carcinomen des Drüsenmagens bei ratten durch orale Gabe von N-methyl-N-nitroso-N'-acetylharnstoff (AcMNH). *Z. Krebsforsch.* 75:23-33.

- Druckrey, H., C. Landschütz, R. Preussmann, and S. Ivankovic. 1971. [In German.] Erzeugung von Magenkrebs und neurogenen Malignomen durch orale Gabe von Methyl-nitrosobiuret (MNB) an Ratten. *Z. Krebsforsch.* 75:229-239.
- Dubelman, S., and R. Shapiro. 1977. A method for the isolation of cross-linked nucleosides from DNA: Application to cross-links induced by nitrous acid. *Nucleic Acids Res.* 4:1815-1827.
- Egan, B., R. J. Waxweiler, L. Blade, J. Wolfe, and J. K. Wagoner. 1979. A preliminary report of mortality patterns among foundry workers. *J. Environ. Pathol. Toxicol.* 2(5):259-272.
- Ehling, U. H., R. B. Cumming, and H. V. Malling. 1968. Induction of dominant lethal mutations by alkylating agents in male mice. *Mutat. Res.* 5:417-428.
- Eisenbrand, G., B. Spiegelhalder, and R. Preussmann. 1980. Nitrate and nitrite in saliva. *Oncology* 37:227-231.
- Eisenbrand, G., B. Spiegelhalder, and R. Preussmann. 1981. Analysis of human biological specimens for nitrosamine contents. Pp. 275-283 in W. R. Bruce, P. Correa, M. Lipkin, S. R. Tannenbaum, and T. D. Wilkins, eds. *Gastrointestinal Cancer: Endogenous Factors, Banbury Report 7.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Elder, J. B., P. C. Ganguli, and I. E. Gillespie. 1979. Letter to the Editor: Gastric cancer in patients who have taken cimetidine. *Lancet* 2:245.
- Elespuru, R. K., and W. Lijinsky. 1976. Mutagenicity of cyclic nitrosamines in *Escherichia coli* following activation with rat liver microsomes. *Cancer Res.* 36:4099-4101.
- El-Merzabani, M. M., A. A. El-Aaser, and N. I. Zakhary. 1979. A study on the aetiological factors of biharzial bladder cancer in Egypt. I. Nitrosamines and their precursors in urine. *Eur. J. Cancer* 15:287-291.
- Emerick, R. J. 1974. Consequences of high nitrate levels in feed and water supplies. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 33:1183-1187.
- Emerick, R. J., L. B. Embry, and R. W. Seerley. 1965. Rate of formation and reduction of nitrite-induced methemoglobin in vitro and in vivo as influenced by diet of sheep and age of swine. *J. Anim. Sci.* 24:221-230.
- Emmelot, P. 1964. Introduction. Pp. 1-7 in P. Emmelot and O. Mühlbock, eds. *Cellular Control Mechanisms and Cancer, Proceedings of the Unio Internationalis Contra Cancrum Conference, September 9-13, 1963, Amsterdam, Holland.* Elsevier Publishing Company, Amsterdam, London, and New York.

- Endo, H., H. Noda, N. Kinoshita, N. Inui, and Y. Nishi. 1980. Formation of a transplacental mutagen, 1,3-di(4-sulfamoylphenyl) triazene, from sodium nitrite and sulfanilamide in human gastric juice and in the stomachs of hamsters. *J. Natl. Cancer Inst.* 65:547-551.
- Enoki, Y., H. Tokui, I. Tyuma, and T. Okuda. 1969. Oxygen equilibria of partially oxidized hemoglobin. *Respir. Physiol.* 7:300-309.
- Ewing, M. C., and R. M. Mayon-White. 1951. Cyanosis in infancy from nitrates in drinking water. *Lancet* 1:931-934.
- Fajen, J. M., G. A. Carson, D. P. Rounbehler, T. Y. Fan, R. Vita, U. E. Goff, M. H. Wolf, G. S. Edwards, D. H. Fine, V. Reinhold, and K. Biemann. 1979. N-Nitrosamines in the rubber and tire industry. *Science* 205:1262-1264.
- Fandre, M., R. Coffin, G. Dropsy, and J.-P. Bergel. 1962. [In French.] Epidémie de gastroentérite infantile á Escherichia coli O 127 B8 avec cyanose méthémoglobinémique. *Arch. Fr. Pediatr.* 19:1129-1131.
- Farber, E., and R. Cameron. 1980. The sequential analysis of cancer development. *Adv. Cancer Res.* 31:125-226.
- Fine, D. H. 1980a. Exposure assessment to preformed environmental N-nitroso compounds from the point of view of our own studies. *Oncology* 37:199-202.
- Fine, D. H. 1980b. N-Nitroso compounds in the environment. *Adv. Environ. Sci. Technol.* 10:39-123.
- Fong, Y. Y., and W. C. Chan. 1976. Effect of ascorbate on amine-nitrite carcinogenicity. Pp. 461-464 in E. A. Walker, P. Bogovski, and L. Griçuite, eds. *Environmental N-Nitroso Compounds Analysis and Formation*, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.
- Food and Drug Administration. 1980a. Re-Evaluation of the Pathology Findings of Studies on Nitrite and Cancer: Histologic Lesions in Sprague-Dawley Rats. Final Report submitted by the Universities Associated for Research and Education in Pathology, to the Food and Drug Administration, Public Health Service, U.S. Department of Health and Human Services, Washington, D.C. 231 pp.
- Food and Drug Administration. 1980b. Evaluation of the MIT Nitrite Feeding Study to Rats. Report by the Interagency Working Group on Nitrite Research. Food and Drug Administration, Public Health Service, Department of Health and Human Services, Washington, D.C. 76 pp. + attachments.

- Foster, A. B., M. Jarman, D. Manson, and H. R. Schulten. 1980. Structure and reactivity of nitrosocimetidine. *Cancer Lett.* 9:47-52.
- Foulds, L. 1958. The natural history of cancer. *J. Chronic Dis.* 8:2-37.
- Fox, R. R., B. A. Diwan, and H. Meier. 1975. Transplacental induction of primary renal tumors in rabbits treated with 1-ethyl-1-nitrosourea. *J. Natl. Cancer Inst.* 54:1439-1448.
- Frankel, A. D., B. K. Duncan, and P. E. Hartman. 1980. Nitrous acid damage to duplex deoxyribonucleic acid: Distinction between deamination of cytosine residues and a novel mutational lesion. *J. Bacteriol.* 142:335-338.
- Franklin, M. A., and S. C. Skoryna. 1971. Studies on natural gastric flora: Survival of bacteria in fasting human subjects. *Can. Med. Assoc. J.* 105:380-386.
- Franza, B. R., Jr., N. S. Oeschger, M. P. Oeschger, and P. S. Schein. 1980. Mutagenic activity of nitrosourea antitumor agents. *J. Natl. Cancer Inst.* 65:149-154.
- Freund, H. A. 1937. Clinical manifestations and studies in parenchymatous hepatitis. *Ann. Intern. Med.* 10:1144-1155.
- Fussgaenger, R. D., and H. Ditschuneit. 1980. Lethal exitus of a patient with N-nitrosodimethylamine poisoning, 2.5 years following the first ingestion and sign of intoxication. *Oncology* 37:273-277.
- Garcia, H., and W. Lijinsky. 1973. Studies of the tumorigenic effect in feeding of nitrosamino acids and of low doses of amines and nitrite to rats. *Z. Krebsforsch.* 79:141-144.
- Garner, R. C., C. Pickering, and C. N. Martin. 1979. Mutagenicity of methyl-, ethyl-, propyl- and butylnitrosourea towards Escherichia coli WP2 strains with varying DNA repair capabilities. *Chem. Biol. Interactions* 26:197-205.
- Geleperin, A., V. J. Moses, and G. Fox. 1976. Nitrate in water supplies and cancer. *Ill. Med. J.* 149:251-253.
- Gilbert, P., J. Rondelet, F. Poncelet, and M. Mercier. 1980. Mutagenicity of p-nitrosophenol. *Food Cosmet. Toxicol.* 18:523-525.
- Gislason, J., and H. K. Dahle. 1980. [In Norwegian.] Nitrat og nitritt i vårt miljø og kosthold. *Nor. Vet. Tidsskr.* 92:557-567.
- Goldfarb, S., and T. D. Pugh. In press. The origin and significance of hyperplastic hepatocellular islands and nodules in hepatic carcinogenesis. *J. Environ. Pathol. Toxicol.*

- Goldfarb, S., S. D. Vesselinovitch, T. D. Pugh, N. Mihailovich, H. Koen, and Y. He. 1981. Tumor progression during diethylnitrosamine (DEN) induced mouse hepatocarcinogenesis. Proc. Am. Assoc. Cancer Res. 22:124. Abstract 491.
- Gombar, C. T., D. E. Jensen, and P. N. Magee. 1981. Methylation of DNA in vivo by the nitroso-derivative of cimetidine. Proc. Am. Assoc. Cancer Res. 22:81. Abstract 320.
- Graw, J. J., H. Berg, and D. Schmähl. 1974. Carcinogenic and hepatotoxic effects of diethylnitrosamine in hedgehogs. J. Natl. Cancer Inst. 53:589.
- Green, L., D. Ralt, and S. R. Tannenbaum. In press. Nitrate, nitrite and N-nitroso compounds: Biochemistry, metabolism, toxicity and carcinogenicity. In A. Neuberger and T. H. Jukes, eds. Biochemistry of Nutrition, Vol. 2. University Park Press, Baltimore, Maryland.
- Greenberg, M., W. B. Birnkrant, and J. J. Schiftner. 1945. Outbreak of sodium nitrite poisoning. Am. J. Public Health 35:1217-1220.
- Greenblatt, M. 1973. Ascorbic acid blocking of aminopyrine nitrosation in NZO/BI mice. J. Natl. Cancer Inst. 50:1055-1056.
- Greenblatt, M., and W. Lijinsky. 1972a. Failure to induce tumors in Swiss mice after concurrent administration of amino acids and sodium nitrite. J. Natl. Cancer Inst. 48:1389-1392.
- Greenblatt, M., and W. Lijinsky. 1972b. Nitrosamine studies: Neoplasms of liver and genital mesothelium in nitrosopyrrolidine-treated MRC rats. J. Natl. Cancer Inst. 48:1687-1696.
- Greenblatt, M., and S. S. Mirvish. 1973. Dose-response studies with concurrent administration of piperazine and sodium nitrite to strain A mice. J. Natl. Cancer Inst. 50:119-124.
- Greenblatt, M., and K. Rijhsinghani. 1969. Comparative cytopathologic alterations induced by alkylnitrosamines in nasal epithelium of the Syrian hamster. J. Natl. Cancer Inst. 42:421-433.
- Greenblatt, M., S. Mirvish, and B. T. So. 1971. Nitrosamine studies: Induction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice. J. Natl. Cancer Inst. 46:1029-1034.
- Greenblatt, M., V. R. C. Kommineni, and W. Lijinsky. 1973. Null effect of concurrent feeding of sodium nitrite and amino acids to MRC rats. J. Natl. Cancer Inst. 50:799-802.

- Guslandi, M. 1979. Letter to the Editor: Gastric cancer in patients who have taken cimetidine. *Lancet* 1:1406.
- Guttenplan, J. B. 1978. Mechanisms of inhibition by ascorbate of microbial mutagenesis induced by N-nitroso compounds. *Cancer Res.* 38:2018-2022.
- Guttenplan, J. B. 1979. Comutagenic effects exerted by N-nitroso compounds. *Mutat. Res.* 66:25-32.
- Guttenplan, J. B., F. Hutterer, and A. J. Garro. 1976. Effects of cytochrome P-448 and P-450 inducers on microsomal dimethylnitrosamine demethylase activity and the capacity of isolated microsomes to activate dimethylnitrosamine to a mutagen. *Mutat. Res.* 35:415-422.
- Gwatkin, R., and P. J. G. Plummer. 1946. Toxicity of certain salts of sodium and potassium for swine. *Can. J. Comp. Med. Vet. Sci.* 10:183-190.
- Haenszel, W. 1967. Epidemiology of gastric cancer. Pp. 3-28 in G. McNeer and G. T. Pack, eds. *Neoplasms of the Stomach*. J. B. Lippincott, Philadelphia, Pennsylvania; and Toronto, Canada.
- Haenszel, W., and P. Correa. 1975. Developments in the epidemiology of stomach cancer over the past decade. *Cancer Res.* 35:3452-3459.
- Haenszel, W., M. Kurihara, M. Segi, and R. K. C. Lee. 1972. Stomach cancer among Japanese in Hawaii. *J. Natl. Cancer Inst.* 49:969-988.
- Haenszel, W., J. W. Berg, M. Segi, M. Kurihara, and F. B. Locke. 1973. Large-bowel cancer in Hawaiian Japanese. *J. Natl. Cancer Inst.* 51:1765-1779.
- Haenszel, W., P. Correa, C. Cuello, N. Guzman, L. C. Burbano, H. Lores, and J. Munoz. 1976a. Gastric cancer in Colombia. II. Case-control epidemiologic study of precursor lesions. *J. Natl. Cancer Inst.* 57:1021-1026.
- Haenszel, W., M. Kurihara, F. B. Locke, K. Shimuzu, and M. Segi. 1976b. Stomach cancer in Japan. *J. Natl. Cancer Inst.* 56:265-274.
- Halver, J. E., C. L. Johnson, and L. M. Ashley. 1962. Dietary carcinogens induce fish hepatoma. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 21:390.
- Harada, M., H. Ishiwata, Y. Nakamura, A. Tanimura, and M. Ishidate. 1975. Studies on in vivo formation of nitroso compounds (I). Changes of nitrite and nitrate concentrations in human saliva after ingestion of salted Chinese cabbage. *J. Food Hyg. Soc.* 16:11-18.

- Hard, G. C. 1979. Effect of age at treatment on incidence and type of renal neoplasm induced in the rat by a single dose of dimethylnitrosamine. *Cancer Res.* 39:4965-4970.
- Hard, G. C., and W. H. Butler. 1970. Cellular analysis of renal neoplasia: Light microscope study of the development of interstitial lesions induced in the rat kidney by a single carcinogenic dose of dimethylnitrosamine. *Cancer Res.* 30:2806-2815.
- Hard, G. C., and W. H. Butler. 1971. Morphogenesis of epithelial neoplasms induced in the rat kidney by dimethylnitrosamine. *Cancer Res.* 31:1496-1505.
- Hartman, P. E. 1980. Bacterial mutagenesis: Review of new insights. *Environ. Mutagen.* 2:3-16.
- Hawker, P. C., T. J. Muscroft, and M. R. B. Keighley. 1980. Letter to the Editor: Gastric cancer after cimetidine in patient with two negative pre-treatment biopsies. *Lancet* 1:709-710.
- Hawksworth, G., M. J. Hill, G. Gordillo, and C. Cuello. 1975. Possible relationship between nitrates, nitrosamines and gastric cancer in south-west Colombia. Pp. 229-234 in P. Bogovski and E. A. Walker, eds. *N-Nitroso Compounds in the Environment*, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Hayakawa, H., K. Kumura, and M. Sekiguchi. 1978. Role of uracil-DNA glycosylase in the repair of deaminated cytosine residues of DNA in *Escherichia coli*. *J. Biochem.* 84:1155-1164.
- Hendy, R., and P. Grasso. 1977. Hepatotoxic response to single or repeated injections of N-nitrosopyrrolidine in the rat. *Chem. Biol. Interact.* 18:309-326.
- Herron, D. C., and R. C. Shank. 1980. Methylated purines in human liver DNA after probable dimethylnitrosamine poisoning. *Cancer Res.* 40:3116-3117.
- Hicks, R. M., C. L. Walters, I. Elsebai, A.-B. El Aasser, M. El Merzabani, and T. A. Gough. 1977. Demonstration of nitrosamines in human urine: Preliminary observations on a possible etiology for bladder cancer in association with chronic urinary tract infections. *Proc. R. Soc. Med.* 70:413-417.
- Higginson, J. 1966. Etiological factors in gastrointestinal cancer in man. *J. Natl. Cancer Inst.* 37:527-545.
- Hill, M. J., G. Hawksworth, and G. Tattersall. 1973. Bacteria, nitrosamines and cancer of the stomach. *Br. J. Cancer* 28:562-567.

- Hince, T. A., and S. Neale. 1974. A comparison of the mutagenic action of the methyl and ethyl derivatives of nitrosamides and nitrosamidines on Escherichia coli. *Mutat. Res.* 24:383-387.
- Hoch-Ligeti, C., M. F. Argus, and J. C. Arcos. 1968. Combined carcinogenic effects of dimethylnitrosamine and 3-methylcholanthrene in the rat. *J. Natl. Cancer Inst.* 40:535-549.
- Hoffmann, D., R. Raineri, S. S. Hecht, R. Maronpot, and E. L. Wynder. 1975. A study of tobacco carcinogenesis. XIV. Effects of N'-nitrosonornicotine and N'-nitrosoanabasine in rats. *J. Natl. Cancer Inst.* 55:977-981.
- Hoffmann, D., J. D. Adams, K. D. Brunnemann, and S. S. Hecht. In press. Formation, occurrence and carcinogenicity of N-nitrosamines in tobacco products. In R. A. Scanlan and S. R. Tannenbaum, eds. *N-Nitroso Compounds*, ACS Symposium Series. American Chemical Society, Washington, D.C.
- Hollstein, M., J. McCann, F. A. Angelosanto, and W. W. Nichols. 1979. Short-term tests for carcinogens and mutagens. *Mutat. Res.* 65:133-226.
- Hook, G. E. R., J. K. Haseman, and G. W. Lucier. 1975. Induction and suppression of hepatic and extrahepatic microsomal foreign-compound-metabolizing enzyme systems by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Chem. Biol. Interact.* 10:199-214.
- Howe, G. R., J. D. Burch, A. B. Miller, G. M. Cook, J. Esteve, B. Morrison, P. Gordon, L. W. Chambers, G. Fodor, and G. M. Winsor. 1980. Tobacco use, occupation, coffee, various nutrients, and bladder cancer. *J. Natl. Cancer Inst.* 64:701-713.
- Høyem, T. 1974. Nitrate and nitrite contents in Norwegian food. Pp. 466-470 in *Proceedings of the IV International Congress of Food Science and Technology*, Vol. 3. Copies of Proceedings are available from Instituto de Agroquímica y Tecnología de Alimentos, c/ Jaime Roig, II, Valencia - 10, Spain.
- Huang, D. P., J. H. C. Ho, and T. A. Gough. 1978a. Analysis for volatile nitrosamines in salt-preserved foodstuffs traditionally consumed by southern Chinese. Pp. 309-314 in G. de The and Y. Ito, eds. *Nasopharyngeal Carcinoma: Etiology and Control*, IARC Scientific Publication No. 20. International Agency for Research on Cancer, Lyon, France.
- Huang, D. P., J. H. C. Ho, D. Saw, and T. B. Teoh. 1978b. Carcinoma of the nasal and paranasal regions in rats fed Cantonese salted marine fish. Pp. 315-328 in G. de Thé and Y. Ito, eds. *Nasopharyngeal Carcinoma: Etiology and Control*, IARC Scientific Publication No. 20. International Agency for Research on Cancer, Lyon, France.

