Microbiological Quality of Foods

Proceedings of a Conference Held at
Franconia, New Hampshire

Edited by

L. W. SLANETZ, C. O. CHICHESTER, A. R. GAUFIN, Z. J. ORDAL

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Proceedings of a Conference Held at Franconia, New Hampshire
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Edited by:

L. W. Slanetz
Department of Microbiology
University of New Hampshire
Durham, New Hampshire

C. O. Chichester
Department of Food Science and Technology
University of California
Davis, California

A. R. Gaufin
Department of Zoology and Entomology
University of Utah
Salt Lake City, Utah

Z. J. Ordal
Department of Food Technology
University of Illinois
Urbana, Illinois

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Contributors

Numbers in parentheses indicate pages on which the
authors' contributions begin.

R. ANGELOTTI, Robert A. Taft Sanitary Engineering Center, U.S. Public
Health Service, Cincinnati, Ohio (149).

M. D. APPLEMAN, Department of Bacteriology, University of Southern
California, University Park, Los Angeles, California (162).

J. C. AYRES, Department of Dairy and Food Industry, Iowa State Uni-
versity, Ames, Iowa (132).

M. S. BERGDOLL, Food Research Institute, University of Chicago, Chicago,
Illinois (54).

C. W. BOHRER, National Canners Association, Washington, D. C. (198).

W. L. BROWN, Technical Research, John Morrell & Co., Ottumwa, Iowa
(243).

L. BUCHBINDER, Bureau of Laboratories, Department of Health, New
York, New York (15).

E. P. CASMAN, Division of Microbiology, Food and Drug Administration,
U.S. Department of Health, Education, and Welfare, Washington, D. C.
(50).

J. H. B. CHRISTIAN, Commonwealth Scientific and Industrial Research
Organization, Division of Food Preservation, Ryde, N.S.W. Australia
(223).

G. M. DACK, Food Research Institute, University of Chicago, Chicago,
Illinois (41).

R. PAUL ELLIOTT, Western Regional Research Laboratory, Albany, Cali-
fornia (171).

J. B. EVANS, Department of Botany and Bacteriology, North Carolina
State College, Raleigh, North Carolina (159).

M. J. FANELLI, Microbiological Research Department, Campbell Soup
Company, Camden, New Jersey (205).

E. M. FOSTER, Department of Bacteriology, University of Wisconsin, Madison, Wisconsin (35).

M. J. FOTER, Robert A. Taft Sanitary Engineering Center, U.S. Public Health Service, Cincinnati, Ohio (94).

H. E. GORESLINE, Armed Forces Food and Container Institute, U.S. Army Quartermaster Research and Engineering Command (179).

M. F. GUNDERSON, Microbiological Research Department, Campbell Soup Company, Camden, New Jersey (23 and 205).

M. INGRAM, Low Temperature Research Station, Cambridge, England (229).

E. H. KAMPELMACHER, National Institute of Public Health, Utrecht, Netherlands (84).

T. KAWABATA, Department of Food Control, National Institute of Health, Shinagawa-ku, Tokyo, Japan (63 and 71).

KEITH H. LEWIS, Division of Environmental Engineering and Food Protection, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio (252).

W. L. MALLMANN, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan (1).

E. M. MRAK, University of California, Davis, California (265).

C. F. NIVEN, JR., American Meat Institute Foundation, Chicago, Illinois (119).

F. R. PEABODY, Department of Bacteriology, Michigan State University, East Lansing, Michigan (113).

CARL S. PEDERSON, Department of Food Science and Technology, New York State Agricultural Experiment Station, Cornell University, Geneva, New York (237).

A. C. PETERSON, Microbiological Research Department, Campbell Soup Company, Camden, New Jersey (205).

G. SAKAGUCHI, Department of Food Control, National Institute of Health, Shinagawa-ku, Tokyo, Japan (63 and 71).

M. A. SHIFFMAN, Milk and Food Section, Department of Health, City of Philadelphia, Pennsylvania (20).

J. H. SILLIKER, Department of Pathology, St. James Hospital, Chicago Heights, Illinois (102).

GLENN G. SLOCUM, Division of Microbiology, U.S. Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C. (6).

LOUIS DS. SMITH, Department of Botany and Bacteriology, Montana State College, Bozeman, Montana (77).

F. S. THATCHER, Department of National Health and Welfare, Ottawa, Canada (10).

C. T. TOWNSEND, National Canners Association, Berkeley, California (32).

R. H. VAUGHN, Department of Food Science and Technology, University of California, Davis, California (193).

C. VINTON, John Morrell & Co., Ottumwa, Iowa (243).

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Other Participants

H. B. ALLEN
Fish and Wildlife Service
Washington, D. C.

C. O. BALL
Department of Food Science
Rutgers University
New Brunswick, New Jersey

CLARA H. BARTLEY
Department of Microbiology
University of New Hampshire
Durham, New Hampshire

W. R. CHESBRO
Department of Microbiology
University of New Hampshire
Durham, New Hampshire

C. O. CHICHESTER
Department of Food Science and
   Technology
University of California
Davis, California

G. K. CROWELL
Bureau of Food and Chemistry
State Department of Health
Concord, New Hampshire

R. H. DEIBEL
Division of Bacteriology
American Meat Institute
Chicago, Illinois

H. M. EL-BISI
Department of Food Technology
University of Massachusetts
Amherst, Massachusetts

P. R. ELLIKER
Department of Microbiology
Oregon State University
Corvallis, Oregon

W. B. ESSELEN
Department of Food Science and
   Technology
University of Massachusetts
Amherst, Massachusetts

W. C. FRAZIER
Department of Bacteriology
University of Wisconsin
Madison, Wisconsin

MILDRED M. GALTON
Veterinary Public Health Laboratory
   Unit
Communicable Disease Center
Department of Health, Education, and
   Welfare
Atlanta, Georgia

A. R. GAUFIN
Department of Zoology and
   Entomology
University of Utah
Salt Lake City, Utah

I. GERRING
Environmental Science and
   Engineering Study Section
National Institutes of Health
Bethesda, Maryland

D. P. GLICK
Biological Sciences
Fort Detrick
Frederick, Maryland

S. A. GOLDBLITH
Department of Nutrition, Food
   Science and Technology
Massachusetts Institute of Technology
Cambridge, Massachusetts

R. A. GREENBERG
Research Laboratory
Swift & Company
Chicago, Illinois

R. E. HALBINGER
University of Buenos Aires
Buenos Aires, Argentina

N. H. HANSEN
Danish Meat Research Institute
Roslikde, Denmark

P. A. HARTMAN
Department of Bacteriology
Iowa State University
Ames, Iowa

F. W. HARTMANN
Division of Laboratories
California State Department of
   Public Health
Berkeley, California

W. J. HAUSLER, JR.
State Hygiene Laboratory
State University of Iowa
Iowa City, Iowa

BETTY HOBBS
Food Hygiene Laboratory
Central Public Health Laboratory
London, England

J. W. HUNNELL
Commercial Development
The Pillsbury Company
New Albany, Indiana

A. HURST
Unilever Research Laboratory
Bedford, England

J. M. JAY
Department of Biology
Wayne State University
Detroit, Michigan

A. JEPSEN
Department of Bacteriology
The Royal Veterinary and
   Agricultural College
Copenhagen, Denmark

A. LANE
Difco Laboratories
Detroit, Michigan

J. LISTON
Food Science
College of Fisheries
University of Washington
Seattle, Washington

W. LITSKY
Department of Microbiology
University of Massachusetts
Amherst, Massachusetts

W. C. LOY
Research and Technical Division
Wilson & Co., Inc.
Chicago, Illinois

B. MALIN
Applications Research
Lilly Research Laboratories
Indianapolis, Indiana

T. F. MANCUSO
Ohio State Department of Health
Columbus, Ohio

A. G. MCLEOD
Bureau of Food Control
Winnipeg, Manitoba, Canada

M. MILNER
Food Conservation Division
UNICEF
United Nations, New York

D. A. A. MOSSEL
Laboratory of Bacteriology
Central Institute for Nutrition
   and Food Research
Utrecht, The Netherlands

J. O. MUNDT
Department of Bacteriology
University of Tennessee
Knoxville, Tennessee

H. B. NAYLOR
Department of Bacteriology
Cornell University
Ithaca, New York

H. RAJ
Food Science
College of Fisheries
University of Washington
Seattle, Washington

H. B. ROBINSON
Research Grants Branch
Division of Environmental
   Engineering and Food Protection
Bureau of State Services
Washington, D. C.