- Ichinotsubo, D., E. A. MacKinnon, C. Liu, S. Rice, and H. F. Mower. 1981. Mutagenicity of nitrosated cimetidines. *Carcinogenesis* 2:261-264.
- Ii, Y., P. Pour, and J. Althoff. 1979. Comparative studies of neoplastic response to a single dose of nitroso compounds. *J. Cancer Res. Clin. Oncol.* 94:1-5.
- Inai, K., Y. Aoki, and S. Tokuoka. 1979. Chronic toxicity of sodium nitrite in mice, with reference to its tumorigenicity. *Gann* 70:203-208.
- Interagency Regulatory Liaison Group. 1979. Scientific Bases for identification of potential carcinogens and estimation of risks. *J. Natl. Cancer Inst.* 63:241-268.
- Ishidate, M. 1977. Personal communication as cited in H. Endo, M. Ishizawa, T. Endo, K. Takahashi, T. Utsumomiya, N. Kinoshita, K. Hidaka, and T. Baba. 1977. A possible process of conversion of food components to gastric carcinogens. Pp. 1591-1607 in H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds. *Origins of Human Cancer, Book C: Human Risk Assessment.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Ishiwata, H., P. Boriboon, Y. Nakamura, M. Harada, A. Tanimura, and M. Ishidate. 1975. Changes of nitrite and nitrate concentrations in human saliva after ingestion of vegetables or sodium nitrate. *J. Food Hyg. Soc.* 16:19-24.
- Ishiwata, H., H. Mizushiro, A. Tanimura, and T. Murata. 1978. Metabolic fate of the precursors of N-nitroso compounds (III). Urinary excretion of nitrate in man. *J. Food Hyg. Soc.* 19:318-322.
- Ivankovic, S. 1979. Teratogenic and carcinogenic effects of some chemicals during prenatal life in rats, Syrian golden hamsters, and minipigs. *Natl. Cancer Inst. Monogr.* 51:103-115.
- Ivankovic, S., and H. Druckrey. 1968. [In German; English summary.] [Transplacental induction of malignant tumours of the nervous-system. I. Ethyl-nitroso-urea (ENU) in BD-IX-rats.] *Z. Krebsforsch.* 71:320-360.
- Ivankovic, S., and R. Preussmann. 1970. [In German.] Transplazentare Erzeugung maligner Tumoren. *Naturwissenschaften* 57:460.
- Ivankovic, S., R. Preussmann, D. Schmähl, and J. Zeller. 1973. [In German; English summary.] [Prevention of nitrosamide-induced hydrocephali by ascorbic acid after prenatal administration of ethylurea and nitrite in rats.] *Z. Krebsforsch.* 79:145-147.

- Ivankovic, S., R. Preussmann, D. Schmähl, and J. W. Zeller. 1975. Prevention by ascorbic acid of in vivo formation of N-nitroso compounds. Pp. 101-102 in P. Bogovski and E. A. Walker, eds. N-Nitroso Compounds in the Environment, IARC Scientific Publication No. 9. International Agency for Research on Cancer, Lyon, France.
- Iwaoka, W. T., C. A. Krone, J. J. Sullivan, E. H. Meaker, C. A. Johnson, and L. S. Miyasato. 1981. A source of error in mutagen testing of foods. *Cancer Lett.* 11:225-230.
- Jaffé, E. R. 1981. Methaemoglobinaemia. *Clin. Haematol.* 10:99-122.
- Jaffé, E. R., and P. Heller. 1964. Methemoglobinemia in man. *Prog. Hematol.* 4:48-71.
- Jägerstad, M., and R. Nilsson. 1977. Intake of nitrate and nitrite of some Swedish consumers as measured by the duplicate portion technique. Pp. 283-287 in B. J. Tinbergen and B. Krol, eds. Proceedings of the Second International Symposium on Nitrite in Meat Products, September 7-10, 1976, Zeist, the Netherlands. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Jägerstad, M., A. Nordén, and R. Nilsson. 1976. Dietary intake of nitrate and nitrite using the duplicate-sampling portion technique. *Ambio.* 6:276-277.
- Jeggo, P., M. Defais, L. Samson, and P. Schendel. 1977. An adaptive response of E. coli to low levels of alkylating agent: Comparison with previously characterised DNA repair pathways. *Mol Gen. Genet.* 157:1-9.
- Jensen, C. W., and H. D. Anderson. 1941. Rate of formation and disappearance of methemoglobin following oral administration or injection of sodium nitrite. *Proc. S. D. Acad. Sci.* 21:37-40.
- Jensen, D. E., and P. N. Magee. 1981. Methylation of DNA by nitrosocimetidine in vitro. *Cancer Res.* 41:230-236.
- Joint Iran-International Agency for Research on Cancer Study Group. 1977. Esophageal cancer studies in the Caspian Littoral of Iran: Results of population studies--A prodrome. *J. Natl. Cancer Inst.* 59:1127-1138.
- Jones, C. A., P. J. Marlino, W. Lijinsky, and E. Huberman. In press. The relationship between the carcinogenicity and mutagenicity of nitrosamines in a hepatocyte-mediated mutagenicity assay. *Carcinogenesis.*

- Joosens, J. V., and J. Geboers. 1981. Nutrition and gastric cancer. Proc. Nutr. Soc. 40:37-46.
- Kakizoe, T., T.-T. Wang, V. W. S. Eng, R. Furrer, P. Dion, and W. R. Bruce. 1979. Volatile N-nitrosamines in the urine of normal donors and of bladder cancer patients. Cancer Res. 39:829-832.
- Karran, P., T. Lindahl, and B. Griffin. 1979. Adaptive response to alkylating agents involves alteration in situ of O⁶-methylguanine residues in DNA. Nature 280:76-77.
- Kawabata, T., M. Ino, and H. Ohshima. 1979a. Formation of methylguanidine, a mother substance of N-nitrosomethylcyanamide and N-nitrosomethylurea, in smoked-dried skipjack sticks, katsuo-bushi. Bull. Jpn. Soc. Sci. Fish. 45:971-976.
- Kawabata, T., H. Ohshima, J. Uibu, M. Nakamura, M. Matsui, and M. Hamano. 1979b. Occurrence, formation, and precursors of N-nitroso compounds in Japanese diet. Pp. 195-209 in E. C. Miller, J. A. Miller, I. Hirono, T. Sugimura, and S. Takayama, eds. Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis. Japan Scientific Society Press, Tokyo, Japan; and University Park Press, Baltimore, Maryland.
- Kiese, M. 1974. Methemoglobinemia: A Comprehensive Treatise. CRC Press, Cleveland, Ohio. 259 pp.
- Kinosita, R. 1969. Studies on factors affecting chemical carcinogenesis of mouse stomach. Gann Monogr. 8:263-268.
- Kodama, F., M. Umeda, and T. Tsutsui. 1976. Mutagenic effect of sodium nitrite on cultured mouse cells. Mutat. Res. 40:119-124.
- Kohl, D. H. 1973. Public Health Consequences of High Nitrate Concentrations in Surface Water. Report prepared for the Illinois Institute of Environmental Quality, March 1973, Illinois Institute of Environmental Quality, Springfield, Illinois.
- Kolonel, L. N., J. H. Hankin, J. Lee, S. Chu, A. Nomura, and M. W. Hinds. 1981. Nutrient intakes in relation to cancer incidence in Hawaii. Br. J. Cancer 44:332.
- Konetzka, W. A. 1974. Mutagenesis by nitrate reduction in Escherichia coli. P. 37 in Abstracts of the Annual Meeting of the American Society for Microbiology 1974, May 12-17, 1974, Chicago, Ill. American Society for Microbiology, Washington, D.C. Abstract G106.

- Koppang, N., and H. Rimeslatten. 1976. Toxic and carcinogenic effects of nitrosodimethylamine in mink. Pp. 443-452 in E. A. Walker, P. Bogovski, and L. Gričiute, eds. Environmental N-Nitroso Compounds: Analysis and Formation, IARC Scientific Publication No. 14. International Agency for Research on Cancer, Lyon, France.
- Kotaka, T., and R. L. Baldwin. 1964. Effects of nitrous acid on the dAT copolymer as a template for DNA polymerase. *J. Mol. Biol.* 9:323-339.
- Kröger, M., and B. Singer. 1979. Ambiguity and transcriptional errors as a result of methylation of N-1 of purines and N-3 of pyrimidines. *Biochemistry* 18:3493-3500.
- Kunz, W., K. E. Appel, R. Rickart, M. Schwarz, and G. Stöckle. 1978. Enhancement and inhibition of carcinogenic effectiveness of nitrosamines. Pp. 261-283 in H. Remmer, H. M. Bolt, P. Bannasch, and H. Popper, eds. University Park Press, Baltimore, Maryland.
- Künzer, W., and D. Schneider. 1953. [In German; English summary.] Zur Aktivität der reduzierenden Fermentsysteme in den Erythrozyten junger Säuglinge. *Acta Haematol.* 9:346-353.
- Langenbach, R., R. Gingell, C. Kuszynski, B. Walker, D. Nagel, and P. Pour. 1980. Mutagenic activities of oxidized derivatives of N-nitrosodipropylamine in the liver cell-mediated and Salmonella typhimurium assays. *Cancer Res.* 40:3463-3467.
- Larimer, F. W., D. W. Ramey, W. Lijinsky, and J. L. Epler. 1978. Mutagenicity of methylated N-nitrosopiperidines in Saccharomyces cerevisiae. *Mutat. Res.* 57:155-161.
- Lathia, D., and M. Rütten. 1979. Cumulative catalytic effects of some physiologically active ions on the rate of nitrosation of N-methylaniline in vitro. *Nutr. Cancer* 1(2):19-22.
- Lawley, P. D. 1980. DNA as a target of alkylating carcinogens. *Br. Med. Bull.* 36:19-24.
- Leaver, D. D., P. F. Swann, and P. N. Magee. 1969. The induction of tumours in the rat by a single oral dose of N-nitrosomethylurea. *Br. J. Cancer* 23:177-187.
- Lee, D. H. K. 1970. Nitrates, nitrites, and methemoglobinemia. *Environ. Res.* 3:484-511.

- Lee, K., B. Gold, and S. S. Mirvish. 1977. Mutagenicity of 22 N-nitrosamides and related compounds for Salmonella typhimurium TA1535. *Mutat. Res.* 48:131-138.
- Li, M., P. Li, and B. Li. 1980. Recent progress in research on esophageal cancer in China. *Adv. Cancer Res.* 33:173-249.
- Lijinsky, W., and M. D. Reuber. 1980. Tumours induced in Fischer 344 rats by the feeding of disulfiram together with sodium nitrite. *Food Cosmet. Toxicol.* 18:85-87.
- Lijinsky, W., and M. D. Reuber. 1981. Carcinogenic effect of nitrosopyrrolidine, nitrosopiperidine and nitrosohexamethylenimine in Fischer rats. *Cancer Lett.* 12:99-103.
- Lijinsky, W., and H. W. Taylor. 1975. Induction of urinary bladder tumors in rats by administration of nitrosomethyldodecylamine. *Cancer Res.* 35:958-961.
- Lijinsky, W., and H. W. Taylor. 1978. Relative carcinogenic effectiveness of derivatives of nitrosodiethylamine in rats. *Cancer Res.* 38:2391-2394.
- Lijinsky, W., M. Greenblatt, and C. Kommineni. 1973. Feeding studies of nitrilotriacetic acid and derivatives in rats. *J. Natl. Cancer Inst.* 50:1061-1063.
- Lijinsky, W., M. D. Reuber, and W. B. Manning. 1980. Potent carcinogenicity of nitrosodiethanolamine in rats. *Nature* 288:589-590.
- Lin, J. Y., H.-I. Wang, and Y.-C. Yeh. 1979. The mutagenicity of soy bean sauce. *Food Cosmet. Toxicol.* 17:329-331.
- Lindahl, T. 1979. DNA glycosylases, endonucleases for apurinic/aprimidinic sites, and base excision-repair. *Progr. Nucleic Acid Res. Mol. Biol.* 22:135-192.
- Lipkin, M. 1975. Biology of large bowel cancer: Present status and research frontiers. *Cancer* 36:2319-2324.
- Litman, R. M. 1961. Genetic and chemical alterations in the transforming deoxyribonucleic acid DNA of *Pneumococcus* caused by ultraviolet light and by nitrous acid. *J. Chim. Phys.* 58:997-1004.

- Lucier, G. W., B. R. Sonawane, and O. S. McDaniel. 1977. Glucuronidation and deglucuronidation reactions in hepatic and extrahepatic tissues during perinatal development. *Drug Metab. Dispos.* 5:279-287.
- Maekawa, A., S. Odashima, and M. Nakadate. 1976. Induction of tumors in the stomach and nervous system of the ACI/N rat by continuous oral administration of 1-methyl-3-acetyl-1-nitroso-urea. *Z. Krebsforsch.* 86:195-207.
- Magee, P. N., and J. M. Barnes. 1956. The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. *Br. J. Cancer* 10:114-122.
- Magee, P. N., and J. M. Barnes. 1959. The experimental production of tumours in the rat by dimethylnitrosamine (N-nitrosodimethylamine). *Acta Unio Int. Contra Cancrum* 15:187-190.
- Magee, P. N., and J. M. Barnes. 1962. Induction of kidney tumours in the rat with dimethylnitrosamine (N-nitrosodimethylamine). *J. Pathol. Bacteriol.* 84:19-31.
- Mager, J., S. Bornstein, and A. Halbreich. 1965. Enhancement of the polyuridylic acid-directed phenylalanine polymerization in liver-microsome preparations from rats treated with carbon tetrachloride or dimethylnitrosamine. *Biochim. Biophys. Acta* 95:682-684.
- Makiura, S., Y. Kamamoto, S. Sugihara, K. Hirao, Y. Hiasa, M. Arai, and N. Ito. 1973. Effect of 1-naphthyl isothiocyanate and 3-methylcholanthrene on hepatocarcinogenesis in rats treated with diethylnitrosoamine. *Gann* 64:101-104.
- Malling, H. V. 1971. Dimethylnitrosamine: Formation of mutagenic compounds by interaction with mouse liver microsomes. *Mutat. Res.* 13:425-429.
- Mancuso, T. F., A. Ciocco, and A. A. El-Attar. 1968. An epidemiological approach to the rubber industry: A study based on departmental experience. *J. Occup. Med.* 10:213-232.
- Marquardt, H., F. Rufino, and J. H. Weisburger. 1977a. Mutagenic activity of nitrite-treated foods: Human stomach cancer may be related to dietary factors. *Science* 196:1000-1001.

- Marquardt, H., F. Rufino, and J. H. Weisburger. 1977b. On the aetiology of gastric cancer: Mutagenicity of food extracts after incubation with nitrite. *Food Cosmet. Toxicol.* 15:97-100.
- Maruyama, S., S. Shimizu, and K. Muramatsu. 1979. Dietary intake of nitrate and urinary excretion of nitrate in the population of several areas in Nagano Prefecture. *J. Food Hyg. Soc. Jpn.* 20:276-282.
- Matsukura, N., T. Kawachi, K. Sasajima, T. Sano, T. Sugimura, and N. Ito. 1977. Induction of liver tumors in rats by sodium nitrite and methylguanidine. *Z. Krebsforsch. Klin. Onkol.* 90: 87-94.
- McCann, J., E. Choi, E. Yamasaki, and B. N. Ames. 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. *Proc. Natl. Acad. Sci. USA* 72:5135-5139.
- McLean, A. E. M., and P. N. Magee. 1970. Increased renal carcinogenesis by dimethyl nitrosamine in protein deficient rats. *Br. J. Exp. Pathol.* 51:587-590.
- McLean, A. E. M., and H. G. Verschuuren. 1969. Effects of diet and microsomal enzyme induction on the toxicity of dimethyl nitrosamine. *Br. J. Exp. Pathol.* 50:22-25.
- McMahon, R. E., J. C. Cline, and C. Z. Thompson. 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. *Cancer Res.* 39:682-693.
- McMichael, A. J., R. Spirtas, and L. L. Kupper. 1974. An epidemiologic study of mortality within a cohort of rubber workers, 1964-72. *J. Occup. Med.* 16:458-464.
- Medina, D. 1975. Tumor progression. Pp. 99-119 in F. F. Becker, ed. *Cancer: A Comprehensive Treatise, Vol. 3; Biology of Tumors: Cellular Biology and Growth.* Plenum Press, New York.
- Meinsma, L. 1964. [In Dutch; English summary.] [Nutrition and cancer.] *Voeding* 25:357-365.
- Meselson, M., and K. Russell. 1977. Comparisons of carcinogenic and mutagenic potency. Pp. 1473-1481 in H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds. *Origins of Human Cancer, Book C: Human Risk Assessment.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

- Metcalf, W. K. 1961. Sensitivity of haemoglobin to oxidation in various conditions. *Nature* 190:543.
- Mettlin, C., S. Graham, R. Priore, J. Marshall, and M. Swanson. 1981. Diet and cancer of the esophagus. *Nutr. Cancer* 2:143-147.
- Miller, E. C., and J. A. Miller. 1976. The metabolism of chemical carcinogens to reactive electrophiles and their possible mechanisms of action in carcinogenesis. Pp. 737-762 in C. E. Searle, ed. *Chemical Carcinogens*, ACS Monograph No. 173. American Chemical Society, Washington, D.C.
- Mirvish, S. S. 1971. Kinetics of nitrosamide formation from alkylureas, N-alkylurethans, and alkylguanidines: Possible implications for the etiology of human gastric cancer. *J. Natl. Cancer Inst.* 46:1183-1193.
- Mirvish, S. S. 1975. Formation of N-nitroso compounds: Chemistry, kinetics, and in vivo occurrence. *Toxicol. Appl. Pharmacol.* 31: 325-351.
- Mirvish, S. S. 1977. N-Nitroso compounds: Their chemical and in vivo formation and possible importance as environmental carcinogens. *J. Toxicol. Environ. Health* 2:1267-1277.
- Mirvish, S. S., and D. A. Cairnes. 1981. Identification of the compound in a fish product yielding methylurea (MU) on nitrosation-denitrosation as creatinine (CRN). *Proc. Am. Assoc. Cancer Res.* 22:140. Abstract 555.
- Mirvish, S. S., and C. Chu. 1973. Chemical determination of methylnitrosourea and ethylnitrosourea in stomach contents of rats, after intubation of the alkylureas plus sodium nitrite. *J. Natl. Cancer Inst.* 50:745-750.
- Mirvish, S. S., M. Greenblatt, and V. R. C. Kommineni. 1972a. Nitrosamine formation in vivo: Induction of lung adenomas in Swiss mice by concurrent feeding of nitrite and methylurea or ethylurea. *J. Natl. Cancer Inst.* 48:1311-1315.
- Mirvish, S. S., L. Wallcave, M. Eagen, and P. Shubik. 1972b. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. *Science* 177: 65-68.
- Mirvish, S. S., J. Sams, T. Y. Fan, and S. R. Tannenbaum. 1973. Kinetics of nitrosation of the amino acids proline, hydroxyproline, and sarcosine. *J. Natl. Cancer Inst.* 51:1833-1839.

- Mirvish, S. S., A. Cardesa, L. Wallcave, and P. Shubik. 1975. Induction of mouse lung adenomas by amines or ureas plus nitrite and by N-nitroso compounds: Effect of ascorbate, gallic acid, thiocyanate, and caffeine. *J. Natl. Cancer Inst.* 55:633-636.
- Mirvish, S. S., C. Chu, and D. B. Clayson. 1978. Inhibition of [³H]thymidine incorporation into DNA of rat esophageal epithelium and related tissues by carcinogenic N-nitroso compounds. *Cancer Res.* 38:458-466.
- Mirvish, S. S., O. Bulay, R. G. Runge, and K. Patil. 1980a. Study of the carcinogenicity of large doses of dimethylnitramine, N-nitroso-L-proline, and sodium nitrite administered in drinking water to rats. *J. Natl. Cancer Inst.* 64:1435-1442.
- Mirvish, S. S., K. Karlowski, D. F. Birt, and J. P. Sams. 1980b. Dietary and other factors affecting nitrosomethylurea (NMU) formation in the rat stomach. Pp. 271-279 in E. A. Walker, M. Castegnaro, L. Griciute, and M. Börzsönyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Mizrahi, I. J., and G. C. de Vries. 1965. Instability of poly-ribosomes derived from rats pretreated with the hepatocarcinogen dimethylnitrosamine. *Biochem. Biophys. Res. Commun.* 21:555-561.
- Modan, B., F. Lubin, V. Barell, R. A. Greenberg, M. Modan, and S. Graham. 1974. The role of starches in the etiology of gastric cancer. *Cancer* 34:2087-2092.
- Mohr, U., and J. Hilfrich. 1972. Effects of a single dose of N-diethylnitrosamine on the rat kidney. *J. Natl. Cancer Inst.* 49:1729-1731.
- Mohr, U., J. Althoff, D. Schmähl, and F. W. Krüger. 1970. The carcinogenic effect of dibutylnitrosamine in Syrian and Chinese hamsters. *Z. Krebsforsch.* 74:112-113.
- Monson, R. R., and K. K. Nakano. 1976. Mortality among rubber workers. I. White male union employees in Akron, Ohio. *Am. J. Epidemiol.* 103:284-296.
- Montes, G., C. Cuello, G. Gordillo, W. Pelon, W. Johnson, and P. Correa. 1979. Mutagenicity activity of gastric juice. *Cancer Lett.* 7:307-312.