P. A. ROHDE
Technical Services
Baltimore Biological Laboratories
Baltimore, Maryland

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PREFACE

Foodborne diseases and problems relating to the sanitary and micro-
biological quality of foods continue to be of major interest and concern
in this and other countries of the world. Such foodborne diseases as
salmonellosis and staphylococcal food poisoning actually appear to be on
the increase in the United States. New problems have been created due
to recent developments in the processing and handling of foods. Little
is known about the role of foods in the transmission of virus diseases.
There is still no general agreement as to the types of microbiological tests
and the standards that should be established to maintain the safety and
quality of many foods.

The specific aims of this conference on the Microbiological Quality of
Foods were to review the present state of knowledge of foodborne diseases
and to discuss the use and efficiency of microbiological tests and standards
for food quality from the academic, regulatory, and industrial stand-
points. Problems related to the use of microorganisms as an index of food
quality were given special attention. This included a consideration of
total counts, coliforms, fecal streptococci, and the detection of specific
pathogens. A particular effort was made to point out and emphasize
areas needing further research.

The conference was jointly sponsored by the Environmental Sciences
and Engineering Study Section of the National Institutes of Health and
the Department of Microbiology, University of New Hampshire. It was
supported by Research Grant EF106 provided by the Division of En-
vironmental Engineering and Food Protection, Bureau of State Services,
Public Health Service. The following members of the above Study Section
assisted in developing plans for the program: Dr. C. O. Chichester,
University of California, Davis; Dr. A. R. Gaufin, University of Utah;
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assistance and advice received from Dr. H. E. Goresline, Quartermaster
Food and Container Institute; Dr. K. H. Lewis, Taft Sanitary Engineer-
ing Center; Dr. W. L. Mallmann, Michigan State University; Dr. C. F.
Niven, Jr., American Meat Institute Foundation; and Dr. G. G. Slocum,
Food and Drug Administration. The assistance of this group in the
development and planning of this conference was most helpful.

Ninety-five selected speakers and participants attended the conference,

44 from universities or research institutes, 35 from federal or state government agencies, and 17 from industry. Seven foreign countries were represented. Many of the foremost authorities in the field of food microbiology presented papers or participated in the conference discussions. Special thanks are due the speakers for the excellent papers they presented and for their cooperation in preparing their manuscripts for publication. The published Proceedings of this conference should be of interest to everyone concerned with problems relating to the microbiological quality of foods.

University of New Hampshire   L. W. SLANETZ
Durham, New Hampshire   Director of Conference
October, 1963

Introductory Remarks

WESLEY E. GILBERTSON

Division of Environmental Engineering and Food Protection, Public Health Service, U.S. Department of Health, Education, and Welfare, Washington, D. C.

In this conference, I find the sense of considerable gratification. Some of you may recall as I do, a meeting at the Sanitary Engineering Center a short five years ago, where the topic of discussion was whether or not research proposals in the food sciences were eligible for support under the grant programs at the National Institutes of Health. At this meeting, Dr. Gordon Seger of the National Institutes of Health corrected a popular misconception that NIH grant funds were exclusively for medical research. With this clarification, the Environmental Sciences and Engineering Study Section, which is co-sponsoring this meeting today, undertook the stimulation of research in this important area, and we have seen grant support for food research grow from the modest two or three projects at that time, to the present level which exceeds $2,000,000 a year.

I am glad that the ESE Study Section chose to sponsor a symposium in this important area. Surely a conference, structured as this one, to probe in depth the many factors of the food microbiology problem, cannot fail to provide us with greater clarity in our thinking and a better basis for the judgments we must make. I should like to commend Dr. Slanetz and his planning committee for their fine work in developing the pattern of discussion. I am impressed with the range of topics and with the speakers who will present them. I appreciate this opportunity to renew acquaintances and to meet many of you who heretofore I have known only through the medium of your work.

There is another factor about this meeting which I mention in a more personal way. This conference is being supported by a grant from the Division of Environmental Engineering and Food Protection. This is significant because it reflects a development of comparatively recent date. Although control of environmental factors has been the very foundation of public health in the past, it has occupied to a large extent an ancillary position to other programs. Environmental health is now emerging as a discrete entity in the public health picture with stature comparable to other major program components. In the Public Health

Service we now have a functional grouping of five divisions of the Bureau of State Services which are concerned with air, water, food, radiation, and occupational health to achieve a more cohesive approach to the total problem of environmental health. There is legislation before both Houses of the Congress which, if enacted, will permit this grouping to be given full Bureau status. In the planning for the establishment of this Bureau, an early decision was reached that environmental health divisions should conduct grant programs to foster and develop extramural research in the respective program areas. Thus, the ability of the Division of Environmental Engineering and Food Protection to support this conference is important, not just from the standpoint of the conference, but from the broader view of enhancing the potential for bringing research in the food sciences and other facets of environmental health to a status more commensurate with present day demands for scientific information. This step, with its attendant responsibilities of program planning and budget justification, should give greater visibility to these areas and permit more accurate assessment of the dimensions of the research needs.

In this regard, I should like to take a moment to comment briefly on our needs for scientific information. The Bureau of State Services deals substantially with the ultimate product of research, the translation of scientific information into the kinds of practice that bring about environmental changes conducive to better health. Thus, the needs for information in the field of food microbiology are for the kinds of data which will clarify problems and permit development of effective countermeasures for the hazards resulting from interaction between microbes, foods, and man.

Development of counter measures is required in the fulfillment of one of our important functions—the provision of technical assistance to state and local health agencies and industry in the control of public health problems.

In the course of developing program guides and similar documents, we become at times acutely conscious of vacuums in scientific information. In such instances, we can only make professional judgments based on the best information available. In so doing, we seek the counsel of the best people we can get—people who can reflect viewpoints of all sides of a problem. For example, in the past few months we released a new manual on sanitation for the food service industry. This was developed by our Milk and Food Program staff with the substantial assistance of a special advisory committee composed of people from public health, research, and industry. They brought together elemental facts derived from the research and observations of many disciplines. In this endeavor and

in other similar projects, we draw heavily on the "bank account" of scientific knowledge. We hope that those of you in research will continue to make regular deposits so that this account may be kept in balance.

The complexities of modern life dictate an expanding research effort to provide the kinds of information required to cope with the changing environment. As the population expands, wastes increase, the metropolitan and rural environments tend to merge, and technology produces new materials and products; the experimental background becomes progressively deficient, and new factors must be studied and equated with classical concepts. To meet this challenge, we intend to press for more adequate support for both the intramural and extramural research programs, including development of Centers for Environmental Health recommended in the report of the Committee on Environmental Health Problems, chaired by Dr. Paul M. Gross of Duke University. (Dr. Mrak and Dr. Dack, members of the Food Subcommittee, are scheduled speakers on the Symposium.) There is an item in our present supplemental appropriation bill to provide the funds needed for acquisition of a site for the center and for the development of structural plans.

Time does not permit a detailed discussion of the specific functions of such a center but I should like to take time to cite to you some of the committee's general conclusions:

"That a national need exists for establishment and maintenance of a vigorous and integrated effort to maintain controls over the human environment compatible with projections of change in both population and the environment itself.

"That accommodation to the National needs in environmental health will require the establishment of a strong focal center adequately staffed and equipped to prosecute an effective and integrated program within the Public Health Service and to manage and coordinate a strong extramural research, training, and technical support program utilizing the available institutional resources of the nation.

"That an adequate legislative basis for a sufficient national program in environmental health does not exist at present."

In the detailed planning for implementation of the general recommendations, our concept calls eventually for competent Centers **for** Environmental Health in the various broad program areas embraced. These will be closely linked but identifiable units. One of these, we believe, should be a center for food research and technology.

In closing, I should like to stress the importance of identifying and studying the public health problems of our changing environment. I have not alluded to specifics purposely because I feel sure that the speakers who follow will delineate many of the problems in food microbiology in

precise terms. As the picture of environmental health problems change, so must the work relationships on the local, state, and federal levels change with respect to meeting this problem. The Public Health Service recognizes that it alone cannot combat the complex situation that now faces us, but that we must work together with other governmental agencies and the institutions of our country in a joint effort. For this reason, we pledge our support to those of you who desire to study and attack these problems and we urgently seek your assistance. We are vitally interested in what you accomplish at this conference, and we sincerely hope that your aims of determining some of the specific research needs on the microbiological quality of foods will be attained, and that they will stimulate research in solving some of the problems involved with the safety of foods in this country.

Introductory Remarks

DR. G. A. ROHLICH

University of Wisconsin, Madison, Wisconsin

As Chairman of the Environmental Sciences and Engineering Study Section, it is my pleasant opportunity and privilege to be able to speak in its behalf at this conference. As you probably know, the Study Section receives research proposals for review in the field of environmental health and I would like to point out that its membership is made up of a multidisciplinary group because we receive applications not only in the food science field but also in occupational medicine, water supply and pollution control, radiological health, and air pollution.