- Montesano, R., and H. Bartsch. 1976. Mutagenic and carcinogenic N-nitroso compounds: Possible environmental hazards. *Mutat. Res.* 32:179-228.
- Montesano, R., and P. N. Magee. 1970. Metabolism of dimethylnitrosamine by human liver slices in vitro. *Nature* 228:173-174.
- Montesano, R., H. Brésil, and G. P. Margison. 1979. Increased excision of O⁶-methylguanine from rat liver DNA after chronic administration of dimethylnitrosamine. *Cancer Res.* 39:1798-1802.
- Mower, H. F., and J. H. Weisburger. 1978. Production of mutagens by nitrosation of the Japanese fish Sanma Hiraki. *Proc. Am. Assoc. Cancer Res.* 19:89. Abstract 354.
- Mullen, P. W. 1979. Letter to the Editor: Gastric cancer in patients who have taken cimetidine. *Lancet* 1:1406.
- Mundry, K. W., and A. Gierer. 1958. [In German; English summary.] Die Erzeugung von Mutationen des Tabakmosaikvirus durch chemische Behandlung seiner Nucleinsäure. *Z. Vererbungsl.* 89:614-630.
- Muñoz, N., P. Correa, C. Cuello, and E. Duque. 1968. Histologic types of gastric carcinoma in high- and low-risk areas. *Int. J. Cancer* 3:809-818.
- Murphey-Corb, M., H.-L. Kong, and M. L. Murray. 1980. Interaction of mutagenic spermidine-nitrous acid reaction products with uvr- and recA-dependent repair systems in Salmonella. *J. Bacteriol.* 142:191-195.
- Nakahara, W., and F. Fukuoka. 1959. Study of carcinogenic mechanism based on experiments with 4-nitroquinoline N-oxide. *Gann* 50:1-15.
- Napalkov, N. P. 1973. Some general considerations on the problem of transplacental carcinogenesis. Pp. 1-13 in L. Tomatis and U. Mohr, eds. *Transplacental Carcinogenesis*, IARC Scientific Publication No. 4. International Agency for Research on Cancer, Lyon, France.
- Natake, M., G. Danno, T. Maeda, K. Kawamura, and K. Kanazawa. 1979. Formation of DNA-damaging and mutagenic activity in the reaction systems containing nitrite and butylated hydroxyanisole, tryptophan, or cysteine. *J. Nutr. Sci. Vitaminol.* 25:317-332.

- National Academy of Sciences. 1978. Nitrates: An Environmental Assessment. A report prepared for the U. S. Environmental Protection Agency by the Panel on Nitrates of the Coordinating Committee for Scientific and Technical Assessments of Environmental Pollutants, Environmental Studies Board, Commission on Natural Resources, National Research Council. National Academy of Sciences, Washington, D.C. 723 pp.
- Neale, S. 1976. Mutagenicity of nitrosamides and nitrosamidines in micro-organisms and plants. *Mutat. Res.* 32:229-266.
- Newberne, P. M. 1978. Dietary Nitrite in the Rat. Final Report on Contract FDA 74-2181, Food and Drug Administration, Public Health Service, U.S. Department of Health, Education, and Welfare, Rockville, Md. Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.
- Newberne, P. M. 1979. Nitrite promotes lymphoma incidence in rats. *Science* 204:1079-1081.
- Nicholas, B. P., and C. Yanofsky. 1979. Nucleotide sequences of trpA of Salmonella typhimurium and Escherichia coli: An evolutionary comparison. *Genetics* 76:5244-5248.
- Nickerson, M. 1975. Vasodilator drugs. Pp. 727-743 in L. S. Goodman and A. Gilman, eds. *The Pharmacological Basis of Therapeutics*. MacMillan Publishing Co., New York, Toronto, and London.
- Oeda, K., K. Shimizu, and M. Sekiguchi. 1978. An enzyme activity specific for nitrous acid-treated DNA in Escherichia coli. *J. Biochem.* 84:1165-1169.
- Ogiu, T., M. Nakadate, and S. Odashima. 1975. Induction of leukemias and digestive tract tumors in Donryu rats by l-propyl-l-nitrosourea. *J. Natl. Cancer Inst.* 54:887-893.
- Olsen, P., and O. Meyer. 1977. Carcinogenicity study on rats fed on canned heated nitrite-treated meat: Preliminary communication. Pp. 275-278 in B. J. Tinbergen and B. Krol, eds. *Proceedings of the Second International Symposium on Nitrite in Meat Products*, September 7-10, 1976, Zeist, the Netherlands. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Opie, L. H. 1980. Drugs and the heart. II. Nitrates. *Lancet* 1: 750-753.

- Orgeron, J. D., J. D. Martin, C. T. Caraway, R. M. Martine, and G. H. Hauser. 1957. Methemoglobinemia from eating meat with high nitrite content. *Public Health Rep.* 72:189-193.
- Paneque, A., F. F. Del Campo, J. M. Ramírez, and M. Losada. 1965. Flavin nucleotide nitrate reductase from spinach. *Biochim. Biophys. Acta* 109:79-85.
- Pearson, A. M., S. D. Sleight, D. P. Cornforth, and B. T. Akoso. 1980. Effects of nitrosamines, nitrite and secondary amines on tumor development in mice. Pp. 216-218 in *Proceedings of the 26th European Meeting of Meat Research Workers, Vol. 2, Aug. 31-Sept. 5, 1980, Colorado Springs, Colorado.*
- Pegg, A. E. 1977. Formation and metabolism of alkylated nucleosides: Possible role in carcinogenesis by nitroso compounds and alkylating agents. *Adv. Cancer Res.* 25:195-269.
- Pegg, A. E. 1978. Dimethylnitrosamine inhibits enzymatic removal of O⁶-methylguanine from DNA. *Nature* 274:182-184.
- Petukhov, N. I., A. I. Ryvkin, G. G. Gainullin, and V. I. Landysheva. 1972. [In Russian; English summary.] [Water nitrate methaemoglobinemia in children and adolescents.] *Gig. Sanit.* 1972(3):14-18.
- Phillips, W. E. J. 1971. Naturally occurring nitrate and nitrite in foods in relation to infant methaemoglobinaemia. *Food Cosmet. Toxicol.* 9:219-228.
- Phillips, J. C., C. E. Heading, B. G. Lake, S. D. Gangolli, and A. G. Lloyd. 1975. Studies on the metabolism of dimethylnitrosamine in the rat. II. The effects of phenobarbitone and 20-methylcholanthrene on the in vitro and in vivo metabolism and acute toxicity of dimethylnitrosamine in young and mature rats. *Food Cosmet. Toxicol.* 13:611-617.
- Pignatelli, B., J. C. Béréziat, I. K. O'Neill, and H. Bartsch. 1981. Catalytic Role of Some Phenolic Substances in Endogenous Formation of N-Nitroso Compounds. Paper presented at 7th International Meeting on Analysis and Formation of N-Nitroso Compounds, September 28 - October 1, 1981. Meeting sponsored by the International Agency for Research on Cancer, Lyon, France.
- Pisciotta, A. V., S. N. Ebbe, and J. E. Hinz. 1959. Clinical and laboratory features of two variants of methemoglobin M disease. *J. Lab. Clin. Med.* 54:73-87.

- Pitot, H. C., and A. E. Sirica. 1980. The stages of initiation and promotion in hepatocarcinogenesis. *Biochim. Biophys. Acta* 605:191-215.
- Pitot, H. C., L. Barsness, T. Goldsworthy, and T. Kitagawa. 1978. Biochemical characterisation of stages of hepatocarcinogenesis after a single dose of diethylnitrosamine. *Nature* 271:456-458.
- Pound, A. W., and L. J. McGuire. 1978. Influence of repeated liver regeneration on hepatic carcinogenesis by diethylnitrosamine in mice. *Br. J. Cancer* 37:595-602.
- Pound, A. W., L. Horn, and T. A. Lawson. 1973a. Decreased toxicity of dimethylnitrosamine in rats after treatment with carbon tetrachloride. *Pathology* 5:233-242.
- Pound, A. W., T. A. Lawson, and L. Horn. 1973b. Increased carcinogenic action of dimethylnitrosamine after prior administration of carbon tetrachloride. *Br. J. Cancer* 27:451-459.
- Pour, P., F. W. Krüger, J. Althoff, A. Cardesa, and U. Mohr. 1974. Cancer of the pancreas induced in the Syrian golden hamster. *Am. J. Pathol.* 76:349-358.
- Pour, P. M., S. Z. Salmasi, and R. G. Runge. 1978. Selective induction of pancreatic ductular tumors by single doses of N-nitrosobis(2-oxopropyl)amine in Syrian golden hamsters. *Cancer Lett.* 4:317-323.
- Pour, P., R. Gingell, R. Langenbach, D. Nagel, C. Grandjean, T. Lawson, and S. Salmasi. 1980. Carcinogenicity of N-nitrosomethyl (2-oxopropyl)amine in Syrian hamsters. *Cancer Res.* 40:3585-3590.
- Preussmann, R. 1978. Toxicological aspects of food safety -- carcinogenicity and mutagenicity. *Arch. Toxicol. Suppl.* 1:69-84.
- Preussmann, R., M. Habs, and B. L. Pool. 1979. Carcinogenicity and mutagenicity testing of three isomeric N-nitroso-N-methylaminopyridines in rats. *J. Natl. Cancer Inst.* 62:153-156.
- Preussmann, R., B. Spiegelhalder, and G. Eisenbrand. 1980. Reduction of human exposure to environmental N-nitroso-carcinogens. Examples of possibilities for cancer prevention. Pp. 273-285 in B. Pullman, P. O. P. Ts'o, and H. Gelboin, eds. *Carcinogenesis: Fundamental Mechanisms and Environmental Effects*. D. Reidel Publishing Co., the Netherlands.

- Preussmann, R., M. Habs, D. Schmähl, and G. Eisenbrand. In press. Dose-response study on the carcinogenicity of N-nitrosodiethanolamine (NDELA) in male Sprague-Dawley rats. Paper presented at the 7th International Meeting on analysis and formation of N-Nitroso compounds, September 28-October 1, 1981, Tokyo, Japan. Meeting sponsored by the International Agency for Research on Cancer, Lyon, France.
- Procter, B. G., and G. Rona. 1977. A study of the potential carcinogenesis of cooked bacon fed to albino rats. Project No. 4631, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario.
- Radomski, J. L., D. Greenwald, W. L. Hearn, N. L. Block, and F. M. Woods. 1978. Nitrosamine formation in bladder infections and its role in the etiology of bladder cancer. *J. Urol.* 120:48-50.
- Rao, K. V. N., and S. D. Vesselinovitch. 1973. Age- and sex-associated diethylnitrosamine dealkylation activity of the mouse liver and hepatocarcinogenesis. *Cancer Res.* 33:1625-1627.
- Rao, T. K., A. A. Hardigree, J. A. Young, W. Lijinsky, and J. L. Epler. 1977. Mutagenicity of N-nitrosopiperidines with Salmonella typhimurium/microsomal activation system. *Mutat. Res.* 56:131-145.
- Rao, T. K., D. W. Ramey, W. Lijinsky, and J. L. Epler. 1979a. Mutagenicity of cyclic nitrosamines in Salmonella typhimurium: Effect of ring size. *Mutat. Res.* 67:21-26.
- Rao, T. K., J. A. Young, W. Lijinsky, and J. L. Epler. 1979b. Mutagenicity of aliphatic nitrosamines in Salmonella typhimurium. *Mutat. Res.* 66:1-7.
- Rapoport, I. A. 1948. [In Russian.] [The alkylation of the gene molecule.] *Dokl. Akad. Nauk SSSR* 59:1183-1186.
- Reddy, B. S., and K. Watanabe. 1979. Effect of cholesterol metabolites and promoting effect of lithocholic acid in colon carcinogenesis in germ-free and conventional F344 rats. *Cancer Res.* 39:1521-1524.
- Reed, P. I., P. G. Cassell, and C. L. Walters. 1979. Letter to the Editor: Gastric cancer in patients who have taken cimetidine. *Lancet* 1:1234-1235.

- Registrar General's Office for England and Wales. 1978. Occupational Mortality: The Registrar General's Decennial Supplement for England and Wales, 1970-72; XVIII, Mostly Tables, Diagrams and Maps. Office of Population Census and Surveys Series DS 1. HMSO No. [O 11 6906448]. Her Majesty's Stationary Office, London, United Kingdom. 224 pp.
- Rice, S., D. Ichinotsubo, G. Stemmermann, T. Hayashi, N. Palumbo, S. Sylvester, A. Nomura, and H. Mower. 1981. Nitrosation reactions of stomach mucosal tissue of the human and dog. Pp. 185-203 in W. R. Bruce, P. Correa, M. Lipkin, S. R. Tannenbaum, and T. D. Wilkins, eds. Gastrointestinal Cancer: Endogenous Factors, Banbury Report 7. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Richardson, H. L., A. R. Stier, and E. Borsos-Nachtnebel. 1952. Liver tumor inhibition and adrenal histologic responses in rats to which 3'-methyl-4-dimethylaminoazobenzene and 20-methyl-cholanthrene were simultaneously administered. *Cancer Res.* 12: 356-361.
- Roberts, W. K., and J. L. Sell. 1963. Vitamin A destruction by nitrite in vitro and in vivo. *J. Anim. Sci.* 22:1081-1085.
- Robins, P., and J. Cairns. 1979. Quantitation of the adaptive response to alkylating agents. *Nature* 280:74-76.
- Rogers, A. E., O. Sanchez, F. M. Feinsod, and P. M. Newberne. 1974. Dietary enhancement of nitrosamine carcinogenesis. *Cancer Res.* 34:96-99.
- Ross, J. D., and J. F. Desforges. 1959. Reduction of methemoglobin by erythrocytes from cord blood: Further evidence of deficient enzyme activity in the newborn period. *Pediatrics* 23:718-726.
- Rounbehler, D. P., I. S. Krull, E. U. Goff, K. M. Mills, J. Morrison, G. S. Edwards, D. H. Fine, J. M. Fajen, G. A. Carson, and V. Rheinhold. 1979. Exposure to N-nitrosodimethylamine in a leather tannery. *Food Cosmet. Toxicol.* 17:487-491.
- Ruddell, W. S. J. 1981. Letter to the Editor: Cimetidine, gastric pH and nitrosation. *Lancet* 1:784-785.
- Ruddell, W. S. J., E. S. Bone, C. L. Walters, and L. M. Blendis. 1976. Hypochlorhydria, gastric cancer, and gastric juice nitrite concentrations. *Gut* 17:831-832.
- Russell, W. L., E. M. Kelly, P. R. Hunsicker, J. W. Bangham, S. C. Maddux, and E. L. Phipps. 1979. Specific-locus test shows ethylnitrosourea to be the most potent mutagen in the mouse. *Proc. Natl. Acad. Sci. USA* 76:5818-5819.

- Russell, W. L., P. R. Hunsicker, and D. A. Carpenter. 1980. Additional results from specific-locus tests on the supermutagenicity of ethylnitrosourea in the mouse. *Environ. Mutagen.* 2:274.
- Rustia, M. 1975. Inhibitory effect of sodium ascorbate on ethylurea and sodium nitrite carcinogenesis and negative findings in progeny after intestinal inoculation of precursors into pregnant hamsters. *J. Natl. Cancer Inst.* 55:1389-1393.
- Rustia, M., and P. Shubik. 1974. Prenatal induction of neurogenic tumors in hamsters by precursors ethylurea and sodium nitrite. *J. Natl. Cancer Inst.* 52:605-608.
- Saito, T., and T. Sugimura. 1973. Biochemical studies on carcinogenesis in the glandular stomach of rats with N-methyl-N'-nitro-N-nitrosoguanidine. *Gann* 64:373-381.
- Salisbury, J. G., and P. J. O'Connor. 1976. Effect of treatment in vivo with N,N-dimethylnitrosamine or methyl methanesulphonate on the cytoplasmic DNA polymerase of regenerating rat liver. *Nucleic Acids Res.* 3:1561-1568.
- Samson, L., and J. Cairns. 1977. A new pathway for DNA repair in Escherichia coli. *Nature* 267:281-283.
- Sandberg, A. S. 1978. [In Swedish.] Nitrat och nitrit. Tillförsel och omsättning hos människan. Socialstyrelsen redovisar 1978:1. Socialstyrelsen, Stockholm, Sweden, as cited in S. A. Slorach. 1981. Dietary intake, in vivo formation and toxicology of nitrates, nitrites and N-nitroso compounds. *Var Foda* 33, Supp. 2:171-184.
- Sander, J. 1970. [In German.] Die Bedeutung des Nitrit- und Nitratgehalts von Nahrungsmitteln für die Bildung kanzerogener Nitrosamine in menschlichen Magen. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1:orig.* 212:331-335.
- Sander, J., and G. Bürkle. 1969. [In German; English summary.] [Induction of malignant tumors in rats by simultaneous feeding of nitrite and secondary amines.] *Z. Krebsforsch.* 73:54-66.
- Sato, T., T. Fukuyama, T. Suzuki, J. Takayanagi, T. Murakami, N. Shiotsuki, R. Tanaka, and R. Tsuji. 1959. Studies of the causation of gastric cancer. 2. The relation between gastric cancer mortality rate and salted food intake in several places in Japan. *Bull. Inst. Public Health Jpn.* 8:187-198.
- Scherer, E., and P. Emmelot. 1976. Kinetics of induction and growth of enzyme-deficient islands involved in hepatocarcinogenesis. *Cancer Res.* 36:2544-2554.

- Scheutwinkel-Reich, M., G. Ingerowski, and H.-J. Stan. 1980. Microbiological studies investigating mutagenicity of deep frying fat fractions and some of their components. *Lipids* 15:849-852.
- Schmährl, D. 1970. [In German; English summary.] [Experimental investigations in "syncarcinogenesis." VI. Addition of minimum doses of four different liver carcinogens in rats in liver cancer development.] *Z. Krebsforsch.* 74:457-466.
- Schmährl, D. 1980. Combination effects in chemical carcinogenesis. *Arch. Toxicol. Suppl.* 4:29-40.
- Schmährl, D., and H. Osswald. 1967. Carcinogenesis in different animal species by diethylnitrosamine. *Experientia* 23:497-498.
- Schmährl, D., and S. von Stackelberg. 1968. [In German; English summary.] [The influence of lactoflavin, nicotinamide or dipyridamole on the carcinogenic activity of diethylnitrosamine in rats. *Arzneim. Forsch.* 18:318-320.
- Schmährl, D., C. Thomas, and K. König. 1963. [In German; English summary.] [Studies of "syncarcinogenesis". I. Experiments on the induction of cancer in rats by the simultaneous administration of di-ethylnitrosamine and 4-dimethylamino-azobenzene.] *Z. Krebsforsch.* 65:342-350.
- Schmährl, D., C. Thomas, W. Sattler, and G. F. Scheld. 1965. [In German; English summary.] Experimental studies of syncarcinogenesis: III. Attempts to induce cancer in rats by administering di-ethylnitrosamine and CCl₄ (or ethyl alcohol) simultaneously. In addition, an experimental contribution regarding "alcoholic cirrhosis." *Z. Krebsforsch.* 66:526-532.
- Schmährl, D., M. Habs, and S. Ivankovic. 1978. Carcinogenesis of N-nitrosodiethylamine (DNA) in chickens and domestic cats. *Int. J. Cancer* 22:552-557.
- Schuster, H., and G. Schramm. 1958. [In German; English summary.] Bestimmung der biologisch wirksamen Einheit in der Ribosenucleinsäure des Tabakmosaikvirus auf chemischem Wege. *Z. Naturforsch.* 13B:697-704.
- Scott, E. M. 1960. The relation of diaphorase of human erythrocytes to inheritance of methemoglobinemia. *J. Clin. Invest.* 39:1176-1179.
- Scott, E. M., and I. V. Griffith. 1959. The enzymic defect of hereditary methemoglobinemia: Diaphorase. *Biochim. Biophys. Acta* 34:584-586.