We, of course, in reemphasizing what Mr. Gilbertson has said, are very much interested in the broad concept of environmental health and recognize the importance of this particular area. To that end the study section has sponsored several conferences. The first was a general conference entitled "Man Versus Environment." Second, a conference on the "Physiological Aspects of Water Quality." The third conference that we sponsored was occupational medicine. This is the fourth of the conferences in which we have taken direct interest. Each of these has been for purposes which have already been outlined by Dr. Slanetz and Mr. Gilbertson. Principally, we are interested in establishing where we stand in research in a particular field, what are the greatest research needs for the future, and who are the people who can be stimulated to work on such research activities. I think Dr. Slanetz and his committee are to be congratulated on the excellent program they have arranged here.

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Current Status of Microbiological Tests

and Standards for Food Quality

W. L. MALLMANN

Department of Microbiology and Public Health,
Michigan State University, East Lansing, Michigan

This conference has been planned for a discussion of methodology for the detection of microorganisms that either induce infection of man when ingested in food or microorganisms that may be indicators of the wholesomeness of food. It is hoped that from the discussion on methodology and microbial standards for food wholesomeness, the research needs in this area will be apparent and that those in attendance will return to their respective research laboratories with research concepts that may materialize into the desired answers necessary to evaluate (1) the need of microbial standards for food wholesomeness, (2) if a need for microbial standards exists, the selection of the best microbial tests, and (3) a decision as to the best approach to wholesomeness of food control by microbial standards, by regulatory specification for production, or a combination of microbial standards and regulatory specifications of production.

A justification for the application of both microbial standards and sanitary regulations for food processing and handling is found in the dairy industry. Over a period of 60 years, regulatory agencies have applied regulations on milk and dairy products. The developments of regulations were largely activated by the unfortunate experiences of epidemics of disease which were traced directly to improper processing and handling. Bacterial standards were set independently by various agencies with broad areas of supervision, or in many cases, with limited areas of control. The writer, over the past 40 years, has had the opportunity to follow these attempts at regulation at city, state, and federal levels, with all the difficulties encountered by overlapping areas of control where several different bacterial standards and processing regulations came into conflict. Adjacent dairy farms might operate under extremely different require-

ments, where milk was supplied to different cities in the same milk shed. We are hopeful that planned research on procedures will lessen the chances of ill-advised standards and microbiological procedures.

Out of the chaos of conflicting regulations has evolved standards that have made possible a dairy industry unequaled in the world for quality and wholesomeness of milk. The bacterial standards based on attainability of performance, rather than health hazard measurements, have been largely responsible for the high standards of quality maintained throughout the United States. In order to hold bacterial population below maximum allowable standards, good sanitation practice has resulted. Bacterial standards have played an essential role in the development and maintenance of wholesome milk production.

The procedures for the detection of bacteria in milk have changed from time to time as research in methodology demonstrated faults in current technique or demonstrated better methodology. Present methods for total bacterial count or coliform counts are satisfactory, but studies on methodology still continue in attempts by research workers to find simpler methods and greater accuracy for comparative purposes.

It can be assumed with considerable justification that bacterial standards could be applied to highly perishable foods with the same degree of success as that attained with milk and dairy products.

Potential health hazard does exist in highly perishable chilled and frozen foods, especially in precooked products, due to contamination of the products by insanitary practices, as well as multiplication of pathogenic bacteria by holding foods at temperatures favorable for bacterial growth during processing and handling. Fortunately, the history on frozen perishable foods shows little cause for concern. However, the use of frozen foods in the household is such that disease or food poisoning is limited to a few people and the source of the disease or food poisoning is generally unknown and unreported. There is evidence to show that disease or food poisoning does occur in the household, but no evidence that precooked frozen foods are responsible. Most regulatory workers believe some controls are necessary, as evidenced by observation of insanitary practices in processing and handling of perishable foods. Although most food processing plants maintain high standards, there are marginal operators that attempt to get by with inadequate equipment, inadequate housing, and poor housekeeping practices. Sometimes failure to produce a quality product may be due to gross ignorance of possible health hazard, but sometimes the failure is due to attempts at cutting manufacturing and handling costs. These latter groups in all food manufacturing areas make regulatory requirements necessary. The precooked frozen food industry is no exception, as demonstrated by Proctor and Phillips (1). An examination was

made of over 100 types of precooked frozen food. Total bacterial counts varied from less than 50,000 to over 1,000,000 per gram; however, microscopic counts ranged from $<0.5 \times 10^6$ to 40×10^6 per gram which indicates a large population at the time of processing. Litsky et al. (2) examined 134 frozen meat, fish, and poultry pies bacteriologically. Total counts ranged from 2000 to $>11 \times 10^6$ with a median of 15,000 bacteria per gram. Eighty-four per cent had counts below 100,000 per gram. Only 16 samples had coliform counts in excess of 100, and 56 had 10 or less. In recent studies by Mallmann and Peabody all meat pies examined from many processors had surprisingly low populations, which would demonstrate an improvement in processing and handling that has occurred since examinations were made in 1957.

It is well known that salmonellae and staphylococci grow rapidly in most readily perishable food when incubated at room temperature. Proctor and Phillips (1) demonstrated that when Staphylococcus aureus and Salmonella enteritidis were added to cooked food and then frozen that at least 10% of the inoculated organisms survived. They also demonstrated that when defrosting of cooked frozen foods occurred at 30°C, the surviving organisms multiplied. These results demonstrate the potential health hazard of precooked frozen foods. Even though there have been few reports of infection and food poisoning from precooked frozen foods, a potential hazard exists, and there is a very definite need for some form of regulatory control.

In a review, Elliott and Michener (3) present a long list of suggested microbiological standards for various chilled and frozen food products. Whereas standards are largely based on total counts and coliform organisms, there are some suggestions as to incidence of both coagulase-positive staphylococci and salmonellae. In some areas, codes and regulations have been formulated but these have been largely confined to regulations in processing and handling. So far, there has been little attempt to evaluate chilled and frozen foods by microbiological means.

The applications of bacterial tests for total count, coliform organisms, enterococci, staphylococci, and salmonellae have been, for the most part, merely the use of tests devised for clinical tests, and water or milk analysis. In most instances investigators have applied these tests to food in the same manner as that used in the original application. The fact that nutrients or spices in the food may have influenced the behavior of the media used has not been considered.

Taylor et al. (4) found that the most probable number technique using selenite F enrichment medium, although satisfactory for clinical use, failed to detect the largest number of salmonellae in foods. In a series of experiments, a cystine selenite F enrichment medium in combination with

brilliant green agar for isolation was found to be the best method for detection and enumeration of salmonellae.

These studies on methodology for the detection and isolation of salmonellae from foods demonstrates the need of better techniques for the detection of these organisms. Because the proposed procedures detected more salmonellae than other methods does not necessarily indicate that still better techniques cannot be developed.

Incidentally, the studies by Taylor *et al* (4) were applied to dried egg albumin known to contain salmonellae. Whether the same technique could be equally effective when precooked frozen meat, fish, and poultry pies were tested, remains to be determined.

Another demonstration of the need of methodology was reported by Mallmann and Peabody (5) in a comparison of the surface plating technique with the multiple tube dilution procedure (MTD). They found that the surface plating procedure (drop-plate) yielded higher counts of coliform than the multiple tube dilution technique, particularly when marginal waters were used where the coliform organisms were under adverse conditions. More recently, Mallmann has been making comparison of a shake tube method with the MTD technique on raw waters from various water treatment plants. In many instances the confirmed coliform organism counts were 10-fold higher with the shake tube method. The medium used for the MTD technique was lauryl tryptose broth and the medium for the shake tube procedure was lauryl tryptose agar. The nutrients in each medium were identical. In the shake tube procedure, as well as in surface plating, the organisms are confined in what is designated a "restricted environment."

Again research in methodology has yielded an answer to the problem of detecting microorganisms that have been weakened by a sojourn in an unfavorable climate.

How effective are the present methods for the quantitative detection of either specific pathogens or indicator organisms for application in water and milk examination? How effective would these methods be when applied to food analysis? Would effective methods applied to precooked frozen meat be equally effective if applied for example to precooked chow mein or spaghetti and meat balls?

Assuming that effective methods were available, then the problem of sampling arises. What constitutes a representative sample of a batch of precooked frozen pies? What size sample should be used for microbiological assay? How many replications of a sample should be made to have a statistically valid evaluation?

Should the microbiological assay be designed to yield data on the viable cell count of the food product at the consumer purchase level, or should

the counts be representative of the condition that existed at the time of processing?