- Scott, E. M., and D. D. Hoskins. 1958. Hereditary methemoglobinemia in Alaskan Eskimos and Indians. *Blood* 13:795-802.
- Selenka, F., and D. Brand-Grimm. 1976. [In German; English summary.] [Nitrate and nitrite in human food calculation of the daily intake and its range.] *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe B* 162:449-466.
- Sell, J. L., and W. K. Roberts. 1963. Effects of dietary nitrite on the chick: Growth, liver, vitamin A stores and thyroid weight. *J. Nutr.* 79:171-178.
- Sen, N. P., D. C. Smith, C. A. Moodie, and H. C. Grice. 1975. Failure to induce tumours in guinea-pigs after concurrent administration of nitrite and diethylamine. *Food Cosmet. Toxicol.* 13:423-425.
- Shank, R. C. 1975. Toxicology of N-nitroso compounds. *Toxicol. Appl. Pharmacol.* 31:361-368.
- Shank, R. C., and P. M. Newberne. 1976. Dose-response study of the carcinogenicity of dietary sodium nitrite and morpholine in rats and hamsters. *Food Cosmet. Toxicol.* 14:1-8.
- Shuval, H. I., and N. Gruener. 1971. Epidemiological and toxicological aspects of nitrates and nitrites in the environment. Paper presented at the Environmental Session of the 99th Annual Meeting of the American Public Health Association, October 13, 1971, Minneapolis, Minn. 22 pp. + figures and tables.
- Shuval, H. I., and N. Gruener. 1972. Epidemiological and toxicological aspects of nitrates and nitrites in the environment. *Am. J. Public Health* 62:1045-1052.
- Simon, J., J. M. Sund, M. J. Wright, A. Winter, and F. D. Douglas. 1958. Pathological changes associated with the lowland abortion syndrome in Wisconsin. *J. Am. Vet. Med. Assoc.* 132:164-169.
- Simon, C., H. Manzke, H. Kay, and G. Mrowetz. 1964. [In German; English summary.] Über Vorkommen, Pathogenese und Möglichkeiten zur Prophylaxe der durch Nitrit verursachten Methämoglobinämie. *Z. Kinderheilkd.* 91:124-138.
- Singer, B. 1979. N-Nitroso alkylating agents: Formation and persistence of alkyl derivatives in mammalian nucleic acids as contributing factors in carcinogenesis. *J. Natl. Cancer Inst.* 62:1329-1339.

- Singer, G. M., and W. Lijinsky. 1976. Naturally occurring nitrosatable compounds. I. Secondary amines in foodstuffs. *J. Agric. Food Chem.* 24:550-553.
- Singer, G. M., and H. W. Taylor. 1976. Carcinogenicity of N'-nitrosornicotine in Sprague-Dawley rats. *J. Natl. Cancer Inst.* 57:1275-1276.
- Singley, T. L., III. 1962. Secondary methemoglobinemia due to the adulteration of fish with sodium nitrite. *Ann. Inter. Med.* 57:800-803.
- Sinios, A., and W. Wodsak. 1965. [In German.] Die Spinatvergiftung des SÄuglings. *Dtsch. Med. Wochenschr.* 90: 1856-1863.
- Sokolowski, J. H., U. S. Garrigus, and E. E. Hatfield. 1960. Some effects of varied levels of potassium nitrate ingestion by lambs. *J. Anim. Sci.* 19:1295.
- Solt, D. B., A. Medline, and E. Farber. 1977. Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. *Am. J. Pathol.* 88:595-618.
- Statens Levnedsmiddelinstitut [State Food Institute]. 1981. [In Danish.] Nitrat og Nitrit i Kødvarer. Rapport fra en Intern Arbejdsgruppe [Report from the Internal Working Group]. Søborg, Denmark. 63 pp.
- Stemmermann, G. N., H. Mower, D. Ichinotsubo, L. Tomiyasu, M. Mandel, and A. Nomura. 1980. Mutagens in extracts of human gastric mucosa. *J. Natl. Cancer Inst.* 65:321-326.
- Stemmermann, G. N., H. Mower, S. Rice, D. Ichinotsubo, L. Tomiyasu, T. Hayashi, A. Nomura, and M. Mandel. 1981. Mutagens in extracts of gastrointestinal mucosa. Pp. 175-183 in W. R. Bruce, P. Correa, M. Lipkin, S. R. Tannenbaum, and T. D. Wilkins, eds. *Gastrointestinal Cancer: Endogenous Factors*, Banbury Report 7. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Stephany, R. W., and P. L. Schuller. 1980. Daily dietary intakes of nitrate, nitrite and volatile N-nitrosamines in the Netherlands using the duplicate portion sampling technique. *Oncology* 37:203-210.
- Sugimura, T., and T. Kawachi. 1973. Experimental stomach cancer. *Methods Cancer Res.* 7:245-308.

- Sugimura, T., and T. Kawach: 1978. Experimental stomach carcinogenesis. Pp. 327-341 in M. Lipkin and R. A. Good, eds. *Gastrointestinal Tract Cancer*. Plenum Medical Book Company, New York and London.
- Sugiyama, K., T. Tanaka, and H. Mori. 1979. [In Japanese; English summary.] Carcinogenicity examination of sodium nitrate in mice. *Gifu Daigaku Igakubu Kiyo* 27:1-6.
- Swann, P. F., and A. E. M. McLean. 1971. Cellular injury and carcinogenesis: The effect of a protein-free high-carbohydrate diet on the metabolism of dimethylnitrosamine in the rat. *Biochem. J.* 124:283-288.
- Takayama, S. 1969. Induction of tumors in ICR mice with N-nitroso-piperidine, especially in forestomach. *Naturwissenschaften* 56: 142.
- Takayama, S., and T. Imaizumi. 1969. Carcinogenic action of N-nitrosodibutylamine in mice. *Gann* 60:353.
- Tanaka, T. 1973. Transplacental induction of tumours and malformations in rats treated with some chemical carcinogens. Pp. 100-111 in L. Tomatis and U. Mohr, eds. *Transplacental Carcinogenesis*, IARC Scientific Publication No. 4. International Agency for Research on Cancer, Lyon, France.
- Tannenbaum, S. R. 1981. Endogenous formation of N-nitroso compounds. Pp. 269-273 in W. R. Bruce, P. Correa, M. Lipkin, S. R. Tannenbaum, and T. D. Wilkins, eds. *Gastrointestinal Cancer: Endogenous Factors*, Banbury Report 7. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Tannenbaum, S. R., M. C. Archer, J. S. Wishnok, P. Correa, C. Cuello, and W. Haenszel. 1977. Nitrate and the etiology of gastric cancer. Pp. 1609-1625 in H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds. *Origins of Human Cancer: Book C. Human Risk Assessment*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Tannenbaum, S. R., D. Moran, W. Rand, C. Cuello, and P. Correa. 1979. Gastric cancer in Colombia. IV. Nitrite and other ions in gastric contents of residents from a high-risk region. *J. Natl. Cancer Inst.* 62:9-12.
- Tatematsu, M., M. Takahashi, M. Hananouchi, T. Shirai, M. Hirose, S. Fukushima, and N. Ito. 1976. Protective effect of mucin on experimental gastric cancer induced by N-methyl-N'-nitrosoguanidine plus sodium chloride in rats. *Gann* 67:223-229.

- Taylor, H. W., and W. Lijinsky. 1975. Tumor induction in rats by feeding heptamethyleneimine and nitrite in water. *Cancer Res.* 35:812-815.
- Taylor, H. W., W. Lijinsky, P. Nettesheim, and C. M. Snyder. 1974. Alteration of tumor response in rat liver by carbon tetrachloride. *Cancer Res.* 34:3391-3395.
- Taylor, T. V., D. Lee, A. G. Howatson, J. Anderson, and I. B. MacLeod. 1979. Letter to the Editor: Gastric cancer in patients who have taken cimetidine. *Lancet* 1:1235-1236.
- Tessman, I. 1959. Mutagenesis in phages ϕ X174 and T4 and properties of the genetic material. *Virology* 9:375-385.
- Thomas, H. F., D. L. Brown, P. E. Hartman, E. H. White, and Z. Hartman. 1979a. Aryl-monoalkyl and cyclic triazenes: Direct-acting mutagens. *Mutat. Res.* 60:25-32.
- Thomas, H. F., P. E. Hartman, M. Mudryj, and D. L. Brown. 1979b. Nitrous acid mutagenesis of duplex DNA as a three-component system. *Mutat. Res.* 61:129-151.
- Thorp, F., Jr. 1938. Further observations on oat hay poisoning. *J. Am. Vet. Med. Assoc.* 92:159-170.
- Tomatis, L. 1979. Prenatal exposure to chemical carcinogens and its effect on subsequent generations. *Natl. Cancer Inst. Monogr.* 51:159-184.
- Tomatis, L., J. Hilfrich, and V. Turusov. 1975. The occurrence of tumours in F₁, F₂ and F₃ descendants of BD rats exposed to N-nitrosomethylurea during pregnancy. *Int. J. Cancer* 15:385-390.
- Tremp, E. 1980. [In German; English summary.] Die Belastung der schweizerischen Bevölkerung mit Nitraten in der Nahrung. *Mitt. Geb. Lebensmittelunters. Hyg.* 71:182-194.
- Turek, B., D. Hlavsová, J. Tuček, J. Waldman, and J. Černa. 1980. The fate of nitrates and nitrites in the organism. Pp. 625-632 in E. A. Walker, M. Castegnaro, L. Gričute, and M. Börzsönyi, eds. N-Nitroso Compounds: Analysis, Formation and Occurrence, IARC Scientific Publication No. 31. International Agency for Research on Cancer, Lyon, France.
- Turner, C. A., and E. W. Kienholz. 1972. Nitrate toxicity. *Feedstuffs* 44:28-29.

- U.S. Department of Agriculture. 1970. Approval of substances for use in the preparation of product; Part 318.7 in Meat Inspection Regulations: Revision Pursuant to Wholesome Meat Act. Fed. Regist. 35:15588-15592.
- U.S. Public Health Service. 1951-1978. Survey of Compounds Which Have Been Tested for Carcinogenic Activity, NIH Publication No. 80-453 (formerly Public Health Service Publication No. 149). National Institutes of Health, U.S. Public Health Service, U.S. Department of Health and Human Services, Washington, D.C. U.S. Government Printing Office, Washington, D.C.
- van Logten, M. J., E. M. den Tonkelaar, R. Kroes, J. M. Berkvens, and G. J. van Esch. 1972. Long-term experiment with canned meat treated with sodium nitrite and glucono- δ -lactone in rats. Food Cosmet. Toxicol. 10:475-488.
- Vesselinovitch, S. D., K. V. N. Rao, and N. Mihailovich. 1979. Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis. Natl. Cancer Inst. Monogr. 51:239-250.
- Vogel, E., and F. H. Sobels. 1976. The function of Drosophila in genetic toxicology testing. Pp. 93-142 in A. Hollaender, ed. Chemical Mutagens: Principles and Methods for Their Detection, Vol. 4. Plenum Press, New York and London.
- Wakabayashi, K., M. Nagao, T. Kawachi, and T. Sugimura. 1981. Co-mutagenic effect of norharman with N-nitrosamine derivatives. Mutat. Res. 80:1-7.
- Walker, E. A., N. Castegnaro, L. Garren, G. Toussaint, and B. Kowalski. 1979. Intake of volatile nitrosamines from consumption of alcohols. J. Natl. Cancer Inst. 63:947-951.
- Walker, R. 1975. Naturally occurring nitrate/nitrite in foods. J. Sci. Food Agric. 26:1735-1742.
- Walton, G. 1951. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. Am. J. Publ. Health 41:986-995.
- Weisburger, J. H. 1979. Mechanism of action of diet as a carcinogen. Cancer 43:1987-1995.
- Weisburger, J. H., and R. Raineri. 1975. Dietary factors and the etiology of gastric cancer. Cancer Res. 35:3469-3474.

- Weisburger, J. H., H. Marquardt, N. Hirota, H. Mori, and G. M. Williams. 1980. Induction of cancer of the glandular stomach in rats by an extract of nitrite-treated fish. *J. Natl. Cancer Inst.* 64:163-167.
- Wood, M., A. Flaks, and D. B. Clayson. 1970. The carcinogenic activity of dibutyl nitrosamine in IF x C₅₇ mice. *Eur. J. Cancer* 6:433-440.
- World Health Organization. 1962. Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants. Pp. 68-75 in the Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives, World Health Organization Technical Report Series No. 228. World Health Organization, Geneva, Switzerland.
- World Health Organization. 1978. Nitrates, Nitrites, and N-Nitroso Compounds, Environmental Health Criteria No. 5. World Health Organization, Geneva, Switzerland. Available from WHO Publications Centre, Albany, New York. 107 pp.
- Wright, A. S. 1980. The role of metabolism in chemical mutagenesis and chemical carcinogenesis. *Mutat. Res.* 75:215-241.
- Wynder, E. L., and I. J. Bross. 1961. A study of etiological factors in cancer of the esophagus. *Cancer* 14:389-413.
- Wynder, E. L., J. Onderdonk, and N. Mantel. 1963. An epidemiological investigation of cancer of the bladder. *Cancer* 16:1388-1407.
- Yahagi, T., M. Nagao, Y. Seino, T. Matsushima, T. Sugimura, and M. Okada. 1977. Mutagenicities of N-nitrosamines on Salmonella. *Mutat. Res.* 48:121-130.
- Yang, C. S. 1980. Research on esophageal cancer in China: A review. *Cancer Res.* 40:2633-2644.
- Yanofsky, C. 1967. Gene structure and protein structure. *Harvey Lect.* 61:145-168.
- Zaldívar, R. 1977. Nitrate fertilizers as environmental pollutants: Positive correlation between nitrates (NaNO₃ and KNO₃) used per unit area and stomach cancer mortality rates. *Experientia* 33:264-265.
- Zaldívar, R., and H. Robinson. 1973. Epidemiological investigation on stomach cancer mortality in Chileans: Association with nitrate fertilizer. *Z. Krebsforsch.* 80:289-295.

- Zaldívar, R., and W. H. Wetterstrand. 1975. Further evidence of a positive correlation between exposure to nitrate fertilizers (NaNO_3 and KNO_3) and gastric cancer death rates: Nitrites and nitrosamines. *Experientia* 31:1354-1355.
- Zimmermann, F. K. 1977. Genetic effects of nitrous acid. *Mutat. Res.* 39:127-147.
- zur Hausen, H. 1976. Biochemical approaches to detection of Epstein-Barr virus in human tumors. *Cancer Res.* 36:678-680.

CHAPTER 10

ESTIMATION OF RISK TO HUMAN HEALTH

| | <u>Page</u> |
|--|-------------|
| THE RISK OF BOTULISM..... | 10-4 |
| Factors that Affect the Risk of Botulism..... | 10-4 |
| Some Practical Experience with Reduction of Nitrate and Nitrite in Meats..... | 10-6 |
| Estimating the Risk of Botulism..... | 10-7 |
| Discussion and Conclusions..... | 10-8 |
| THE BIOLOGICAL BASIS FOR ESTIMATING THE RISK OF CANCER FROM THE ADDITION OF NITRATE AND NITRITE TO FOODS..... | 10-9 |
| Discussion and Conclusions..... | 10-10 |
| ESTIMATION OF THE RISK OF CANCER FOR HUMANS EXPOSED TO NITRATE, NITRITE, AND N-NITROSO COMPOUNDS..... | 10-11 |
| Quantitative Risk Assessment Based on Lifetime Feeding Studies on Animals..... | 10-11 |
| Discussion and Summary..... | 10-33 |
| OVERALL SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS..... | 10-34 |
| REFERENCES..... | 10-36 |

NOTE

Dr. Rolf Preussmann, an advisor to the Committee on Nitrite and Alternative Curing Agents in Food, does not believe that the scientific data are sufficiently reliable to be used for estimating the risk for humans exposed to N-nitroso compounds.

CHAPTER 10

ESTIMATION OF RISK TO HUMAN HEALTH

Discussions in the previous chapters of this report have focused on two major issues: first, the beneficial effects of adding nitrite to foods, e.g., its ability to control the outgrowth of Clostridium botulinum spores, thereby offering protection against botulism, and second, the potential adverse effects, especially carcinogenesis, resulting from exogenous and endogenous exposure to nitrate, nitrite, and N-nitroso compounds. In this chapter, the committee discusses factors that affect the risk of botulism. In addition, it has attempted to estimate the risk of cancer that might result from exposure to nitrate, nitrite, and N-nitroso compounds and the reduction in such risk if nitrite were to be omitted from certain foods.

Quantitative risk assessment is a developing rather than a precise science. The numerical estimates in this chapter are based on a series of assumptions made by the committee. Another group using different assumptions could arrive at very different conclusions. The attempt at quantitation has been made to provide information to policymakers about the relative risks, depending on the extent of exposure to N-nitroso compounds. The absolute numbers are not meant to be a basis for policy formation.

In the discussion that follows, the committee first outlines the type of information that may be needed to estimate with accuracy the risks of botulism and cancer. Regrettably, such information is difficult to define precisely, and the available information is all too often incomplete or unreliable. This is the first hindrance to the performance of a quantitative risk assessment: the inadequacy of the data base.

The discussion proceeds to a description of mathematical models that may be used for the numerical estimation of risk. Use of these models requires a set of assumptions concerning (1) how and whether one may predict the effects of an exposure to low doses when data have been gathered from experiments performed at high doses (high to low dose extrapolation) and (2) how and whether one may predict effects in humans when data have been gathered from experiments performed in laboratory animals (interspecies conversion). Although experts may favor one model over another, they generally agree that effects may occur at lower doses and that effects observed in well-designed studies on laboratory animals probably have predictive value for effects in humans. Obviously, the necessity to use a set of assumptions where data do not exist and mathematical models to predict the outcome where the precise effects are not known introduces

uncertainty into the validity of the risk estimates. Using data from animal studies and the limited information on humans, the committee has attempted to quantitate not only the risk of carcinogenesis from exposure to nitrite and N-nitroso compounds, but also the diminution in risk if nitrite were to be removed from cured meats. The validity and limitations of the numerical estimates are discussed side-by-side in order to emphasize the uncertainty that surrounds such computations. In addition to the limitations of the various mathematical models used in estimating risks, the accuracy and reliability of the data used by the committee for human exposures from exogenously and endogenously derived nitrate, nitrite, and N-nitroso compounds are also uncertain because of the inherent weaknesses in current assay methods. Also, in some cases, averages were derived from incomplete data bases. Furthermore, accurate information on the intake of these chemicals by the U.S. population is not available.

THE RISK OF BOTULISM

Factors that Affect the Risk of Botulism

Foodborne botulism is caused by the ingestion of products in which botulinum toxin has been produced by C. botulinum. Thus, calculation of the potential risk requires consideration of two major questions: What is the likelihood that a food contains botulinum toxin? What is the likelihood that it will be ingested?

Nitrite may affect the risk of botulism by altering either or both of these probabilities. It affects the former by its inhibition of C. botulinum and may affect the latter by inhibiting other microorganisms which, had they proliferated, would have altered the palatability of the food.

The information that is needed to estimate the increase in the frequency with which foods would become toxic if nitrite were omitted from cured meats includes:

- The number, clustering, and location of spores within the contaminated product. These factors would affect the abuse time required for toxin production and the compatibility of the environment for C. botulinum growth.
- The type of spore that has contaminated the product. Different types require different minimum growth temperatures and have different growth characteristics.
- The combined effects of the treatment used and the characteristics of each type of product on control of the growth of C. botulinum and other microorganisms. Such characteristics include pH, water activity, redox potential, nitrite and sodium chloride

concentrations, and processing temperatures (see Chapter 3 for definitions of these terms).

- The frequency of faulty processing (e.g., insufficient levels of added salt or short cooking time), which affects the ability of the specific treatments mentioned above to achieve their objective.

- The frequency with which the products are stored at suboptimal temperatures in the food distribution system, in the retail outlet, and by the consumer. For example: What proportion of which products are stored at what temperatures, and for how long? What is the proportion of retail display cabinets with temperatures higher than desirable, and by how much are they too warm? What is the proportion of home refrigerators with temperatures higher than desirable, and which products are most frequently mishandled? See Bryan (1980) for a discussion of this subject.

The limited information pertaining to some of these issues (e.g., Bryan, 1980; Holley, 1978) is not sufficient to permit a calculation of the probability that a product contains toxin.

Various factors affect the likelihood that a toxic product will be eaten or that it will cause botulism if ingested.

- The type of *C. botulinum* contaminating the product. For example, proteolytic strains are likely to lead to a breakdown of protein and render the product aesthetically unacceptable, whereas non-proteolytic strains will not (Smith, 1977).

- The degree to which the food and drug laws are effective in facilitating the identification of potentially toxic foods and preventing them from reaching consumers. (See Johnston and Krumm, 1980.)

- The method of cooking. Since botulinum toxin is sensitive to heat, the method of preparation (cooking temperature and duration) will affect the amount of toxin present at the time of ingestion (Woodburn et al., 1976).

- The dose of toxin ingested and individual susceptibility to it (Sakaguchi, 1979).

The probability of a fatality resulting from the ingestion of toxin will be dependent upon the speed of diagnosis and delivery of medical care. The number of persons that may consume a portion of toxic food is generally rather small. Between 1950 and 1977, an average of 2.4 cases were affected in an "outbreak" of botulism. However, one outbreak involved 58 cases (Center for Disease Control, 1979a,b). In recent years, there has been a decrease in fatalities attributed to botulism (Center for Disease Control, 1979a,b, 1980,

1981). The fatality rate is also somewhat dependent on the type of toxin ingested (Center for Disease Control, 1979a).

The ideal data to use in estimating the risk resulting from the omission of nitrite from foods would accrue from epidemiological studies that compare outbreaks of botulism due to consumption of meat products to which nitrite has been added and outbreaks due to consumption of similar, but nitrite-free meat products that have been handled (and abused) by producers, distributors, and consumers in an identical fashion. But no such foods exist. Thus, data of this type are not available and may never be obtainable because it is difficult to discriminate between the effects of nitrite and the effects of other contributory factors such as the pH or the salt content of the meat or the manner in which the meat has been handled.

Some Practical Experience with Reduction of Nitrate and Nitrite in Meats

Since the control of botulism is one of the objectives for the addition of nitrite to meats, it is useful to examine some recent experiences with removing nitrate and reducing the use of nitrite. Since December 1975, Norway has discontinued the addition of nitrate to cured meats and has limited residual nitrite levels to 5 mg/kg in some categories of meats to which no nitrite is added, such as fresh meats and sausages and commercially sterile canned meat items (Høyem, 1977). Fresh emulsion products to which no nitrite is added include frankfurters. These products constitute nearly one-half of the Norwegian meat market. In practice, residual nitrite is permitted in these products in concentrations up to 10 mg/kg (Nelson, personal communication, 1979). Currently, addition of nitrite is permitted in a limited number of cured products in Norway. Since 1973, when a reduction in its use was proposed, the total amount of nitrate and nitrite used as food additives in Norway has decreased by more than 80% (Ringén, personal communication, 1981). As shown in Table 5-1, a 1976 survey of Norwegian cured meat products reflects this decrease in nitrite (American Meat Institute, 1976). One large meat processor in that country has ceased to use nitrate and nitrite completely, even in products for which nitrite addition is permitted (Ringén, personal communication, 1981).

In Norway, no outbreak of botulism has been known to result from these reductions of nitrate and nitrite in cured meat products. However, caution must be exercised when applying such information to predict the impact of reducing nitrite in foods in the United States because there are several important differences between the two countries. In Norway, dietary patterns are different, the mean daily atmospheric temperature is lower, and the population is only 4 million.

In the United States, approximately 60% of the meat and meat products consumed are unprocessed and have no nitrate or nitrite added to

them. Of the processed meat, about 50% has no added nitrate or nitrite (see Chapter 3). Although there are some data concerning the incidence of botulism (Center for Disease Control, 1979a; Tompkin, 1980), it is difficult to compare the number of cases of botulism attributable to such products between 1899 and 1977 with the number attributable to cured products to which nitrite had been added. During much of that period, transportation and refrigeration were much less adequate than they are at present, the detection and reporting of foodborne illnesses were probably deficient, and slaughtering and processing were done on a much more local scale, thus shortening distribution chains (see Chapter 2).

Food handling and refrigeration practices in modern retail stores (Buege, 1980) and in restaurants (Bryan, 1980) are sometimes substandard. Yet, only one outbreak of botulism known to be caused by meat products either with or without nitrate- or nitrite-containing preservatives can be attributed to mishandling by these establishments (Tompkin, 1980). Panalaks *et al.* (1973, 1974) found that 108 (~36%) of 297 cured meat products analyzed contained residual nitrite in concentrations less than 7 mg/kg. Among these 108 products were numerous items in all eight product categories listed in Table 3-7. Therefore, it seems reasonable to assume that many cured meat products with low residual nitrite levels are currently on the market.

Alternative meat products that mimic cured meat, such as nitrite-free bacon, are also permitted in the United States (U.S. Department of Agriculture, 1981a,b). Although they are produced on an extremely small scale, these specialty products to which no nitrite has been added have not been known to cause an outbreak of botulism (Center for Disease Control, 1980, 1981; Tompkin, 1980).

Estimating the Risk of Botulism

Despite the lack of adequate data, efforts have been made to determine the increment in the risk of botulism (e.g., the extra number of cases or deaths that might result) if nitrite and nitrate were no longer used in cured products. The Nitrite Task Force of the Food and Drug Administration (1979a) attempted to estimate the increased risk of botulism that is likely to result from the omission of nitrite from cured meats. This group used data on the observed number of deaths from the outbreaks of botulism caused by the consumption of commercially processed smoked fish containing type E *C. botulinum* in the early 1960's. By applying various factors to adjust for the relative amount of cured meats (as compared to smoked fish) consumed, differences in the prevalence and types of *C. botulinum* spores in the products, and food handling practices of smoked fish consumers versus cured meat eaters, the task force arrived at an estimate of the increase in mortality. The committee believes that

this estimate should be regarded as speculative because of the limited data base from which it was derived, the considerable uncertainty about the validity of the assumptions, and the questionable basis for the calculations.

In order to use epidemiological data to assess the absolute risk of botulism or reduction in risk due to the addition of nitrite and other ingredients in the curing process, it is essential to obtain reliable data on the incidence of or mortality from botulism. Despite the existence of a sophisticated system for reporting botulism in the United States, the difficulty in diagnosing this disease (Center for Disease Control, 1979a; Sakaguchi, 1979) would lead one to suspect that cases of botulism are misdiagnosed and attributed to other causes. Microbiological characterization of C. botulinum or botulinum toxin during autopsy of cases of sudden and unexpected death could assist in determining if misdiagnoses contribute significantly to the underreporting of botulism. Such concerns were raised by the results of an unconfirmed study conducted in Switzerland (O. Sonnabend, 1981; W. Sonnabend, 1981). The Center for Disease Control (1979a,b, 1980, 1981) has reported that only three deaths due to botulism have been traced to ingestion of fresh meats or commercially produced meat and poultry products in the United States during the past 55 years.

Discussion and Conclusions

The critical effect of nitrite in combating botulism is its inhibition of the outgrowth of C. botulinum spores. However, other factors, e.g., salt, pH, and thermal processing, also contribute to the suppression of spore outgrowth, cell growth, and, thus, toxin production. If a product is not stored at optimal temperatures, the contribution of nitrite to the suppression of spore outgrowth varies with the type of product and other conditions prevalent at the time of the abuse (see Chapter 3). However, there are no data on its impact on the entire range of variables in any one product, on these variables in all classes of products, or in different situations in which the product may be abused.

Because of the lack of adequate information on the frequency of various events in the sequence leading to the production of botulinum toxin and, thus, botulism, e.g., contamination and length and conditions of abuse, it is impossible to predict the likelihood of products becoming toxic or becoming sufficiently unpalatable to be ingested. Thus, although nitrite prolongs the period that a contaminated product can be stored at suboptimal temperatures without becoming toxic, it is impossible to predict the extent to which this extended endurance reduces the incidence of botulism. Moreover, epidemiological data cannot be used to determine the increased risk of botulism that might result if nitrite were omitted from products to which it is currently added because the nature of products and methods of handling them vary in many respects.

THE BIOLOGICAL BASIS FOR ESTIMATING THE RISK OF CANCER FROM THE
ADDITION OF NITRATE AND NITRITE TO FOODS

As discussed in earlier chapters of this report, humans are exposed to nitrate, nitrite, nitrogen oxides, and N-nitroso compounds in the diet and in other environmental media. Since nitrate, nitrite, and nitrogen oxides can react with other substances in the diet and in the human body to produce N-nitroso compounds, most of which produce cancer in animals, it is desirable to determine the increased risk arising from their addition to foods as well as the risk posed by their natural occurrence in foods. These risks could be estimated with confidence if there were accurate information on exposure to these substances, if their metabolism and pharmacokinetics in humans were well understood, and if the total body burden of N-nitroso compounds were known.

In order to estimate exposure to nitrate, nitrite, and N-nitroso compounds, it is necessary to know:

- The average and the range of the amounts of nitrate and nitrite ingested, the nature and size of the population subgroups consuming different amounts, and the proportion of the intake resulting from deliberate addition of these compounds to foods.
- The characteristics and sizes of groups with high intakes of nitrosatable substrates such as certain drugs.
- Individual variability in the metabolism of these compounds, e.g., in salivary nitrate reduction or endogenous synthesis of nitrate.
- The amount and pattern of nitrate, nitrite, and nitrosatable substrates in the stomach at any one time in healthy and diseased individuals; the physiological conditions in the stomach; and the probability for the generation of N-nitroso compounds (as discussed by Ohshima and Bartsch, 1981). Or, alternatively, experimental evidence pertaining to the amounts and types of all N-nitroso compounds produced in the normal and abnormal stomach over a wide range of intakes and dietary patterns.
- The sizes of population subgroups with abnormal stomach conditions or other gastrointestinal disorders.
- Exogenous and endogenous exposure to other nitrosating agents, e.g., nitrogen oxides and preformed nitrosamines, and to the modifiers of nitrosation, e.g., thiocyanate, ascorbic acid, and α -tocopherol.

In order to make an adequate assessment of the significance of such exposure to human health, it is necessary to know:

- How humans metabolize the various N-nitroso compounds formed in vivo as a result of nitrate and nitrite ingestion, and the tissue distribution (dose) of carcinogenic metabolites.

- The tissue distribution of detoxication, activation, and repair mechanisms for each of the N-nitroso compounds.

- The tissue specificity, latent period between exposure and tumor formation in humans exposed at various ages, and the carcinogenic potency of each N-nitroso compound formed in vivo.

- The fatality rate of the various types of tumors induced (if the risk is to be expressed as possible deaths).

Discussion and Conclusions

The major sources of environmental exposure to nitrate, nitrite, and N-nitroso compounds have been specified and, to the extent possible, quantified in Chapters 5, 6, and 7 (see Tables 5-20, 5-21, 6-1, and 7-17). As pointed out earlier, the data base is clearly limited and considerable uncertainty surrounds any attempt to assign confidence limits to these estimates. Similarly, Chapter 8 summarizes the current knowledge pertaining to metabolism and pharmacokinetics and provides estimates of the endogenous exposure to nitrate, nitrite, and N-nitroso compounds (Tables 8-3 and 8-4). It is apparent that nitrate and nitrite can participate in the formation of N-nitroso compounds. It also appears that nitrosation (kinetically correlated with in vitro experiments) can occur in the human body (Ohshima and Bartsch, 1981) and that the process can be blocked by ascorbic acid and α -tocopherol (see Table 8-3). However, little is known about the nitrosating potential of naturally occurring nitrate and nitrite and of nitrite added to cured meats, and knowledge concerning the formation of various N-nitroso compounds in humans is extremely limited. Moreover, differences in the rates of metabolism and the potency of these compounds as tested in animals and the variability in individual susceptibility to their effects make it difficult to provide reliable estimates of their total body burden and, consequently, the risk to human health.

Nonetheless, bearing these limitations in mind and using a number of clearly delineated assumptions, the committee has attempted to compute the risk of cancer for humans by using data on tumor incidence from two experiments in which rats were fed a nitrosamine, and discusses the feasibility of using data from one in which mice were given nitrite and an amine. In another series of calculations to assess the risk of carcinogenesis and mortality for various population groups, the committee used data on tumor incidence in animals; estimates of exogenous exposure to nitrate, nitrite, and N-nitroso compounds; and estimates of total exposure to N-nitroso compounds

based on data concerning the formation of nitrosamines in a human subject. In addition, it estimated the diminution in risk if nitrite were removed from cured meats by assuming certain well-delineated characteristics of exposure for these population groups.

The assumptions and methodology used in these computations and their limitations are the focus of the next section.

ESTIMATION OF THE RISK OF CANCER FOR HUMANS EXPOSED TO NITRATE, NITRITE, AND N-NITROSO COMPOUNDS

This section has been prepared primarily for use by those readers who are familiar with quantitative risk assessment and who may wish to compare the methodology used by the committee with other methods.

The risks estimated in this chapter should be regarded as rough approximations of doses that might be relatively innocuous for humans. It would be misleading to equate these estimates with predictions derived from data obtained from the dose ranges actually used in the animal experiments.

Quantitative Risk Assessment Based on Lifetime Feeding Studies on Animals

In this report, quantitative risk assessment refers specifically to the estimation of the probability or the risk that various toxic end points will result from a range of doses or levels of exposure over a lifetime. The rationale and the details of the methodology for such assessments can be found in a recent report prepared by the Scientific Committee of the Food Safety Council (Food Safety Council, 1980) and in many papers (e.g., Cornfield, 1977; Cornfield *et al.*, 1978; Crump *et al.*, 1977; Hartley and Sielken, 1977; Hoel *et al.*, 1975; Krewski and Van Ryzin, in press; Mantel and Bryan, 1961; Rai and Van Ryzin, 1979, 1981; Van Ryzin, 1980).

Data from lifetime feeding studies are generally derived from a group of animals (n_0) tested at dose $d_0 = 0$, i.e., a control group, and groups of animals (m) tested at doses $d_1 < d_2 < \dots < d_m$ with n_1, n_2, \dots, n_m animals, respectively. The toxic responses, $x_0, x_1, x_2, \dots, x_m$, are recorded for each dose for each toxic end point. For example, x_i = the number of animals exhibiting the toxic end point under study at dose d_i , where $i = 0, 1, 2, \dots, m$. Using these data, one can attempt to predict the probability that such a toxic end point will occur in humans at doses to which they might be exposed.

As mentioned earlier, the estimation of risk involves two steps--high- to low-dose extrapolation and species conversion--both of which are accompanied by many uncertainties.

High to Low Dose Extrapolation. To understand this step, it is necessary to comprehend the concepts of a dose-response model, a specified level of risk and its corresponding dose, the statistical estimation of dose-response curves from the experimental data, and the resulting estimate of a dose corresponding to a specified risk.

A dose-response model is a mathematical equation that relates the dose, d , to the rate of occurrence (incidence) of response or the probability (P) that an animal exposed to that dose will exhibit a specific toxic end point during its lifetime. This function is typically written as $P(d)$ (Figure 10-1).

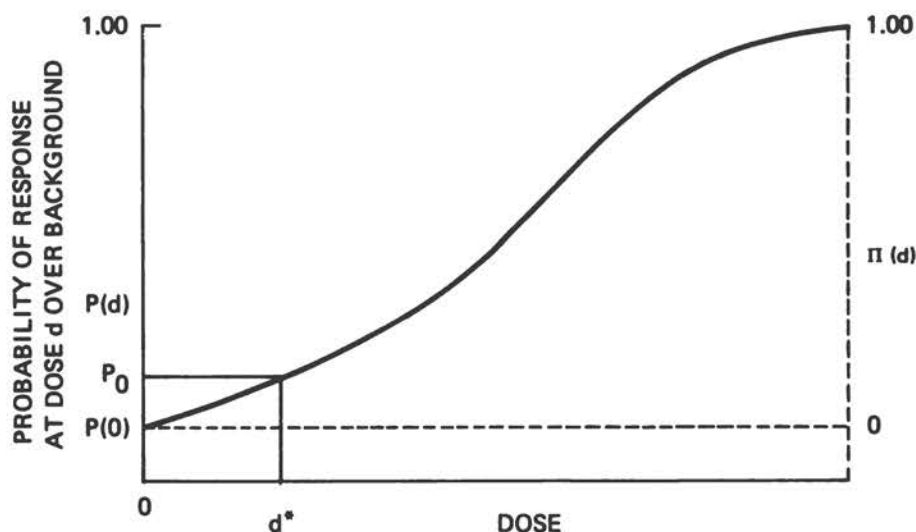


FIGURE 10-1. A dose-response curve. P_0 = specific level of risk; $P(0)$ = background or spontaneous response rate; d^* = the dose that corresponds to a specified risk; $\Pi(d)$ = probability of toxic response at dose d .

The quantity $P(0)$ represents the background or the spontaneous rate of response. $P(d)$ is usually assumed to be an increasing function of d , reflecting the concept that an increase in the dose of a toxic substance increases the probability of a toxic response.

The specified level of risk is based on the probability of a toxic response at dose d , minus the spontaneous rate of response $P(0)$, divided by the probability of no response from the background:

$$\Pi(d) = [P(d) - P(0)] / [1 - P(0)],$$

where $\Pi(d)$ represents the amount of increased risk, or probability of toxic response, due to exposure to the dose, d , alone, i.e., without the background exposure. In Figure 10-1, the curve $\Pi(d)$ is represented by the vertical dashed line. For toxic end points such as liver tumors, the specified risk level, P_0 , may be 10^{-6} , which corresponds to a frequency of approximately three cases per year in the United States. If one assumes that approximately 220 million people whose average lifespan is approximately 72 years are exposed annually at this level, this would lead to an effective rate of 3 million people per year at risk, i.e., $3 = (10^{-6} \times 3,000,000)$ cases of cancer per year. Once the specified risk level, P_0 , is known, the equation $\Pi(d^*) = P_0$ yields the corresponding dose level d^* ¹. The dose d^* is that level which would, for the average subject exposed to a chronic dose d^* (usually expressed as mg/kg body weight/day, %, or ppm), result in an increased risk of P_0 over the background level of risk. This dose d^* is illustrated in Figure 10-1.

The statistical problem of extrapolating from high to low dose can now be easily described. By using a suitable statistical estimation technique, usually the maximum likelihood estimation, estimates of the function $P(d)$, denoted $\hat{P}(d)$, and the background $P(0)$, denoted by $\hat{P}(0)$, can be obtained. These lead to an estimate of $\Pi(d)$, given by the equation $\hat{\Pi}(d) = [\hat{P}(d) - P(0)] / [1 - \hat{P}(0)]$. This estimate of $\hat{\Pi}(d)$ depends on all the response data given by the observed rates of response $x_0/n_0, x_1/n_1, \dots, x_m/n_m$ at doses d_0, d_1, \dots, d_m , respectively. Having estimated this, one can estimate the resulting dose, denoted by \hat{d}^* , by solving the equation $\hat{\Pi}(\hat{d}^*) = P_0$. The estimated dose \hat{d}^* for a specific risk P_0 based on the actual data from the experiment represents the experimentally determined estimate of that dose which would lead to an increased risk of P_0 . This process is shown in Figure 10-2, where $\Pi(d)$, the dashed line, represents the true unknown dose-response curve, and $\hat{\Pi}(d)$, the solid line, represents the estimate of that curve based on the data. The figure has been included for illustrative purposes only; $\hat{\Pi}(d)$ is not always less than $\Pi(d)$ in the low dose region.