During the 3 days of this conference, it is hoped that discussions will result that may be helpful in resolving research needs in the public health aspects of precooked frozen food, as follows:

1. The need for microbiological procedures and standards of attainment.

2. The selection of microorganisms that might serve as yardsticks of wholesomeness.

3. The need for methodology for the detection of specific pathogens or the desired indicator microorganisms.

4. A discussion of the broad field of precooked frozen foods as to diversity of products, the application of microbial standards, and the use of processing and handling sanitation codes.

REFERENCES

1. B. E. Proctor and A. W. Phillips. Frozen pre-cooked foods. *Am. J. Public Health* 38, 44–49 (1948).
2. Warren Litsky, I. S. Ferguson, and C. R. Fellers. A bacteriological survey of commercial frozen beef, poultry and tuna pies. *J. Milk Food Technol.* 20, 215–219 (1957).
3. R. Paul Elliott and David Michener. Microbiological standards and handling codes for chilled and frozen foods: A review. *Appl. Microbiol.* 9, 452–468 (1961).
4. W. I. Taylor, J. H. Silliker, and H. P. Andrews. Isolation of salmonellae from food samples. 1. Factors affecting the choice of media for the detection and enumeration of salmonellae. *Appl. Microbiol.* 6, 189–193 (1958).
5. W. L. Mallmann and F. R. Peabody. Coliforms determined by surface plating technique. *Water Sewage Works* 109, 146–149 (1962).

Food Microbiological Problems

from the Federal Regulatory Standpoint

GLENN G. SLOCUM

Division of Microbiology, U. S. Food and Drug Administration,
Department of Health, Education, and Welfare, Washington, D. C.

The Food, Drug, and Cosmetic Act is the principal federal statute designed to assure American consumers that foods in interstate commerce are clean and wholesome. However, meat and poultry products are subject to the jurisdiction of the Meat Inspection Act and Poultry Products Inspection Act enforced by the U.S. Department of Agriculture, while the control of foods on interstate carriers is the responsibility of the Public Health Service of the Department of Health, Education, and Welfare. My remarks are confined necessarily to problems within the purview and experience of the Food and Drug Administration (FDA), but will, to some extent, impinge upon areas which are responsibilities of other agencies.

The principal concern of regulatory officials at all levels of government, and of industry and research scientists as well, is the safety or wholesomeness of foods. Unfortunately, we are not in a position to judge accurately the safety of our food supply in the United States. It is generally agreed that the recorded incidence of foodborne diseases, most of which are bacterial infections and intoxications, is a relatively small proportion of the true incidence. The number of cases reported annually in the decade 1951–1960 ranged from about 7,000 to 12,000 (see Table I). Estimates of the probable incidence range from 300,000 to one million cases a year. It is evident from the fact that one state alone reports about one-third of all recorded food poisoning episodes, and 5 or 6 states report about two-thirds of the total, that a large proportion of foodborne disease occurring in this country goes unrecorded and, to a large extent, unrecognized. Like the floating iceberg, the bulk of the problem is submerged and we can determine little of the nature and characteristics of the hidden structure by looking at the visible part.

TABLE I
SUMMARY OF DISEASE OUTBREAKS

	Water		Milk and milk products		Other foods		Total	
Year	Out.[a]	Cases	Out.	Cases	Out.	Cases	Out.	Cases
1951	7	3,960	12	90	256	7,182	277	11,344
1952	14	530	6	833	143	6,828	163	8,191
1953	11	719	4	97	194	9,914	209	10,730
1954	7	452	9	200	234	11,704	250	12,390
1955	2	22	3	302	193	9,633	198	9,957
1956	9	719	31	873	210	11,133	250	12,725
1957	4	131	8	67	250	11,085	262	11,283
1958	4	445	13	441	236	9,925	253	10,811
1959	7	206	11	49	322	10,595	340	10,850
1960	11	1,784	5	48	182	7,434	198	9,266

[a] Outbreaks.

We need then, a vastly improved system for the detection, investigation, and reporting of foodborne illness and for dissemination of the collected information for the guidance of regulatory and industry officials. Without adequate information on the extent of the problem, the agents responsible, the food vehicles, the sources and routes of contamination, and the defects in food processing or handling which contribute to the health hazards, it is difficult to devise adequate control measures.

Available evidence makes it quite clear that progress in the control of foodborne disease has lagged significantly behind the control of water- and milkborne disease. The latter have become virtually insignificant as sources of human illness whereas there is no evidence of a similar decline in foodborne illness. Dr. Buchbinder (1) has recently compared the status of control of foods with water and milk control. Certainly, the absence of clear-cut epidemiological evidence for the role of other foods similar to that formerly existing for water and milk has contributed substantially to the relative lack of progress.

As mentioned earlier, FDA is primarily concerned with foods in interstate commerce. The proportion of recorded cases of foodborne illness traceable to interstate foods is relatively small. But events of recent years have caused us to question this record and to suspect that the problem is of larger proportions than indicated by reported statistics.

Four outbreaks of salmonellosis have been traced to foods nationally distributed in the United States within the past 5 or 6 years. In addition, outbreaks of typhoid fever, *Salmonella reading*, and, recently, *Salmonella*

hartford infections of undetermined origin have had the characteristics of common food source incidents. The presence of salmonellae in egg products, poultry, meats, and animal feeds is well known and the list of infected foods will undoubtedly grow as the scope of the search is widened. Desiccated coconut has been implicated in salmonellosis in Australia and England but not in the United States to date. However, FDA has detected and detained numerous shipments of imported coconut containing salmonellae. Annual summaries of the National Office of Vital Statistics have shown a rather steady increase in the number of cases of salmonellosis (excluding typhoid fever), from 882 cases in 1948 to 6929 cases in 1960 (see Table II). It is hard to escape the conclusion that salmonellosis is

TABLE II

REPORTED CASES OF SALMONELLOSIS
(Except Typhoid Fever)

Year	Number of cases	Year	Number of cases
1948	882	1955	5447
1949	1243	1956	6704
1950	1233	1957	6693
1951	1733	1958	6363
1952	2596	1959	6606
1953	3946	1960	6929
1954	5375		

an increasing problem and that interstate foods may play an important role in the dissemination of these organisms.

Staphylococcal intoxications from interstate foods are also infrequent. However, the common occurrence of coagulase-positive staphylococci in raw milk and occasional outbreaks from domestic and imported cheeses, and from dried milk remind us of a continuing potential hazard. Similarly, the frequent incidence of such staphylococci in cooked foods subsequently exposed to food handlers involves a potential threat, particularly where such foods are subjected to later mishandling. We need to know much more about the incidence and sources of enterotoxigenic strains of staphylococci, and measures for their elimination or control.

The role of *Clostridium perfringens* in food poisoning in the United States is just now being rediscovered. We have been convinced for several years that this type of food poisoning is relatively common but unrecognized in this country. While commercially prepared foods in interstate

commerce have not, to our knowledge, been implicated in outbreaks, the possibility of such an occurrence is not remote.

Several months ago Dr. Dack made the interesting observation that the commercially prepared foods involved recently in botulism cases were not canned products but such diverse items as a cheese spread, pot pies, and smoked fish. Such episodes are rare but should serve to remind us that changes in methods of food preservation, processing, and packaging may involve unanticipated hazards.

The smoked fish episode is of special interest. Two persons died after eating smoked fish (ciscoes) packed under vacuum in a plastic bag. Type E *Clostridium botulinum* and toxin were found in remnants of the fish. Although *C. botulinum* was not detected in other samples of fish from the same source, we demonstrated experimentally that smoked fish inoculated with the organism and held with or without vacuum became toxic within 5 days at 10°C but remained nontoxic after 30 days at 5°C. Toxin was present in fish prior to the development of obvious spoilage. Schmidt (2) has recently demonstrated production of toxin by certain strains of type E in another medium at 3.3°C after about 30 to 45 days. Since vacuum packing extends the apparent shelf-life of smoked fish up to a month or 6 weeks and the organism is capable of growth in the normal refrigeration zone, the potential hazard is obvious. We are now attempting to evaluate the problem more critically.

Oysters and clams from polluted waters have recently been implicated in the spread of infectious hepatitis, and local outbreaks of the disease have been traced to infected food handlers. The possibility of transmission of enteric viruses through fecal pollution of foods has long been acknowledged and needs epidemiological and laboratory investigation.

These are some of the food microbiological problems in the health field from the federal regulatory point of view. The trend towards centralization of food production in large interstate plants and to production of an increasing variety of nonsterile, prepared foods used with a minimum of processing by consumers, makes it imperative, we believe, to judge the safety and sanitary quality of food production more and more upon a microbiological basis.

The numbers and types of microorganisms vary widely from product to product with variations in raw materials, processing procedures, and sanitary conditions and practices. Thorough and competent bacteriological studies in each food plant are necessary to detect and eliminate sources of contamination and greatly facilitate the interpretation of the findings on finished products. FDA has embarked on such a program. We believe that substantial improvement in the microbiological quality of foods can be effected. Ultimately, emphasis by industry and regulatory officials

upon better microbiological control of food production should be most effective in reducing the risk of foodborne disease.

REFERENCES

1. L. Buchbinder. *Public Health Rep.* 76, 515–520 (1961).
2. C. F. Schmidt, R. V. Lechowich, and J. F. Folinazzo. *J. Food Sci.* 26, 626–630 (1961).

Discussion by F. S. Thatcher

Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada

Food-control problems in the United States as reviewed by Dr. Slocum seem so remarkably similar to Canadian experience that I surmise he will have touched upon principles that have a high degree of applicability in many countries. The Canada Food and Drugs Act differs pointedly, in one respect, from its United States counterpart. All foods in Canada are subject to the provisions of the Canadian Act. The need to demonstrate interstate or inter-provincial movement of foods is not involved.

As I appraise Dr. Slocum's remarks, the problems reside in four main areas. For convenience I will name these as scientific, jurisdictional, practical, and subjective. These will be referred to later.

The Changing Food Industry

Another aspect of regulatory concern touched upon by Dr. Slocum was the need to be aware of change: "change in the methods of food preservation, processing, and packaging." I would like to enlarge a little on this aspect, for it illustrates well the niceties of balance that must be established between all four areas, if food control efforts are to be effective.

It is fundamental to consider that any factor which acts selectively against any component of a mixed microbial population will change the comparative distribution and specific dominance of organisms within that population, and in a manner largely unpredictable. Dr. Slocum has illustrated this by his reference to botulism from vacuum-packed smoked fish. We have published on the same aspect and upon the development of staphylococci and enterotoxin in anaerobically incubated bacon in the absence of organoleptically detectable spoilage.

It is important for the food microbiologist to evaluate in perspective his knowledge that the bacterial cell is a complex agent of synthesis and catabolism with the expression of these properties conditioned by environment as well as by the genetic makeup of the cell.

Many species are known to have multiple avenues of genetic modification available to them. Selection pressures may be exerted on all such changes, and

upon the comparative dominance of particular groups of microorganisms, through environmental and ecological factors which man continuously alters by the use of chemicals, by antibiotics, by evacuation of air, or more recently by use of irradiation, or by changing agricultural and industrial practices. The extent of risk from any potential disease hazard is augmented by the production of foods in massive volume and by the efficiency with which foods are distributed throughout the community.

The pace of change in the food industry is rapid, but problems of potential public health significance seem to emerge just as quickly as new technological developments. It is questionable whether the mechanisms or extent of public health aspects of control within the food industry have kept abreast of the technological developments in that industry. Tardy recognition of the problems, cost, and availability of competent personnel are all involved.

It became necessary in recent months to enact and enforce a regulation requiring that egg products shall not be sold in Canada unless free from salmonellae. This was done because the frequency of human salmonellosis in Canada seemed to have approximately doubled in each of the last 2 years, while in the same period the recovery of the serotype *Salmonella thompson* from human sources had increased from 0.5% of the total to 25%. This serotype had obviously become widely disseminated. Our laboratory found that *S. thompson* was the most common serotype found in egg products, much of which was produced in plants certified by another government agency. Some 70% of the packages of a specific type of widely distributed egg-containing food was found to contain *S. thompson*. We could find no other widely distributed source of this serotype. Epidemiological evidence implicating this type of food is now positive and increasing in extent. This illustrates the potential hazard from modern processing and marketing, and the need for marketing developments to be matched by laboratory testing. While the industries concerned have, in general, shown commendable collaboration in correcting the problem, and the manufacturers of powdered egg within a few weeks showed substantial advance in response to the regulation, nevertheless, the role of eggs as a source of *Salmonella* had been understood by industry for several years, but control testing had been unusual, and processes aimed at destruction of salmonellae used only sparingly.

Scientific Problems

Some of the scientific problems touched upon by Dr. Slocum include the need to determine the full extent of the food poisoning problem which seems to be increasing on a wide front. We need to learn and correct the causes of these increases. Methodology demands more attention. Adequate and simple methods for determination of salmonellae in foods are now available, but no laboratory has yet developed a fully satisfactory test for the presence of enterotoxin in foods, though general use of serological methods for detecting the toxins in laboratory cultures or filtrates is to be anticipated. Only recently has Dr. Hobbs' proof of involvement of *Clostridium perfringens* in food poisoning led to recognition and detection of the problem on this continent. Even the fecal coliforms, the enterococci, *Staphylococcus* and *Micrococcus* need more study or a new basis for agreement on suitable methods for each.

The role of foods as a vehicle for transmission of viruses within the human population is still largely ignored.

Jurisdictional Problems

I do not wish to attribute to Dr. Slocum anything he did not wish to imply, but his reference to multiple agencies having various areas of responsibilities in food control led my mind to the jurisdictional problem which probably exists to some degree in most countries. In Canada, the Federal Departments of Health, Agriculture, and Fisheries have defined roles to play. In the provinces, Health and Agriculture operate under their own respective legislation. Municipalities may legislate for specific foods within the purview of the pertinent Provincial Act. All must conform to the provisions of the Food and Drugs Act and its regulations. To be effective, all such agencies must have effective rapport with the food industries. Liaison and practical integration of effort are one obvious problem. The desire of competent people to achieve the same end is the most potent cohesive force here. A second problem arises when different objectives are pursued narrowly. Where, for example, a dominant interest in production and sales conflicts with appraisal of health hazard then a veritable Solomon may be needed for effective solution at the council table, though would the decision be that of a Solomon if it failed to rule in favor of health?

Practical Problems

The practical aspects, of course, involve money, people, and facilities. If the safety and sanitary quality of foods are to be measured microbiologically in a manner to give minimal consumer protection, then more of all three will be required, and by both government and industry. A practical requirement, too, is that legislative demands or standards shall be attainable under conditions of good commercial production. The volume of foods that can be analyzed, and the number of commodities that can be subject to standards that are enforced pose intensely practical problems to the regulatory agency.

Subjective Problems

It is in these latter aspects that the fourth problem area lies—the subjective one. This refers to the decisions to be made that embody all the foregoing considerations, and ask for answers to such questions as: What group or numbers of what organisms constitute a health hazard, or imply a risk of enteric infection? What degree of accuracy in a method, a sampling procedure, or interpretation of a finite standard is necessary to provide effective public health control? What is adequate sanitation in a food plant in terms of microbiological parameters? Should produce from a country with low enteric disease rates be required to meet the standard desirable for foods from a country where enteric disease is endemic at high rates? Which are the commodities that require priority in control effort, and which of these are best rendered safe and wholesome by introduction of microbiological standards or by insistance upon the practice of good factory sanitation?

It is the responsibility of all who work in the field of public health to become aware of changes or trends that may modify the kind or degree of health hazard to which the public may become exposed. Decisions may need to be made concerning the significance of such potential hazards, often before extensive experience or sufficient data are available to serve as a guide. On occasion this calls for subjective judgement of far-reaching responsibility.

Microbiological Control Action in Canada

I do not propose to offer an immediate solution to all of these questions. I can summarize briefly what the small team, of which I am a member, tries to do in appreciation of these problems.

Scientific effort is directed to research and investigation to a greater extent than to surveys and routine determinations in relation to standards. The motivation for such research is to find the facts that will enable intelligent decisions to be made regarding potential health hazards, and preferably before these will have become a public problem. Our published studies of staphylococci in dairy products, the role of toxins and their determination, the public health aspects of the effect of irradiation on bacteria, salmonellae in processed foods, the microbiology of frozen precooked foods, the effect of antibiotics on food flora, the effect of the vacuum-pack process on toxin production have all been initiated under such stimulus. The findings from all of these studies have influenced control policy and action.

Major control effort is based on a policy of "control at the source" with emphasis on factory sanitation. It is high time that food plant sanitation should be treated as the specialized aspect of bacteriology that it is, and should be examined as such. But neither government nor industry is adequately equipped for such a development.

Microbiological standards are used largely to reinforce the sanitation effort or to provide protection not afforded by sanitary practice alone. This last applied in promulgation of our standards for egg products. Sanitation alone could not guarantee freedom from salmonellae. Efforts to facilitate international movement of foods with public safety imply some need for international standards. The movement of shellfish between Canada and the United States is already governed by reciprocal standards that involve parameters of sanitation and microbial numbers.