¹The dose d^* , which corresponds to a specific risk $P_0 = 10^{-8}$ when combined in the probit model with an arbitrary slope of unity, was called the "virtually safe dose" by Mantel and Bryan (1961). The committee chose not to use the word "safe" because it may be understood differently by different people. For example, if the chance of a skiing accident is 1 in 10,000, that may be safe to one person, unsafe to another. "Safety" is a regulatory/political/societal decision, which scientists alone cannot determine.

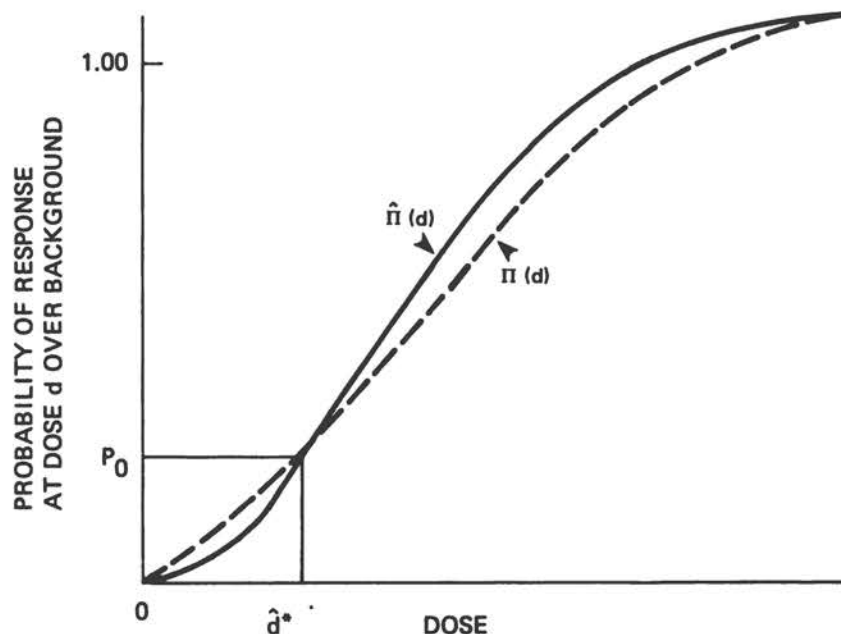


FIGURE 10-2. Calculation of a dose \hat{d}^* that corresponds to a specified risk, P_0 . $\Pi(d)$ = probability of toxic response at dose d ; $\hat{\Pi}(d)$ = estimate of dose-response curve.

Note that this process of estimating the dose d^* for a specific risk involves certain statistical uncertainties since it depends on data that vary from one experiment to another. There is no one mathematical model that is best for the estimation of risk to humans. Therefore, a further, and perhaps far more serious source of error, is that these estimates of dose will vary considerably, depending on the mathematical model used for the dose-response function $\Pi(d)$ and the manner in which the background response is incorporated. Because of the uncertainty of the risk estimates based on mathematical models, the committee has based its calculations of these doses on three mathematical models: the one-hit model, the multistage model, and the multihit model.

Each of the models is described in terms of the function $\Pi(d)$. Thus, the complete model, when the background effect is incorporated, would be given by the equation:

$$P(d) = P(0) + [1 - P(0)] \Pi(d). \quad (1)$$

The method used in equation (1) for incorporating the background response, $P(0)$, was first described by Abbott (1925), and is known as Abbott's correction. That equation assumes that the response due to the background exposure is statistically independent of the response due to the additional dose. That is, equation (1) may be rewritten as:

$$1 - P(d) = [1 - P(0)] [1 - \Pi(d)], \quad (2)$$

which states that the probability that there is no toxic response at dose d is the product of the probability of no response from background exposure and the probability of no response from the additional dose. This is an important assumption because it means that the outcome at the low doses represented by the dose-response curve will be determined by the mathematical model adopted for $\Pi(d)$. However, suppose that equation (1) is not assumed, but, instead, one assumes that the additional dose reacts additively with a postulated "effective" background dose, $d_0 > 0$ (Crump et al., 1976; Peto, 1978). Specifically, suppose that:

$$P(d) = f(d + d_0) \quad (3)$$

for a given dose-response function, $f(d)$. Then the shape of the curve for the incremental risk function, $\Pi(d) = [P(d) - P(0)] / [1 - P(0)]$, will be approximately linear for small additional doses if the slope of the curve for function f at d_0 is positive. That is, for small doses, it is mathematically easy to show that:

$$\Pi(d) = \text{approx. } c \text{ or } d, \quad (4)$$

where $c = f'(d_0) / [1 - P(0)]$, and $f'(d_0) > 0$ is the first derivative or slope of f at d_0 . The function $f'(d_0)$ would always be positive if the underlying dose-response curve $f(d)$ continues to increase and has no threshold, i.e., there is no point $d' > 0$, or there is no value greater than zero on the dose scale. Thus, $f(d) = 0$ if $d \leq d'$.

Equation (4), known as low dose linearity, has important consequences for high to low dose extrapolation. Its implications for the risk estimates for N-nitroso compounds and nitrite are discussed later in this chapter.

The three models that are used to calculate $\Pi(d)$ can be shown as follows:

One-Hit Model:

$$\Pi_1(d) = 1 - e^{-\theta d}, \quad (5)$$

where $\theta > 0$ is a constant.

Multistage Model:

$$\Pi_2(d) = 1 - e^{-(\theta_1 d + \theta_2 d^2 + \dots + \theta_k d^k)}, \quad (6)$$

where $\theta_1 \geq 0$, $\theta_2 \geq 0$, ..., $\theta_k \geq 0$ are constants and $k =$ number of stages.

Multihit Model:

$$\Pi_3(d) = \int_0^{\theta d} (u^h - 1) e^{-u} du / \Gamma(h) \quad (7)$$

where $\theta > 0$ is a constant, h is the number of "hits", and the gamma function is $\Gamma(h) = \int_0^{\infty} (u^h - 1) e^{-u} du$, where u is the variable of integration.

The mathematical reasoning for each of these models is not discussed here. For detailed descriptions of the one-hit model, see Hoel et al. (1975) or Rai and Van Ryzin (1979); for the multistage model, see Armitage and Doll (1961) and Crump et al. (1976); and for the multihit model, see Rai and Van Ryzin (1979, 1981). Each model is based on various biological assumptions, which are explained in the papers cited. A critical variable in high to low dose extrapolation is the applicability of each of these equations at low doses. Depending on the value of the constants, this variability in applicability at low doses can be severe. For low doses, whenever $k > 1$ and $h > 1$, then:

$$\Pi_1(d) > \Pi_2(d) > \Pi_3(d), \quad (8)$$

when $k = h = 1$, $\Pi_1(d) = \Pi_2(d) = \Pi_3(d) =$ equation (5) for all doses, and all three models are identical. Equation (8) is important because if \hat{d}_1^* , \hat{d}_2^* , and \hat{d}_3^* represent estimates of the dose corresponding to a small specified level of risk P_0 , it can be shown in most cases that:

$$\hat{d}_1^* < \hat{d}_2^* < \hat{d}_3^*. \quad (9)$$

The results concerning the relative order of such estimates were examined in approximately 20 data sets by Krewski and Van Ryzin (in press). These authors discussed the three models mentioned here along with three additional dose-response models: the logistic, Weibull, and probit models. They demonstrated that the results of the Weibull and logistic models are usually close to \hat{d}_3^* , whereas that of the probit model exceeds \hat{d}_3^* . Thus, for the dose estimates presented in this report, the relative order illustrated in equation (9) will suffice for our purposes. At very low specified levels of risk, the spread between \hat{d}_1^* and \hat{d}_3^* may often be several orders of magnitude.

In addition to using different data sets to estimate \hat{d}^* at various levels of risk for the three models, the committee has calculated the "goodness-of-fit" of the experimental data for each of the models. Also provided are the results of a statistical test to determine if the one-hit model fits the data. It is important to evaluate all this information when incorporating the assumed degree of linearity into quantitative risk assessments.

As mentioned above, the one-hit model always leads to a linear extrapolation for low doses, typically providing higher estimates of risk and lower estimates of doses than the other models. Whether such estimates are accurate depends, in part, on how the background rate of response interacts with the increased rate of response. As mentioned earlier, if this inaction is additive for the range of doses used, the assumption that the response will be linear at low doses is appropriate [equations (3) and (4)].

Estimates of risk assuming low dose linearity may be a prudent course, regardless of the statistical considerations. However, the role of DNA repair mechanisms at low doses should also be considered when making such estimates. For example, when the biological evidence indicates that a carcinogen under study may act through direct-acting mechanisms such as DNA binding, rather than as a promoter or modifier, it may be prudent to use linear extrapolation, regardless of the shape of the dose-response curve (Chapter 11 in Food Safety Council, 1980). When there is little or no support for dose-wise additivity for direct-acting genotoxic substances, estimates based on the multihit model or the multistage models may be more appropriate because both models allow for nonlinearity. These models will generally result in a steep dose-response curve, leading to estimates of dose at specified levels of risk that are higher than those obtained with the one-hit model.

Doses for each of the data sets are estimated for risk levels of $P_0 = 10^{-2}$, 10^{-4} , 10^{-6} , and 10^{-8} for each of the three models. In addition, the estimates of the dose to correspond to a risk of 10^{-2} for the multistage and multihit models are multiplied by factors of 10^{-2} , 10^{-4} , and 10^{-6} . Such estimates of the dose are linearized model estimates at risk levels of $P_0 = 10^{-4}$, 10^{-6} , and 10^{-8} , respectively. This amounts to extrapolation with the model up to the risk level of 10^{-2} ,

followed by linear extrapolation to 10^{-4} , 10^{-6} , and 10^{-8} . Whenever the dose-response curve is convex in the low dose range, it constitutes an upper bound on the risk and, hence, a lower bound on the dose. This is illustrated in Figure 10-3 for a risk of $P_0 = 10^{-6}$.

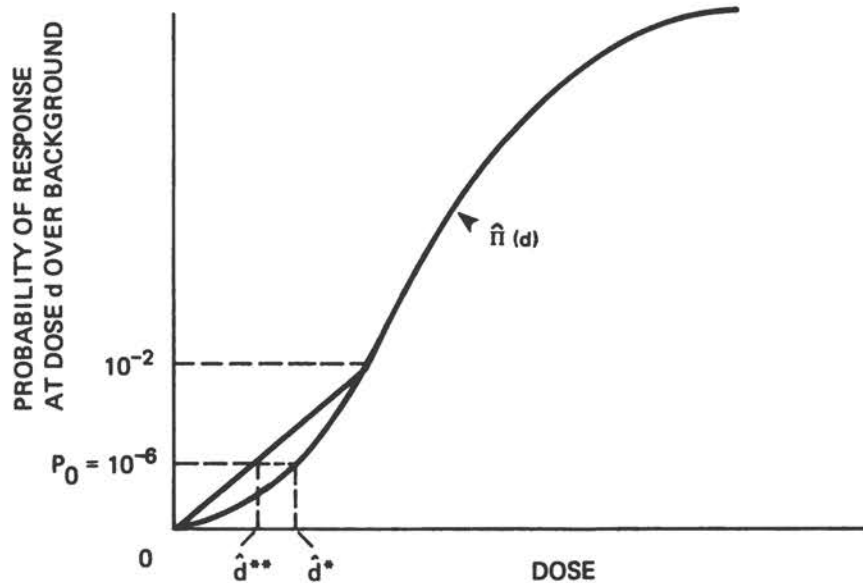


FIGURE 10-3. Linearized model estimation for $\hat{\Pi}(d)$ (probability of toxic response at dose d) at 10^{-6} . \hat{d}^* = dose at 10^{-6} extrapolated from $\hat{\Pi}(d)$, \hat{d}^{**} = dose at 10^{-6} extrapolated from $\hat{\Pi}(d)$ to 10^{-2} , followed by linear extrapolation to 10^{-6} . $\hat{\Pi}(d)$ = estimate of convex dose-response curve.

An important point to be recognized is that estimates of doses at various levels of risk are based on data from tests in animals and represent doses that correspond to various levels of risk for the species of animal studied. The relevance of these data for humans is addressed below.

Recently, attention has been focused on risk extrapolation models that relate the probability of tumor induction to both the dose and the time-to-tumor occurrence (Hartley and Sielken, 1977). These models are important because time-to-tumor occurrence increases with reduction in dose (Druckrey, 1967). Thus, the lower doses may be less capable of inducing tumors during the animal's lifetime than higher doses. Consequently, time-to-tumor occurrence may have a pro-

found effect on the incidence of tumors at varying doses and the subsequent assessment of risk, depending on the latency period of the tumors (Society of Toxicology Task Force Report, 1981). Unfortunately, such models and risk assessment cannot be applied to N-nitroso compounds because there are no adequate data.

Interspecies Conversion. The estimates of the dose for specified levels of risk that are given later in this chapter are presented in dose units used in the original animal experiment, either in mg/kg body weight per day or ppm (mg/kg) in the diet on a dry weight basis, and they apply to the species of animal used in the experiment. To relate these estimates of dose to exposures of humans, it is necessary to do an interspecies conversion. Two methods commonly used for these conversions are:

- Conversion to mg/kg body weight per day. Concentrations of test substances in the animal's diet are usually expressed as ppm or ppb, which is equal to mg/kg and $\mu\text{g}/\text{kg}$ of diet, respectively. The concentration in the original experiment is multiplied by the weight of the diet consumed by the animal daily and then divided by the weight of the animal. This amount (milligrams of test substance per kilogram of body weight per day) is then used directly as a measure of the exposure of humans expressed as mg/kg body weight per day.

- Surface area conversion. The mg/kg body weight dosage given to the animal species is converted to mg/cm^2 body surface area. Exposure of humans is then given in equivalent terms. This type of conversion is often used in cancer chemotherapy and was the method of conversion adopted by the Safe Drinking Water Committee (National Academy of Sciences, 1977).

Opinions differ as to which of the two methods of conversion is preferable. Therefore, both sets of calculations are presented in this report.

Having discussed in general terms the methodology for estimating a dose corresponding to a specific risk, P_0 , the committee has devoted the next two sections to applying this methodology to data from experiments in animals for two N-nitroso compounds (nitrosodimethylamine and nitrosopyrrolidine) and for nitrite in the presence of a fixed level of an amine (piperazine). Following these discussions, the results are summarized and their meaning for risks to humans exposed to nitrate and nitrite is examined. The risk estimates for N-nitroso compounds are then combined with data on the exposure of humans to nitrate and nitrite to arrive at estimates of risk for specific subgroups of the population.

Risk Extrapolations for N-Nitroso Compounds. The committee has applied the methodology described above to N-nitroso compounds using two sets of data containing sufficient dose-response information to fit all three models with $k > 1$ for the multistage model and $h > 1$ for the multihit model. At a minimum, this means we are restricted to data sets with $m > 2$. If $m = 1$, then $k = h = 1$, and only the one-hit model can be used to estimate risk. Thus, extrapolations can be avoided.

Also, because of uncertainty in the estimation procedure whenever the data are insufficient to indicate a statistically significant increase in the dose-response function, it makes little sense to force fit statistics for dose-response functions that continue to increase with dose, such as those given by the functions $\Pi_1(d)$, $\Pi_2(d)$, and $\Pi_3(d)$. Specifically, for all the data sets given in this section and the next one on nitrite, a test for statistical significance for trend was conducted before risk assessment models were used. That is, a test was conducted to determine the justification of $P(0) < P(d_1) < \dots < P(d_m)$ -- an increasing dose-response model. The statistical test, described by Mantel (1963) and Tarone (1975), was carried out to a 5% level of significance in all cases.

Nitrosodimethylamine (NDMA)

The dose-response data in Table 10-1 pertain to liver tumors in rats fed daily doses of NDMA in a chronic toxicity test (Terracini et al., 1967). For this data set, the estimated doses corresponding to risk levels $P_0 = 10^{-2}$, 10^{-4} , 10^{-6} , and 10^{-8} are given for the one-hit, multistage, and multihit models in Table 10-2. The figures in parentheses in Table 10-2 are the linearized model estimates at $P_0 = 10^{-4}$, 10^{-6} , and 10^{-8} for the multistage and multihit models.

In addition to the results in Table 10-2, the estimated constant, h , for the multihit model was $\hat{h} = 1.57$, where ± 0.35 is the standard error of the estimate. The multistage model resulted in a two-stage model for which θ_1 and θ_2 were estimated to be: $\theta_1 = 0.0236$ and $\theta_2 = 0.000521$. All three models had acceptable "goodness-of-fit" statistics at a level of significance of 0.05. Thus, each of the models provides a reasonably good fit in the experimental range for the positive dose levels. The orders of magnitude differences in the estimates at 10^{-4} through 10^{-8} can be explained by the different sensitivity of the models in the low dose range. As suggested in equation (9), the ordering of dose estimates is $d_1^* < d_2^* < d_3^*$.

Finally, a statistical likelihood ratio test of the hypothesis that $h = 1$ (a one-hit model and low dose linearity) versus $h > 1$ (nonlinearity) (Rai and Van Ryzin, 1981) yields a chi-squared value of 3.82, which for one degree of freedom has an observed significance level of 0.051. Thus, the statistical significance of the evidence for rejecting the one-hit model is slightly greater than the tradi-

TABLE 10-1

Liver Tumors in Rats Fed N-Nitrosodimethylamine (NDMA) in the Diet^a

| Group (i) ^b | Dose (d _i) of NDMA in the Diet, ppm | No. of Animals with Liver Tumor (x _i) | No. of Animals on Test (n _i) |
|------------------------|--|--|---|
| 0 | 0 | 0 | 41 |
| 1 | 2 | 1 | 37 |
| 2 | 5 | 8 | 83 |
| 3 | 10 | 2 | 5 |
| 4 | 20 | 15 | 23 |
| 5 | 50 | 10 | 12 |

^aData from Terracini et al., 1967.^bThe first three groups contained males and females; the last three contained females only. The 5 ppm dose was given to two groups of animals, which are combined as Group 2 in this table. Exclusion of males from the calculations would lead to lower estimates of risk and a higher estimate of doses at the various risk levels in Tables 10-2 and 10-3.

TABLE 10-2

Estimated Daily Doses of N-Nitrosodimethylamine (NDMA) Corresponding to Specific Levels of Risk of Liver Tumors in Rats^a

| Model | NDMA in Diet, ppb, by Level of Risk (P ₀) | | | |
|------------------------------|---|--------------------------------|---------------------------------|-------------------------------------|
| | 10 ⁻² (1/100) | 10 ⁻⁴ (1/10,000) | 10 ⁻⁶ (1/million) | 10 ⁻⁸ (1/100 million) |
| One-hit (\hat{d}_1^*) | 303 | 3.0 | 0.03 | 0.0003 |
| Multistage (\hat{d}_2^*) | 421 | 4.2 (4.2) ^b | 0.04 (0.04) | 0.0004 (0.0004) |
| Multihit (\hat{d}_3^*) | 974 | 51.0 (9.7) | 2.7 (0.097) | 0.15 (0.00097) |

^aBased on data from Terracini et al., 1967.^bNumbers in parenthesis are the linearized estimates.

tionally used significance level of 5% ($P = 0.05$). Therefore, the question of what estimates seem reasonable for low dose extrapolation and risk assessment purposes, linear or not, is important. The committee assumed linearity below $P_0 = 10^{-2}$, extrapolated for levels of risk below 10^{-2} with the upper bound on risk in accordance with Figure 10-3, and used these linearized estimates at 10^{-4} , 10^{-6} , and 10^{-8} . This approach is cautious in that it assumes low dose linearity below a risk level of 10^{-2} (0.01) and extrapolates only slightly beyond the observed frequency level 0.027 (1/37) at the lowest observed positive dose level of 2 ppm (2 mg/kg). The multihit and multistage models were used.