The jurisdictional aspect is approached by trying to earn and by reciprocating the respect that still makes the "round-table" discussion a potent democratic instrument.

The practical aspects impose serious limitations to the scope of our activities, and in some fields consumer protection approaches a bare minimum. This aspect will require a greater degree of control testing by industry. Our recent regulation controlling *Salmonella* in egg products seems to have had some success in this regard.

With regard to the subjective problems, I am often guided in seeking the full picture by what is purported to be a statement by Leonardo da Vinci concerning a fine painting: "Every part seeks to unite with the whole, thereby escaping its own incompleteness." The habit of trying to appraise in perspective all pertinent factors bearing upon a specific problem is essential for effec-

tive public health control in a democratic community. In the matter of food control, such perspective demands due recognition of the four problem areas illustrated by Dr. Slocum's address, viz., the scientific, jurisdictional, practical, and subjective.

Food Microbiological Problems
from a Local Regulatory Standpoint

LEON BUCHBINDER

Bureau of Laboratories, Department of Health, New York, New York

It might not be entirely inappropriate to note by way of introduction that not all bacteria in food are bad. As a matter of fact, although our interest in food is fundamentally related to those pathogenic bacteria contained therein which produce disease, we all are aware that some bacteria help make food what it is, others are in food adventitiously, and still others by their kind or number indicate the sanitary quality of the food. The latter actually act for us somewhat like the Judas goat in the abattoir. It is the pathogens and their so-called "indicator organisms" with which we are concerned here.

Experience in New York City indicates that a local health department cannot only influence the microbiological status of food products produced locally but can also affect the microbiological quality of such products from almost anywhere else. In the first instance this can be achieved by inspection and guidance furnished by local sanitarians, combined with the use of the bacteriological laboratory as a guide. As concerns food prepared outside the city, microbiological standards, imposed either by law or by administrative regulation, have been the only tools. The health department is convinced that by the use of these tools the microbiological quality of food can be improved at a distance and the potential hazard of food poisoning thus can be abated.

This started to become clear around 1955–1956 when we began studying and following up findings on frozen ready-to-eat foods on a systematic basis. Previous to that our experience had not been encouraging. This was subsequent to publication by us in 1949 of a market study of frozen ready-to-eat chicken products (1). Our findings had indicated that some of this food was potentially hazardous and that there was much to be desired concerning bacteriological quality. Manufacturers apparently did not know or care about the bacterial flora of these relatively new food

products. Nor did we then get the impression that these producers were terribly concerned about our findings. The climate was bleak. Apparently it had changed by 1955. Our experience since then has been that discussion or correspondence with manufacturers from outside the city or state is generally well received and leads to bacteriological improvement of their products. Whether this is because we are the largest individual market, or because of the prestige of the city, or simply because of the desire of industry to do a good job, is immaterial. The fact is that both local and outside producers now usually listen.

We have hardly ever had to embargo either frozen or fresh ready-to-eat products or even threaten to do so. We have never gone to court. Furthermore, we have tried to be helpful with technical advice. An illustration of the influence of a local department was an experience of some years ago concerning skinned and individually packaged ready-to-eat beef tongues. This food, prepared by a national company, was packaged in a plastic wrapper as the final step in processing. An outbreak of staphylococcus food poisoning was traced to it. Laboratory examination of the peccant food and the product as purchased in stores indicated that it was heavily contaminated with coagulase-positive staphylococci. Our diagnosis was that the product was contaminated by hand during skinning of the tongue just prior to final enclosure in the plastic wrapper. The company was informed at a meeting that according to our knowledge this should be a simple problem for them to solve. The suggestion was made that heat treatment in an appropriate broth immediately prior to packaging would solve the problem. The company demurred and said that it would rather remove its product from the market. Three months later we were informed that it had acceded to our suggestion and would be pleased to have us test its new product. Our finding indicated that the tongues were then in excellent microbiological condition.

Another instance concerned frozen ready-to-eat fish cakes which apparently caused illness after being consumed in a restaurant. Apart from poor heat control in the restaurant, the unheated product, both in the distributor's warehouse and on the manufacturer's premises outside the state, were found to be highly contaminated with coagulase-positive staphylococci. Suggestions and instructions by our Bureau of Food and Drugs resulted in a product that was bacteriologically satisfactory. Most of our experience has been of this type. Excessive counts are found, correspondence with the manufacturer ensues, sometimes an interview takes place. The end result is usually a better product from a bacteriological standpoint.

Our philosophy as a local health department, since reorganization of our efforts in food control which was stimulated by the burgeoning of ready-to-eat foods, both fresh and frozen, in the mid-1950's, has been to

help establish conditions which will reduce the probability of food poisoning by the encouragement of rational processes of food preparation and handling. We have been partially successful until the present. The difficulties facing us in such an attempt are manifold. Some of them have been delineated in a review of food poisoning control which was published last year (*2*). Because of poor reporting of outbreaks it is difficult to ascertain objectively the effectiveness of our efforts as demonstrated by a reduction in the known incidence of foodborne disease. We must rely to a great extent on bacteriological findings which indicate a reduced likelihood of such occurrences. Incidentally, we believe that the greatest value of the laboratory lies not in investigation of outbreaks that have occurred but in controlling critical foods on a routine preventive basis (*2*). Previous to the time mentioned above, almost all of our laboratory efforts had been devoted to the *ex post facto* study of outbreaks.

We believe that if the safety of ready-to-eat food is to be reasonably sure then (*a*) sound sanitary practices must be followed, and (*b*) some microbiological standards must be recognized to check both proper production procedure and handling until the food reaches the consumer. Our beliefs generally coincide with those found in the new Advisory Code concerning proper methods of sanitary control in plants and in product handling and distribution which has been recently developed by the Association of Food and Drug Officials of the United States. It should be noted that producers today are frequently concerned with microbiological levels but primarily from the marketing viewpoint, i.e., shelf-life. It is hardly to be expected that as a group they would take as much interest in standards related to health as do official agencies.

Our first experience with the establishment of bacteriological standards for foods, other than those for milk and other dairy products which are very old, was with fresh crabmeat. New York City is the largest market for this product from the fisheries of the South Atlantic and Gulf Coast States. Studies were made on fresh crabmeat subsequent to a series of foodborne disease outbreaks involving this product which occurred in the eastern part of the country in 1953. Frequent gross bacterial contamination of this food was disclosed. Subsequent tests made at random revealed that only a portion of the industry was able to control the sanitary quality of this desirable but highly perishable food. It was ascertained that with suitable precautions, plate counts of less than 100,000 per gram were obtainable. An attempt was made on an administrative basis to establish this and several other bacteriological standards for crabmeat. However, producers were not uniformly able or willing to make changes in practice which would bring their product within such standards. Furthermore, the producing states, although recognizing the need for regulation, were unable

to obtain legislation for licensing and other control procedures. All this was changed when the New York City Health Code was amended in 1955 and definite bacteriological standards were set. Our law reads: "The Department may exclude . . . crabmeat produced in a plant under permit or approval of an inspection service as herein provided, if such crabmeat is suspected of containing pathogenic organisms or contains bacteria in excess of the following standards: (1) more than 100 per gram of hemolytic *Staphylococcus aureus*; or, (2) more than 100 per gram of coliform organisms; or, (3) more than 1,000 per gram of enterococci; or (4) more than 100,000 per gram in the total bacteria plate count." The law also states that fresh crabmeat cannot be sold unless it has been prepared in a plant under federal inspection or state inspection approved by the Department of Health. The response of the affected states was electric, new legislation being passed almost immediately. In 1953, 63% of all fresh crabmeat samples failed to meet one or more of the bacterial criteria. Noncompliance with standards was reduced gradually during the next 3 years so that the figure stood at 37% in 1956. Since then it has ranged annually from 36 to 39%. Fresh crabmeat today, rarely if ever, produces food poisoning in our city.

The above, as noted, was our first effort to control a food product on a continuing basis by bacteriological criteria. It is also the only instance in which a food except milk has been regulated by Code bacteriological standards. All subsequent work on other foods has been done without such stated Code requirements. It has been relatively simple to use administrative rather than Code bacterial standards. Our experience has generally been that all kinds of food processors accept the authority of the Department of Health to regulate in such a fashion. The effectiveness of this procedure in the instance of the food products and processes so regulated now is well established for us (*3*).

New York City has been concerned with custard baked goods for a number of years. The liability of such products to cause food poisoning was forcibly brought to the attention of the Department of Health by an explosive outbreak involving a large chain bakery operation many years ago. A great deal of energy has been expended on custard baked products since then and today custard baked goods are practically no problem in New York City. Our first major effort was a study of products produced by the city's sixty-five wholesale bakeries (*4*). To summarize briefly, the first trial showed that 47 of the 65 bakeries could be classed as bacteriologically unsatisfactory, i.e., failed to meet a plate count of 100,000 per gram or coliform count standard of 10 per gram. Seventeen of the operators attempted to improve their operation by one, or usually several, of the following means: engaging a bacteriologist, improving sanitation,

improving processing, installing a mechanical handling device, installing pH control. Great bacteriological improvement was found in the products of these bakeries subsequent to the changes. We have since continued to check all wholesale bakeries on a limited scale.

A regular program of study of custard products in retail bakeries which manufacture their own cakes was started in 1958. This program has been continued on an annual basis since. Administrative bacteriological standards similar to those in the Code for crabmeat are used. In 1960, 733 of 744 such bakeries were tested. A total of 1533 samples were taken. It was found that samples from 658 (89.8%) of the bakeries were bacteriologically satisfactory on initial sampling.

A number of large restaurant organizations have gone into the marketing of fresh or frozen ready-to-eat take-home food. These products are sold in their own restaurants or special stores, or to the trade, and even for vending machine operations and automatic industrial feeding units. Such operators have had to be instructed in methods of preparation different from those which they used in their restaurant operations to ensure bacteriological safety during the increased handling and longer shelf-life which these products must endure.

A great deal of effort has been expended by New York City's Department of Health, not only in the application of administrative bacteriological standards, but just as importantly, in these attempts to assist the operator in increasing the safety and durability of his products. Such an instance was noted in the case of the ready-to-eat tongues. Extensive application of what might be called terminal pasteurization has been encouraged. This method has been found very effective as a means of controlling and eliminating endogenous and exogenous microbial contamination which may exist despite reasonable precautions. It has been determined by us that small pieces of meat, such as chicken, turkey, ham, roast beef, and corned beef, and seafood such as shrimp, crabmeat, and lobster meat which frequently are exposed to unsound handling after cooking, can be made "commercially sterile" by treatment in small masses by boiling in self-flavored stock, in less than 30 seconds. Even celery can be treated in this manner (5). Naturally such foodstuffs must be handled aseptically thereafter. It has been found that the texture of food particles is not adversely affected by such treatment, yield is increased, shelf-life safely prolonged, and most significantly, the possible food poisoning hazard eliminated. Our records are replete with instances of before and after bacteriological sampling of food products which have been investigated. The "after" counts are, in the matter of sanitation, quite benign as a rule.

We do not suffer from the delusion that the direct impact of our effort covers more than a relatively small part of the total food consumption

area. Our work has been limited to processors of both fresh and frozen ready-to-eat food prepared and sold in New York City and of foods prepared outside the city, to local bakers of custard goods, and to some restaurateurs. It should be noted that we have practically no control at the present by educational means of the approximately two million home kitchens in the city and very little control of the more than twenty thousand resturants and the some five thousand other food businesses of one kind or another. The problems with these places are not insoluble ones; they are merely very difficult. But no real start has been made on them. The aforementioned review discusses possible approaches. However, it can be stated that New York City will never be able to do it alone and that a national effort is required.

REFERENCES

1. L. Buchbinder, V. Loughlin, M. Walter, and G. Dangler. A survey of frozen precooked foods with special reference to chicken à la king. *J. Milk Food Technol.* 12, 209–214 (1949).
2. L. Buchbinder. Current status of food poisoning control. *Public Health Rep.* 76, 515–520 (1961).
3. A. E. Abrahamson. Frozen foods: Their impact on health. *Assoc. Food Drug Officials U. S. Quart. Bull.* 22, 7–16 (1958).
   A. E. Abrahamson, L. Buchbinder, J. Guenkel, and M. Heller. A study of frozen precooked foods: Their sanitary quality and microbiological standards for control. *Assoc. Food Drug Officials U. S. Quart. Bull.* 23, 63–72 (1959).
4. A. E. Abrahamson, R. Field, L. Buchbinder, and A. V. Catelli. A study of the control of the sanitary quality of custard-filled bakery products in a large city. *Food Res.* 17, 268–277 (1952).
5. A. E. Abrahamson and A. F. Clinton. The control of bacterial population in foods. *Assoc. Food Drug Officials U. S. Quart. Bull.* 24, 31–38 (1960).

Discussion by M. A. Shiffman

Department of Health, City of Philadelphia, Pennsylvania

Dr. Buchbinder has discussed the use of laboratory services in a local regulatory program for food protection. In many of our local agencies, laboratory services have been limited to the endless routine testing of milk and milk products and a few other foods, and the occasional investigation of a food-borne disease outbreak. It would seem that there is a more effective and creative role for the laboratory in a local food program. Dr. Buchbinder has given us some examples of another approach, and the point of attack lies somewhere between the routine and repetitive sampling of a few usual foods, and the intermittent retrospective attempts to determine the food already

responsible for an outbreak. There are food products, long established on the market, that have not been studied or studied only in a cursory manner. To these we may add the new food products. Dr. Buchbinder has spoken of the problems related to foods produced in distant places and for which the local agency has no knowledge of the physical standard of manufacture. Local agency budgets do not allow for the routine testing of all the foods that might warrant such sampling, but these foods should not escape our interest or concern. Laboratory services in food bacteriology should be used as an evaluative or diagnostic tool; that is, to derive information relative to acceptable processing techniques, potential hazard, and sanitation status through surveys of many foods rather than continually repeat samplings of a few foods. The function of the local regulatory laboratory would be one of problem finding, aid in problem solving, and periodic evaluations of improvement of sanitary quality, rather than in the routine control function where the laboratory can supply only *post facto* information. This evaluative approach requires the active correlation of the field inspectional service with the laboratory service. Field studies of the sanitary conditions and methods of processing and handling of the food should be related to the bacteriological findings. This approach uses the ecological concept of the relationship between living organisms and their environment, and uses epidemiologic methods which comprises an orderly approach to the study of causes and effects.

In all this, the role and expectations of the administrator of the food sanitation program is critical. On one hand, the administrator operates within a wide area of discretion. On the other hand, legislative bodies, courts, and even administrators themselves have been interested in clearly defined quantitative limits in order to have a greater uniformity in the interpretation of public health laws. It is suggested by some that the development of objective criteria will result in less need for dependence on individual determinations and subjective judgement. Some administrators have looked to microbiological standards for foods as authorative guides that may substitute for professional judgment and relieve them of troublesome decisions. Wolman has said that administrators are constantly searching for mathematical certainty in the solution of complex problems related to the environment, despite the fact that there is a high degree of uncertainty in the underlying scientific principles that are involved in establishing such standards. It is important that microbiological tests and criteria not become a mere "numbers" game, and that criteria be as objective and meaningful as possible. The criteria are more likely to be objective if the field inspection services are teamed with the laboratory services to correlate and identify the sanitation factors, the food components, and the particular food processes which influence the microbiological quality of the food at each stage.

Finally, Dr. Buchbinder makes the important point that a national effort will be required before a real start can be made to the solution of food microbiological problems at a local level. The basic food ingredients may come from a foreign country or a point far distant from the site of production. The finished food product may be transported and distributed in local food markets, or further handled and served in food outlets distant from the place of manufacture. The very nature of the food chain requires that there be close intergovernmental cooperation. This is especially apparent when it is realized that

no single level of government can muster enough resources to do a complete job of food protection.

Metropolitan health departments may enter the area of the microbiological evaluation of foods. They are particularly well suited to carry on this type of field research. However, some of the benefits to be derived from these efforts will be lost unless there is a method for the information gathered in this way to be readily disseminated. Perhaps one way to do this is for the major metropolitan health departments to set up an information network so that these findings may be made available to each other. At the same time, there should be more use made of reciprocal arrangements between departments, so that a regulatory agency receiving a food product may be informed of the results of physical inspections of the food plant made by the agency where the manufacturer is located.

For the most part, regulatory microbiological standards for food have been based either on arbitrary indexes which have been established and modified through subsequent experience, or have been arrived at retrospectively, based on performance studies of food products already on the market. This does not say that standards derived in this way are not valid. We do not have to wait until all uncertainties are settled before proposing microbiological criteria. Legislative bodies, courts, and administrative practice do not require perfect knowledge; they do require the absence of capriciousness. Nor is the question one of having microbiological criteria or not having such criteria. Whenever the administrator receives a laboratory report, he must make some interpretations as a guide to action. One can not escape from criteria, be they implicit or explicit.