The committee used the numbers in parentheses for the multihit model in Table 10-2 as estimated doses corresponding to specific levels of risk for the species of animal tested (in this experiment, the rat). It then converted these estimates to the daily dose levels for humans and expressed them as mg/kg body weight and mg/cm² body surface. Paget (1965) reported that the ratio of the dose in mg/kg body weight to dose in mg/cm² surface area for rats is 1.43, whereas for humans, it is 9.8. Therefore, the data from rats were converted to body surface area for humans by dividing the data in the first row of Table 10-3 by 6.85 (i.e., $9.8/1.43$) to obtain the estimates in the second row.

N-Nitrosopyrrolidine (NPYR)

The dose-response data in Table 10-4 pertain to malignant liver tumors in rats fed NPYR (Preussmann *et al.*, 1977). In applying the models to these data, there was a lack of fit at the level of significance of 0.05 for each of the models when the highest dose was included. This is primarily because there was a decreased response rate of 37.5% (9/24) at that dose (10.0 mg/kg body weight) as compared to 81.5% (31/38) at the second highest dose (3.0 mg/kg body weight). The usual practice when such reversals occur at the highest dose is to exclude that dose when analyzing the data. With this adjustment, the tests of Mantel (1963) and Tarone (1975) show a definite increase in dose response at the 5% level of significance for the data in Table 10-4.

For the data set in Table 10-4 (excluding the highest dose), the doses that correspond to risk levels $P_0 = 10^{-2}$, 10^{-4} , 10^{-6} , and 10^{-8} are given in Table 10-5 for the one-hit, multistage, and multihit models. The figures in parentheses in that table are the linearized model estimates at $P_0 = 10^{-4}$, 10^{-6} , and 10^{-8} for the multistage and multihit models. The estimated constant, h , for the multihit model was $\hat{h} = (3.14 + 0.74)$, and the multistage model resulted in a two-stage model with θ_1 and θ_2 estimated to be $\theta_1 = 0$ and $\theta_2 = 0.19635$. Both the multistage and multihit models had acceptable "goodness-of-fit" statistics at a level of significance of 0.05, whereas there was a lack of fit for the one-hit model at the 0.05 level of significance.

TABLE 10-3

Estimates for Daily Doses of N-Nitrosodimethylamine (NDMA) that Correspond to Specific Levels of Risk for Humans^a, Based on Multihit Model with Linear Extrapolation from $P_0 = 10^{-2}$

| <u>Basis for Estimate</u> | <u>Doses of NDMA, by Level of Risk (P_0)</u> | | | |
|--|---|-----------------------------|-----------------------------|-----------------------------|
| | <u>10^{-2}</u> | <u>10^{-4}</u> | <u>10^{-6}</u> | <u>10^{-8}</u> |
| ng/kg body weight/day | 58,400 | 584 | 5.84 | 0.058 |
| mg/cm ² body surface/ day ^c | 8,525 | 85.3 | 0.85 | 0.0085 |

^aBased on data from Terracini et al., 1967.

^bUsing 97.4 ng/kg (0.0974 ppb) of NDMA in the diet, and assuming the average daily diet for rats to be 0.015 kg and the average weight of a rat to be 0.25 kg, the amount in ng/kg body weight daily would be 5.84. That is: $\frac{97.4 \times 0.015}{0.25} = 5.84$ ng/kg.

^cThe ratio of the weight of the diet (mg) to body surface area (cm²) for rats is 1.43 and for humans 9.8 (Paget, 1965). Thus, the ratio 6.85 (9.8/1.43) is the factor used to convert the numbers in row 1 to row 2. That is, the numbers in row 2 are obtained by dividing the numbers in row 1 by 6.85.

TABLE 10-4

Malignant Liver Tumors in Rats Fed N-Nitrosopyrrolidine (NPYR)^a

| <u>Group (i)</u> | <u>Dose (d_i) of NPYR in Diet (mg/kg of Body Weight/Day)</u> | <u>No. of Animals with Malignant Liver Tumors (x_i)</u> | <u>No. of Animals on Test (n_i)</u> |
|------------------|--|---|---|
| 0 | 0.0 | 0 | 61 |
| 1 | 0.3 | 0 | 60 |
| 2 | 1.0 | 13 | 62 |
| 3 | 3.0 | 31 | 38 |
| 4 | 10.0 | 9 | 24 |

^aData from Preussmann et al., 1977.

Therefore, the multistage and multihit models give a reasonably good fit in the experimental range for the positive dose levels. The differences in orders of magnitude in the extrapolations, especially at 10^{-4} , 10^{-6} , and 10^{-8} , are due to the different sensitivity of the models in the low dose range. As suggested in equation (9), the ordering of dose estimates is $\hat{d}_1^* < \hat{d}_2^* < \hat{d}_3^*$.

A statistical likelihood ratio test of the hypothesis $h = 1$ (a one-hit model and linearity) versus $h > 1$ (multihit and nonlinearity) (Rai and Van Ryzin, 1981) yields a chi-squared value of 18.46 for one degree of freedom, which is significant at the 0.001 level. Thus, there is strong evidence for nonlinearity in the observed range. A similar result is obtained in a test that rejects $\theta_1 = 0$ (and hence linearity) in the multistage model using the results of Crump et al. (1977). Thus, the question of what estimates seem reasonable for low dose extrapolation and risk assessment is more difficult to answer in this case where linearity in the observed dose-response curve is not substantiated by the data.

The committee used two methods of extrapolation, both of which provide a conservative estimate of the dose that corresponds to a specific risk. In the first method, the linearized estimates for risk level 10^{-2} are based on the multihit model. The second method (Rai and Van Ryzin, 1981) uses a 95% lower confidence limit on h in

TABLE 10-5

Estimated Daily Doses of N-Nitrosopyrrolidine (NPYR) that Correspond to Specified Levels of Risk for Liver Tumors in Rats. Estimates are Based on Data in Table 10-4, with the Highest Dose Excluded^a

| Model | Doses of NPYR, mg/kg Body Weight/Day, by Level of Risk (P_0) | | | |
|------------------------------|--|---|--|--|
| | 10^{-2} | 10^{-4} | 10^{-6} | 10^{-8} |
| One-hit (\hat{d}_1^*) | 3.0×10^{-2} | 3.0×10^{-4} | 3.0×10^{-6} | 3.0×10^{-8} |
| Multistage (\hat{d}_2^*) | 2.3×10^{-1} | 2.3×10^{-2} (2.3×10^{-3}) ^b | 2.3×10^{-3} 2.3×10^{-5} | 2.3×10^{-4} (2.3×10^{-7}) |
| Multihit (\hat{d}_3^*) | 3.1×10^{-1} | 6.5×10^{-2} (3.1×10^{-3}) | 1.5×10^{-2} (3.1×10^{-5}) | 3.4×10^{-3} (3.1×10^{-7}) |

^aBased on data from Preussmann *et al.*, 1977.

^bNumbers in parenthesis are the linearized estimates.

the multihit model, where $1.92 = 3.14 - (1.65)(0.74)$, and uses this value of h for extrapolation with the same model. The first method (A) forces low-dose linearity, whereas the second method (B) allows for a minimal amount of low dose nonlinearity, as suggested by the 95% lower confidence limit on h . Results calculated by both methods are presented in Table 10-6 as unit of dose per unit of body weight and per unit of surface area for risk levels 10^{-2} , 10^{-4} , 10^{-6} , and 10^{-8} .

Comparison of Tables 10-5 and 10-6 indicates that there are considerable differences among dose estimates, especially at the 10^{-6} and 10^{-8} levels, depending on the choice of model and extrapolation method adopted (e.g., linearized, lower confidence limits on certain key parameters, etc.).

Risk Extrapolations for Nitrite Plus Amine. There are no reliable dose-response studies in which tumor induction has been observed to increase with the dose of nitrite alone. The Interagency Working Group on Nitrite Research (Food and Drug Administration, 1980) reanalyzed an earlier study in which rats were fed different doses of sodium nitrite over a 2-year period (see Chapter 9). No significant dose-response curve resulted from this study. Accordingly,

TABLE 10-6

Estimated Daily Doses of N-Nitrosopyrrolidine (NPYR) that Correspond to Various Levels of Risk for Humans^a

| Basis of Estimate and Model Used | Doses of NPYR, by Level of Risk (P_0) | | | |
|--|---|-----------|-----------|-----------|
| | 10^{-2} | 10^{-4} | 10^{-6} | 10^{-8} |
| <u>ng/kg Body Weight/Day:</u> | | | | |
| Method A ^b | 3.1×10^5 | 3,100 | 31 | 0.31 |
| Method B ^c | 8.3×10^4 | 7,220 | 652 | 59 |
| <u>mg/cm² Body Surface/Day:^d</u> | | | | |
| Method A | 4.5×10^4 | 452 | 4.5 | 0.05 |
| Method B | 1.2×10^4 | 1,054 | 95 | 8.6 |

^aBased on data from Preussmann *et al.*, 1977.

^bMethod A: Multihit with linear extrapolation from $P_0 = 10^{-2}$.

^cMethod B: Multihit with 95% lower confidence limit on h.

^dSee footnote c of Table 10-3 for basis of calculation.

the committee has considered an experiment by Greenblatt and Mirvish (1973), in which Strain A mice received a diet containing 6 g piperazine per kilogram of diet and drinking water containing various concentrations of sodium nitrite. The mice received the treatment for 20 weeks, beginning at 10 weeks of age, and were killed when they were 40 weeks old. The surface lung adenomas were then counted. The results were expressed as percent incidence of tumors and as number of tumors per mouse, i.e., tumor multiplicity. The authors found that the yield of tumors per mouse was proportional to the square of the concentration of nitrite. However, since tumor multiplicity is not considered in the mathematical models used to obtain risk estimates, the committee has used the data on tumor incidence (Table 10-7).

Direct risk extrapolation for nitrite and a nitrosatable amine (piperazine) from studies in animals fed for 1 to 40 weeks is shown in Table 10-8 for the one-hit, multistage, and multihit models at risk levels 10^{-2} , 10^{-4} , 10^{-6} , and 10^{-8} . The figures in parentheses are the linearized model estimates for risk levels 10^{-4} , 10^{-6} , and 10^{-8} for the multihit model.

TABLE 10-7

Lung Adenomas in Male Strain A Mice Given Piperazine at 6 g/kg of Diet and Various Levels of Sodium Nitrite in Drinking Water^a

| <u>Group (i)</u> | <u>Dose of Sodium Nitrite in water (g/liter) (d_i)</u> | <u>No. of Mice with Lung Adenoma (x_i)</u> | <u>No. of Mice on Test (n_i)</u> |
|------------------|--|--|--|
| 0 | 0 | 11 | 39 |
| 1 | .05 | 10 | 39 |
| 2 | .25 | 20 | 40 |
| 3 | .50 | 30 | 39 |
| 4 | 1.0 | 37 | 37 |
| 5 | 2.0 | 39 | 40 |

^aData from Greenblatt and Mirvish, 1973.

TABLE 10-8

Estimated Daily Doses of Sodium Nitrite in Drinking Water Which, in the Presence of 6 g of Piperazine per Kilogram of Diet, Correspond to Various Levels of Risk of Lung Adenomas in Strain A Mice^a

| <u>Model</u> | <u>Dose of Sodium Nitrite, mg/liter Drinking Water, by Level of Risk (P₀)</u> | | | |
|---|--|--|---|---|
| | <u>10⁻²</u> | <u>10⁻⁴</u> | <u>10⁻⁶</u> | <u>10⁻⁸</u> |
| One-hit (d ₁ [*]) and Multistage (d ₂ [*]); d ₁ [*] = d ₂ [*] | 4.3 | 4.2 x 10 ⁻² | 4.2 x 10 ⁻⁴ | 4.2 x 10 ⁻⁶ |
| Multihit (d ₃ [*]) | 17 | 8.5 x 10 ⁻¹ (1.7 x 10 ⁻¹) ^b | 4.2 x 10 ⁻² (1.7 x 10 ⁻³) | 2.1 x 10 ⁻³ (1.7 x 10 ⁻⁵) |

^aBased on data from Greenblatt and Mirvish, 1973.

^bNumbers in parenthesis are the linearized estimates.

The estimated constant h for the multihit model was $\hat{h} = (1.54 + 0.69)$, and the multistage model resulted in a one-stage model with $\hat{\theta}$ estimated to be $\hat{\theta}_1 = 2.36371$. Hence, the first row of Table 10-8 for the onehit model also gives the results for the multistage model.

The statistical likelihood ratio test of Rai and Van Ryzin (1981) yields a chi-squared value of 1.08, which for one degree of freedom is not significant, even at 25% level. Therefore, the observed data provide no evidence to reject the one-hit model, and the data in the first row of Table 10-8 are believed to be relevant estimates of the doses for various risks.

The exact meaning of these estimates for humans is difficult to assess since the data from animals cannot be converted directly to humans, either as mg/kg body weight or as mg/cm² body surface, without assuming that humans are concurrently exposed to 6 g of piperazine per kilogram of diet. Such an assumption is unreasonable in view of the exposures of the general population to piperazine. Moreover, piperazine is nitrosated more readily than most common amines that are present in the diet (Greenblatt and Mirvish, 1973).

Therefore, the only conclusion that one can draw from this risk extrapolation is that increased levels of nitrite consumed in the presence of exceedingly high levels of a nitrosatable amine lead to a rather linear dose-response curve for nitrite. By extrapolating such a dose-response curve down to zero dose, it can be seen that a reduction in the frequency with which animals consume nitrite in the diet would lead to a proportional reduction in the incidence of lung adenomas. For example, halving the dose of nitrite would halve the frequency of animals with lung adenomas. Extrapolation and conversion techniques do not have the capability of determining the exact relevance of this observation to humans, with regard to the effect on in vivo formation of N-nitroso compounds resulting from reduced exposure to nitrite in the presence of nitrosatable amines.

Additional Estimates of Risk. In this section, the calculations of risk for N-nitroso compounds are combined with exposure to nitrate and nitrite from food and water (Table 5-20 and 5-21) and exogenous exposure from other sources (Table 8-4, as modified by using data for in-vivo formation, Table 8-3) to arrive at estimates of risk for certain defined subgroups of the population.

Underlying these calculations are the assumptions that nitrosamines are carcinogenic in humans, that NDMA is the main source of exposure for humans, that NDMA is representative of all nitrosamines (even though its potency in animals is greater than that of many other nitrosamines), and that NDMA is as carcinogenic in humans as it is in animals. The committee recognizes that these assumptions may not be valid. Certain alkyl nitrosoureas and other nitrosamides

are more carcinogenic than NDMA, and humans may be more sensitive to their effects than rodents, particularly if the exposure occurred during pre- or neonatal development.

Thus, the estimates were taken from Table 10-3, i.e., a lifetime risk of 10^{-6} is incurred by a person whose daily exposure to nitrosamines is 5.84 ng/kg body weight per day, if converted on a unit of body weight basis, and 0.85 ng/cm² body surface per day, if converted on a unit of body surface area basis.

In addition, to calculate the total amount of nitroso compounds formed endogenously, the committee assumed that amino substrates in the stomach are nitrosated at the same rate as proline and that the daily intake of amines in the diet is 4 g. The committee recognizes that weakly basic amines and amides are more readily nitrosated than proline and that the actual exposure to amines may be considerably higher or lower than 4 g/day.

Using these assumptions and estimates, the committee calculated the risk for seven groups with low to high levels of exposure (Table 10-9). Assuming linearity of the risk at low doses, the low-dose risk for a daily exposure dose (d in μg) is calculated:

$$P(d) = 1,000 \times \theta \times d = (1.81 \times 10^{-5}) d,$$

where $\theta = P_0/d_0 = 10^{-6}/(0.85 \times 65)$ and 0.85×65 is the estimated daily dose of NDMA in ng for a person weighing 65 kg to yield a risk (P_0) of 10^{-6} over a lifetime (Table 10-3). Thus, the lifetime risk for Group 1 of Table 10-9 is estimated by the following calculation:

$$\begin{aligned} P(3.1) &= \frac{1,000 \times 10^{-6} \times 3.1}{0.85 \times 65} \\ &= (1.81 \times 10^{-5}) \times 3.1 = 5.6 \times 10^{-5}. \end{aligned}$$

Similar calculations were also made for the other six groups (Table 10-9).

If we assume that 130 million people in the United States fall into Group 1, then the exposure to nitrosamines from all sources (in vivo and exogenous) would lead to approximately 104 deaths per year (assuming all tumors lead to death), i.e.:

$$104 = \frac{130 \times 10^6 \times 5.6 \times 10^{-5}}{70} .$$

TABLE 10-9

Estimates of Lifetime Risk of Cancer for Various Population Groups Based on Exogenous and In-Vivo Exposure to Nitrosamines. Numbers in Parenthesis Indicate Reduced Exposure if All Nitrite were Removed from Cured Meats

| Source of Exposure ^b | Exposure to Nitrosamines, $\mu\text{g}/\text{Person}/\text{Day}$ ^a | | | | | | |
|---|---|--|--|--|---|--|--|
| | Group 1 Average Diet, Nonsmoker | Group 2 Average Diet, Smoker | Group 3 High Cured Meat Diet ^c | Group 4 Vegetarian, ^d No Beer | Group 5 Nitrate-Rich Water, ^e No Beer, Nonsmoker | Group 6 High Risk ^f | Group 7 Low Risk ^g |
| <u>Endogenous^h</u> | | | | | | | |
| Diet (Total) | 1.3(1.1) | 1.3(1.1) | 2.0(1.1) | 12.0(12.0) | 14.0(13.0) | 16.0(13.0) | 1.3(1.1) |
| <u>Exogenous</u> | | | | | | | |
| Cosmetics | 0.41(0.41) | 0.41(0.41) | 0.41(0.41) | 0.41(0.41) | 0.41(0.41) | 0.82(0.82) | |
| Car Interiors | 0.20(0.20) | 0.20(0.20) | 0.20(0.20) | 0.20(0.20) | 0.20(0.20) | 0.50(0.50) | 0.02(0.02) |
| Beer | 0.97(0.97) | 0.97(0.97) | 0.97(0.97) | 0 | 0 | 3.9(3.9) | 0 |
| Bacon | 0.17(0) | 0.17(0) | 0.68(0) | 0 | 0.17(0) | 0.68(0) | 0 |
| Tobacco smoke | 0 | 17(17) | 0 | 0 | 0 | 35.0(35.0) | 0 |
| Work | 0 | 0 | 0 | 0 | 0 | 250(250) | 0 |
| TOTAL EXPOSURE | 3.1(2.7) | 20.1(19.7) | 4.3(2.7) | 12.6(12.6) | 14.8(13.6) | 307(303) | 1.3(1.1) |
| LIFETIME RISK ⁱ | 5.6×10^{-5} | 3.6×10^{-4} | 7.8×10^{-5} | 2.3×10^{-4} | 2.7×10^{-4} | 5.6×10^{-3} | 2.3×10^{-5} |
| REDUCED LIFE- TIME RISK ^{i,j} | 4.9×10^{-5} | 3.5×10^{-4} | 4.9×10^{-5} | 2.3×10^{-4} | 2.5×10^{-4} | 5.5×10^{-3} | 2.1×10^{-5} |
| RANGE OF LIFETIME RISK ^k | 8.2×10^{-6} to 1.8×10^{-4} | 5.3×10^{-5} to 1.2×10^{-3} | 1.1×10^{-5} to 2.5×10^{-4} | 3.4×10^{-5} to 7.4×10^{-4} | 3.9×10^{-5} to 8.7×10^{-4} | 8.2×10^{-4} to 1.8×10^{-2} | 3.4×10^{-6} to 7.4×10^{-5} |

^aAssuming that the average body weight is 65 kg.

^bTaken from Tables 8-3 and 8-4.

^cAssuming 4 times the average intake of meat and bacon.

^dAssuming 4 times the average intake of vegetables and no meat.

^eAssuming that 160 mg of nitrate is provided by consumption of nitrate-rich water.

^fAssuming 4 times the average intake of meat and bacon and the use of nitrate-rich water (which provides 160 mg of nitrate daily [see Table 5-20]), heavy cosmetic use (twice the average), frequent use of a new automobile, heavy beer consumption (4 times the average), heavy smoker (twice the average), and high occupational exposure.

^gAssuming an average diet, no cosmetics, light automobile use (15 min/day), no beer, no bacon, nonsmoker, and no occupational exposure.

^hAmount of nitrosoproline formed in vivo as a result of various types of exposure (see Table 8-3 and 8-4 for methods used to arrive at these estimates).

ⁱBased on mg/cm^2 body surface area. These amounts should be divided by 6.85 to obtain an estimate for ng/kg body weight.

^jAssuming that all nitrite is removed from cured meats.

^kThe higher extreme is derived by using the one-hit model, i.e., multiplying the lifetime risk by 3.2. The lower extreme is obtained by dividing by 6.85 the lifetime risk estimates calculated for units of body surface area (footnote i), to obtain the estimate for units of body weight.

This would lead to an effective rate of 1.9 (130/70) million people at risk per year.

The Possible Effect of Eliminating Nitrite from Cured Meats. The row under the lifetime risk in Table 10-9 provides rough estimates of reduced risk of cancer if nitrite were removed from cured meats. These estimates were derived from speculative calculations based on reduced exposures to nitrosamines that might be expected if all nitrite were removed from cured meats. If 130 million people in the United States are assumed to fall into Group 1, as before, the reduced number of deaths per year from cancer due to exposure to nitrosamines would be:

$$91 = \frac{130 \times 10^6 \times 4.9 \times 10^{-5}}{70}.$$

Therefore, for Group 1, the reduction of deaths per year due to removal of nitrite from cured meats would be approximately:

$$13 = (104 - 91).$$

Likewise, if we assume that another 50 million people belong to Group 2 (average diet and smoker), removal of nitrite would lead to a reduction of approximately 7.1 deaths per year due to tumors induced by exposure to nitrosamines:

$$7.1 = \frac{(50 \times 10^6)}{70} \times (3.6 - 3.5) \times 10^{-4}.$$

Assuming that another 20 million people in the United States belong to the high cured meat diet group (Group 3), 10 million to the high risk group (Group 6), and 10 million to the low risk group (Group 7), then the following reduction in deaths per year due to cancer from exposure to nitrosamines would result from the removal of nitrite from cured meats:

High Cured Meat Diet (Group 3):

$$8.3 = \frac{(20 \times 10^6)}{70} \times (7.8 - 4.9) \times 10^{-5}$$

High Risk (Group 6):

$$14.3 = \frac{(10 \times 10^6)}{70} \times (5.6 - 5.5) \times 10^{-3}$$

Low Risk (Group 7):

$$0.3 = \frac{(10 \times 10^6)}{70} \times (2.3 - 2.1) \times 10^{-5}$$

Adding the numbers of deaths estimated for Groups 1, 2, 3, 6, and 7 indicates that elimination of nitrite from cured meats would result in a reduction of approximately 43 deaths per year (i.e., $13 + 7.1 + 8.3 + 14.3 + 0.3 = 43$) from cancer if all cancers lead to death in the general mixed population of 130 million nonsmokers eating an average diet, 50 million smokers eating the same diet, 20 million eating a high cured meat diet, 10 million high risk individuals who are exposed occupationally as well as from other sources, and 10 million low risk individuals. This estimate is based on conversion of the dose to unit of surface area for humans. When expressed as unit of dose per unit of body weight, the resulting reduction in the number of deaths would be approximately 6.3.

The calculations given in Table 10-9 and those that follow the table are all presented as specific numbers. However, these numbers should only be considered as crude estimates of risk and that they represent a number based on the specific assumptions made. In general, estimates over a range of values are more realistic and depend on the assumptions made. For example, if one uses the one-hit model estimate from Table 10-2 in developing Tables 10-3 and 10-9, the resulting estimates would be multiplied by a factor of 3.2 ($0.097 \div 0.03$). Thus, the lifetime risks from Group 1 would be 1.8×10^{-4} . Likewise, as pointed out in footnote 1 of Table 10-9, if the ng/kg body weight conversion were used the estimates in Table 10-9 should be divided by 6.85, yielding a lifetime risk for Group 1 of 8.2×10^{-6} . The last row of Table 10-9 shows this range for each population group using these two variations in the risk estimates.

If one applies these same changes in assumptions to the possible effect of eliminating nitrite from cured meats, the estimate of a reduction of deaths per year from cancer would range from approximately 6 to 138 deaths, based on the assumed distribution of exposed population groups given above.

Furthermore, it should be realized that all calculations in Table 10-9 and the reduction in the number of deaths assumed the mid-points of exposure given in Tables 5-20, 5-21, and Table 8-4 and that one could easily widen these ranges. However, the committee believes that the ranges given in Table 10-9 for various population groups and the estimated reduction in cancer deaths (6 to 138 per year) if nitrite were removed from cured meats, are plausible estimates based on the assumptions cited herein.

Another point that must be realized is that the above estimates completely ignore subpopulations that are heterogeneous and that may be more sensitive to the carcinogenic effects of nitrosamines because of a genetic or metabolic abnormality or other condition. If such populations exist, reduced exposure to nitrite for these populations might considerably reduce these risks.

Discussion and Summary

The speculative risk estimates made by the committee indicate that from approximately 6 to 138 cases of cancer could be avoided in the United States annually if nitrite were removed from cured meats. This calculation and conclusion are very speculative since they are based on the following assumptions: (1) that humans are as susceptible as rats to cancer induction by all nitrosamines, including NDMA; (2) that NDMA is the main source of exposure for humans and is, therefore, representative of all nitrosamines; (3) that the potency and low-dose linear behavior of all nitrosamines are similar to those of NDMA; (4) that all nitrosatable amines behave like proline in the stomach; (5) that the exposure levels are those given in Tables 5-20, 5-21, 8-3, and 8-4; and (6) that the population consists of 130 million from Group 1 (the average nonsmoker), 50 million from Group 2 (smokers), 20 million from Group 3 (high cured meat diet), and 10 million each from Groups 6 and 7 (high and low risk). The last assumption, concerning population mix, is not too crucial because among the seven groups, Group 1, the largest group, has the second lowest ratio of lifetime risk to reduced lifetime risk (0.9), as shown in Table 10-9. The lowest ratio is for the high cured meat diet group (0.6).

From these calculations and speculations, it appears that a large reduction in exposure to nitrosamines in work environments, from cigarette smoke, and possibly from certain cosmetics and drugs would have a greater life-saving effect than the removal of nitrite from cured meats. The main reason for this is that exposure to nitrosamines from nitrite in cured meats is small compared to the total exposure from all sources for all population groups, except for the high cured meat diet group considered in Table 10-9. Thus, for most groups the removal of nitrite from cured meats would not have a major effect on the risk over a lifetime. But, if only dietary sources of exposure were considered, the diminution in risk will be proportionally greater if nitrite were removed from cured meat.

To the degree that one places confidence in the estimate made by the Food and Drug Administration Task Force (Food and Drug Administration, 1979b) that 22 deaths from butulism would result from the omission of nitrite in meat products, that number could be compared to the possibility of 6 to 138 deaths estimated above. The committee does not believe that such a comparison is advisable since both numbers are rough estimates.

OVERALL SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Evidence of carcinogenicity provided by well-conducted experiments in animals should be regarded as indicating a potential for carcinogenicity in humans. This is especially true when results of investigations have demonstrated carcinogenicity in more than one species. There are no completely reliable methods for using data obtained from animal experiments to derive the magnitude of tissue- or organ-specific carcinogenic potency of a chemical in humans.

Based on one experiment in rats and assuming low dose linearity, the committee has estimated that the lifetime risk of cancer would be one in a million, if humans were exposed to a daily dose of 5.8 to 19 ng of nitrosodimethylamine per kilogram of body weight or 0.85 to 2.7 ng of nitrosodimethylamine per cm^2 of body surface. In arriving at this estimate, the committee has also assumed that (1) the dietary doses given to rats can be converted to unit of dose per unit of body weight or per unit of body surface area to reflect human exposure and (2) that nitrosodimethylamine is the main source of exposure to nitrosamines for humans and is therefore representative of all nitrosamines, even though its potency in animals is greater than that of many other nitrosamines.

The committee also examined seven hypothetical population groups and estimated that the lifetime risk of cancer from exposure to all sources of nitrosamines would be 820 to 18,000 per million for a high risk group (including occupational exposure), 11 to 250 in a million for a high cured meat diet group, 8 to 180 in a million for an average population of nonsmokers, and 3 to 74 in a million for a low risk group.

The committee wishes to emphasize that the validity of these estimates of risk is limited by some significant gaps in our knowledge: insufficient data about the average and extreme levels of exposure to nitrate, nitrite, and N-nitroso compounds, multiplicity of inadequately characterized variables that determine the extent of endogenous nitrosation, uncertainty about the molecular mechanisms leading to the carcinogenic effect of N-nitroso compounds and their precursors; uncertainty about the comparable ability of humans and laboratory animals to repair genotoxic damage; and the probability that individuals and subgroups of the population vary in the susceptibility to the carcinogenic effects

of N-nitroso compounds. The set of assumptions selected and the mathematical models used in the development of these risk estimates introduce additional uncertainty. Therefore, the committee suggests that the numerical estimates be used solely as rough indicators of the relative risk to each of these population groups. The absolute numbers stated in this chapter are not intended as a guide for policy formation, nor should they be understood by the public to be final and definitive.

Although a reduction in exposure to nitrite is likely to reduce the risk of cancer, there is insufficient evidence to support the plausible assumption that a reduced exposure to nitrate and nitrite will lead to a directly proportional reduction in the risk to human health. There is better evidence for N-nitroso compounds: Studies of nitrosodimethylamine in animals indicate that a directly proportional reduction in risk could result from the reduction of exposure to N-nitroso compounds.

Nitrosamines formed endogenously from nitrite in cured meats provide only a small proportion of the total exposure of the general population to nitrosamines from all sources. Thus, it does not appear that the reduction of nitrite in cured meats will lead to a major decrease in risk to humans arising from total exposure to nitrosamines. However, if only dietary contributors to exposure to N-nitroso compounds are considered, the diminution in risk will be proportionally greater if nitrite were removed from cured meats. These conclusions are based on average exposure to N-nitroso compounds and do not fully consider the effects of exposure to peak levels, which may be a critical factor.

The committee examined various approaches to estimating the risk of botulism and the increment in its incidence that might arise from omitting nitrite from cured products. It found that previous attempts to derive such estimates were based on speculation with which it did not wholly concur. It concluded that a more adequate data base must be developed before one can predict the likelihood of a product becoming toxic and, from this, the incidence of botulism. However, the committee believes that the degree of protection against botulism is likely to decrease if the essential preservative uses of nitrite are substantially reduced without introducing an efficacious, but safer alternative.

In the interest of defining the absolute risk of botulism and the increment in this risk if the use of nitrite were diminished, the committee recommends that investigations be pursued to determine the incidence of and resulting mortality from botulism.

REFERENCES

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18:265-267.
- American Meat Institute. 1976. Nitrite: Good Manufacturing Practices. American Meat Institute, Arlington, Virginia. 44 pp. + appendix.
- Armitage, P., and R. Doll. 1961. Stochastic models for carcinogenesis. Pp. 19-38 in J. Neyman, ed. *Proceedings of the Fourth Berkeley Symposium on Mathematical Statistics and Probability, Vol. 4.* University of California Press, Berkeley and Los Angeles, California.
- Bryan, F. L. 1980. Foodborne diseases in the United States associated with meat and poultry. *J. Food Prot.* 43:140-150.
- Buege, D. 1980. Update -- Nitrite-free processed meats. Pp. 122-135 in the *Proceedings of the 33rd Annual Reciprocal Meats Conference, June 22-25, 1980, Purdue University, West Lafayette, Indiana.* American Meat Science Association and the National Livestock and Meat Board, Chicago, Illinois.
- Center for Disease Control. 1979a. Botulism in the United States, 1899-1977. Handbook for Epidemiologists, Clinicians, and Laboratory Workers. Center for Disease Control, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia. 41 pp.
- Center for Disease Control. 1979b. Botulism -- United States, 1978. *Morbidity and Mortality Weekly Report* 28(7):73-75.
- Center for Disease Control. 1980. Botulism in the United States, 1979. *J. Infect. Dis.* 142:302-305.
- Center for Disease Control. 1981. Botulism -- United States, 1979-1980. *Morbidity and Mortality Weekly Report* 30(10):121-123.
- Cornfield, J. 1977. Carcinogenic risk assessment. *Science* 198: 693-699.
- Cornfield, J., F. W. Carlborg, and J. Van Ryzin. 1978. Setting tolerances on the basis of mathematical treatment of dose-response data extrapolated to low doses. Pp. 143-164 in G. L. Plaa and W. A. M. Duncan, eds. *Proceedings of the First International Congress on Toxicology: Toxicology as a Predictive Science.* Academic Press, New York, San Francisco, and London.

- Crump, K. S., D. G. Hoel, C. H. Langley, and R. Peto. 1976. Fundamental carcinogenic processes and their implications for low dose risk assessment. *Cancer Res.* 36:2973-2979.
- Crump, K. S., H. A. Guess, and K. L. Deal. 1977. Confidence intervals and test of hypotheses concerning dose response relations inferred from animal carcinogenicity data. *Biometrics* 33:437-451.
- Druckrey, H. 1967. Quantitative aspects of chemical carcinogenesis. Pp. 60-78 in R. Truhaut, ed. *Potential carcinogenic hazards from drugs: Evaluation of risks.* UICC Monograph Series, Vol. 7. Springer Verlag, Berlin, Heidelberg, and New York.
- Food and Drug Administration. 1979a. Report: Nitrite Task Force. Unpublished report of the Nitrite Task Force, Bureau of Foods, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, D.C. 43 pp. plus tables and figures.
- Food and Drug Administration. 1979b. Chemical compounds in food-producing animals: Criteria and procedures for evaluating assays for carcinogenic residues. *Fed. Regist.* 44(55):17070-17114.
- Food and Drug Administration. 1980. Report of the Interagency Working Group on Nitrite Research: Evaluation of the MIT Nitrite Feeding Study to Rats. Food and Drug Administration, Public Health Service, U.S. Department of Health and Human Services, Washington, D.C. 76 pp. plus attachments.
- Food Safety Council. 1980. Proposed System for Food Safety Assessment. Final Report of the Scientific Committee of the Food Safety Council. Food Safety Council, Washington, D.C. 160 pp.
- Greenblatt, M., and S. S. Mirvish. 1973. Dose-response studies with concurrent administration of piperazine and sodium nitrite to strain A mice. *J. Natl. Cancer Inst.* 50:119-124.
- Hartley, H. O., and R. L. Sielken, Jr. 1977. Estimation of "safe-doses" in carcinogenic experiments. *Biometrics* 33:1-30.
- Hoel, D. G., D. W. Gaylor, R. L. Kirschstein, U. Saffiotti, and M. A. Schneiderman. 1975. Estimation of risks of irreversible, delayed toxicity. *J. Toxicol. Environ. Health* 1:133-151.
- Holley, R. A. 1978. Botulism Hazard in Cured Meats Treated with Reduced Concentrations of Nitrite. A report prepared for the Industry/Government Committee on Nitrites and Nitrosamines. Food Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada. 20 pp.

- Høyem, T. 1977. Experiences with nitrite and nitrate ban in Norway. Pp. 293-297 in B. J. Tinbergen and B. Krol, eds. Proceedings of the Second International Symposium on Nitrite in Meat Products, September 7-10, 1976, Zeist, the Netherlands. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Johnston, R. W., and G. W. Krumm. 1980. The microbiological safety of canned, cured, perishable meat products. Pp. 295-299 in Proceedings of the 26th European Meeting of Meat Research Workers, Vol. 2, August 31-September 5, 1980, Colorado Springs, Colorado.
- Krewski, D., and J. Van Ryzin. In press. Dose response models for quantal response toxicity data. In M. Csörgö, D. Dawson, J. N. K. Rao, and E. Saleh, eds. Current Topics in Probability and Statistics. Elsevier/North Holland Press, New York and Amsterdam.
- Mantel, N. 1963. Chi-square tests with one degree of freedom; extensions of the Mantel-Haenszel procedure. *Am. Stat. Assoc. J.* 58:690-700.
- Mantel, N., and W. R. Bryan. 1961. "Safety" testing of carcinogenic agents. *J. Natl. Cancer Inst.* 27:455-470.
- National Academy of Sciences. 1977. Drinking Water and Health, Vol. 1. A report prepared for the U.S. Environmental Protection Agency by the Safe Drinking Water Committee, Advisory Center for Toxicology, Assembly of Life Sciences, National Research Council. National Academy of Sciences, Washington, D.C. 939 pp.
- Ohshima, H., and H. Bartsch. 1981. Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. *Cancer Res.* 41:3658-3662.
- Paget, G. E. 1965. Toxicity tests: A guide for clinicians. Pp. 31-39 in A. D. Herrick and M. Cattell, eds. *Clinical Testing of New Drugs*. Revere Publishing Company, Inc., New York.
- Panalaks, T., J. R. Iyengar, and N. P. Sen. 1973. Nitrate, nitrite, and dimethylnitrosamine in cured meat products. *J. Assoc. Off. Anal. Chem.* 56:621-625.
- Panalaks, T., J. R. Iyengar, B. A. Donaldson, W. F. Miles, and N. P. Sen. 1974. Further survey of cured meat products for volatile N-nitrosamines. *J. Assoc. Off. Anal. Chem.* 57:806-812.
- Peto, R. 1978. Carcinogenic effects of chronic exposure to very low levels of toxic substances. *Environ. Health Perspect.* 22:155-159.

- Preussmann, R., D. Schmähl, G. Eisenbrand, and R. Port. 1977. Dose-response study with N-nitrosopyrrolidine and some comments on risk evaluation of environmental N-nitroso compounds. Pp. 261-268 in B. J. Tinbergen and B. Krol, eds. Proceedings of the Second International Symposium on Nitrite in Meat Products, September 7-10, 1976, Zeist, the Netherlands. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.
- Rai, K., and J. Van Ryzin. 1979. Risk assessment of toxic environmental substances using a generalized multihit dose-response model. Pp. 99-117 in N. E. Breslow and A. S. Wittemore, eds. Energy and Health. SIAM Press, Philadelphia, Pennsylvania.
- Rai, K., and J. Van Ryzin. 1981. A generalized multihit dose-response model for low-dose extrapolation. *Biometrics* 37:341-352.
- Sakaguchi, G. 1979. Botulism. Pp. 389-442 in H. Riemann and F. L. Bryan, eds. Food-Borne Infections and Intoxications. Academic Press, New York, San Francisco, and London.
- Smith, L. 1977. Botulism: The Organism, Its Toxins, The Disease. American Lecture Series No. 997. Chales C Thomas, Publisher, Springfield, Illinois. 236 pp.
- Society of Toxicology Task Force. 1981. Re-examination of the ED₀₁ Study. *J. Fundam. Toxicol.* 1:26-128.
- Sonnabend, O. 1981. Different types of Clostridium botulinum (A, D, and G) Found at Autopsy: II. Pathological and Epidemiological Findings in Twelve Sudden and Unexpected Deaths. Paper presented at the Symposium on the Biomedical Aspects of Botulism, March 16-18, 1981. U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland. Abstract.
- Sonnabend, W. 1981. Different types of C. botulinum (A, D, and G) Found at Autopsy in Humans: I. Isolation of the Organisms and Identification of the Toxins. Paper presented at the Symposium on the Biomedical Aspects of Botulism, March 16-18, 1981. U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland. Abstract.
- Tarone, R. E. 1975. Tests for trend in life table analysis. *Biometrika* 62:679-682.
- Terracini, B., P. N. Magee, and J. M. Barnes. 1967. Hepatic pathology in rats on low dietary levels of dimethylnitrosamine. *Br. J. Cancer* 21:559-565.
- Tompkin, R. B. 1980. Botulism from meat and poultry products-- A historical perspective. *Food Technol.* 34:229-236, 257.