However, confidence in the use of microbiological criteria both in the regulatory agencies and the food industries will be proportional to their demonstrated objectivity. Local regulatory agencies which combine field inspection services and laboratory services can do a great deal of field research to help arrive at such objective criteria. Nor can the need for objectivity be lessened by designating a standard or criteria as advisory or administrative. Dr. Buchbinder has shown, from a program point of view, there is little difference between a so-called advisory or administrative standard or an explicit legal standard. When a criterion is used as a basis for decision making, it becomes part of the governmental process. The main difference is that the designation of a standard or criterion as advisory or administrative indicates its tentative nature and its greater ease of modification than is possible for an explicit legal standard.

If and when microbiological criteria for foods come into wide use, we can expect that they will be tested in the courts. Such an experience may be more harrowing than a presentation before one's scientific colleagues.

Food Microbiological Problems

from the Standpoint of Industry

M. F. GUNDERSON

Campbell Soup Company, Camden, New Jersey

The most important formula with respect to the processing of foods is the equation for time-temperature-quantity. How long did you hold it? At what temperature? What was the amount of product involved? Basically, products should be kept hot or cold. Apply enough heat to prevent the multiplication of bacteria (71°C); if cooled, apply enough refrigeration to prevent growth. With respect to the lower temperatures, much depends upon what it is you wish to do. The product that is to be used within 12 or 24 hours should be cooled to a temperature below 4°C and as near 0°C as possible. The product that is to be used at a later time should be maintained in the frozen state, at temperatures of 0°F or lower. It should be recalled that the product has a melting point lower than 32°F, and whenever there is free water present there may be an opportunity for the growth of molds, yeasts, and bacteria.

In our modern time, the laboratory is faced with a new problem and that is the proper choice of laboratory methods and certainly an understanding as to the influence of an added ingredient to the laboratory media. The addition of sucrose or glucose to the agar which is used to determine coliforms will influence the results. Organisms from cheese may produce alpha hemolysis on blood agar. Improper appraisal of these results will do harm. Therefore, the laboratory researcher must be knowledgeable and must know how to interpret results obtained with foods.

Heat processing is an old, venerable art and many of its problems have been long since solved. The emergence of the National Canners Association was brought about by some of these old problems which plagued the industry. The work of Esty, Cameron, O. B. Williams, and C. O. Ball, as well as others, stand out as those solving some of the problems which plagued heat processing. The selection of test strains of heat-resistant bacteria and the technique of the inoculated pack were developed over the

TABLE I

LINE SURVEY-MACARONI AND CHEESE DINNER

	Sample description	Batch	Total (TGE agar)	Number of organisms per gram of sample			
				AFDOUS method		Laboratory method	
				Coliforms	Staphylococci	Coliforms	Staphylococci
1	Macaroni at cooking kettle	2	<10	<3	<3	<10	<10
2	Macaroni just before addition of sauce	2	730	<3	<3	<10	55
3	Cheese sauce going into macaroni tub	2	100	<3	<3	<10	<10
4	Peas from box, Lot #8082	2	35,000	3	<3	<10	240
5	Cheddar cheese	2	>1,000,000 Lactics	<3	<3	<10	>7,500
6	Parmesan cheese at filling line	2	35,000 Primarily lactics	9.1	<3	<10	1,700
7	Macaroni & Cheese from dinner tray	2	620	<3	3.6	<10	25

8	Glazed carrots from dinner tray	2	1,300	9.1	<3	<10	10
9	Peas from dinner tray	2	38,000	15	9.1	30	350
10	Cooked glaze–9:25 A.M. from cooling kettle		35	3.6	<3	<10	<10
11	Cooked carrots at cooking kettle before cooling, 9:30 A.M.		<10	<3	<3	<10	<10
12	Macaroni at cooking kettle	4	<10	<3	<3	<10	<10
13	Macaroni just before addition of sauce	4	300	<3	<3	<10	<10
14	Cheese sauce going into macaroni tub	4	130	<3	<3	<10	<10
15	Peas from box, Lot #8082	4	19,000	<3	3.6	<10	1,600
16	Cheddar cheese	4	>1,000,000 Lactics	<3	<3	<10	45
17	Parmesan cheese at filling line	4	140,000 Primarily lactics	<3	7.3	<10	>3,500
18	Macaroni & cheese from dinner tray	4	310	3.6	<3	<10	<10
19	Glazed carrots from dinner tray	4	800	3.6	9.1	15	<10
20	Peas from dinner tray	4	16,000	43	15	50	120

TABLE II

RANGE, MEAN, AND MEDIAN OF THE TOTAL NUMBERS OF ORGANISMS, COLIFORMS, AND STAPHYLOCOCCI FROM MACARONI
AND CHEESE DINNERS

Sample description and section	Total (TGE agar)[b]	Number of organisms/gram of sample			
		AFDOUS[a] method		Laboratory method	
		Coliforms	Staphylococci	Coliforms	Staphylococci
Dinners from Batch #2					
Mac. and cheese					
Range	880–7,800	<3–9.1	<3–3	<10	170–2,300
Mean	3,800	4.5	<3	<10	840
Median	3,800	<3	<3	<10	530
Carrots					
Range	1,000–1,500	<3–9.1	<3–23	<10–10	45–210
Mean	1,300	4.5	7	<10	82
Median	1,300	<3	<3	<10	50
Peas					
Range	32,000–63,000	7.3–23	<3–3.6	25–60	280–<5,000
Mean	45,000	12.7	<3	34	1,400
Median	44,000	9.1	<3	30	540

[a] Association of Food and Drug Officals of the United States.
[b] Tryptone, glucose, meat extract agar.

Dinners from Batch #4					
Mac. and cheese					
Range	1,000–4,500	<3–3.6	<3–21	<10	310–2,300
Mean	1,800	<3	<3	<10	940
Median	1,200	<3	<3	<10	640
Carrots					
Range	600–960	3.6–43	<3–3.6	<10–20	<10–110
Mean	830	19.2	<3	12	44
Median	870	23	<3	10	35
Peas					
Range	13,000–31,000	21–93	3.6–9.1	20–55	150–380
Mean	21,000	59	6.9	40	280
Median	21,000	43	7.3	35	350
Dinners produced 8/5/60					
(12–3 P.M.)					
Mac. and cheese					
Range	65–5,800	<3–460	<3–43	<10–270	<10–160
Mean	4,300	173	7.9	184	67.5
Median	4,500	140	<3	200	48
Carrots					
Range	1,500–30,000	<3–23	<3–93	<10	<10–40
Mean	9,300	10.3	14.7	<10	23.5
Median	3,900	6.3	3.6	<10	30
Peas					
Range	14,000–33,000	9.1–93	<3–9.3	10–50	35–2,300
Mean	24,000	28.2	4.7	17.5	780
Median	24,000	19	<3	10	260

years. All new products are subjected to this treatment, so that a com-
mercially sterile product has evolved.

Perhaps there are some new problems for this industry since foods of
different character than those previously packed by the canner are com-
ing into being. Sometimes strange results are present when the incubation
tests are subcultured. We have found heat-resistant organisms in curry
powder and in mushrooms so that a different technique will have to be
devised, or means to clean up various ingredients will have to be used.
So perhaps, the heat processing industry will have to look with the same
critical eye to the selection of ingredients as do the industries involved in
frozen or dehydrated foods. All through these considerations, the prob-
lem of time-temperature-quantity prevails.

There are three approaches to the process of canning, freezing, or dry-
ing, and each has its own requirements. It is true that in heat processing
the microbial load is destroyed by the pressure and heat treatment. How-
ever, proper handling of ingredients is still necessary and rules are estab-
lished regarding the time-temperature-quantity holding of all ingredients.
With frozen foods, the problem is somewhat different. We must process
our products rapidly, package and freeze quickly. This insures us that the
product is of good quality and low microbial count. With frozen foods,
industry standards are set for ingredients. Vegetables and fruits are
never lower in count than immediately after blanching or after washing in
a plant where in-plant chlorination is practiced. After the product has
been cleaned in this manner, the re-use of fluming water is deplored.
Where individually quick frozen (I. Q. F.) vegetables and other products
are prepared, clean equipment and containers must be made available.
The take-off belt that leads to the freezing tunnels has at times been
found to contribute the bacterial load of the products being frozen. The
processor can get products of low microbial count. For example, mixed
vegetables can be obtained wherein 6% will be under 50,000 per gram and
71% under 150,000 per gram. These are old data and today much better
results can be expected. Peas can be obtained with an average total count
of 60,000 per gram; however, 68% are under 50,000 per gram. Frozen
carrots will have 60% of the samples under 50,000 per gram. Of I. Q. F.
corn, 60% will be under 50,000 per gram. Lima beans can be obtained
wherein 50% will be under 50,000 per gram and 100% under 125,000 per
gram.

The processor should set standards based upon his investigations for all
ingredients. Plant surveys should be done. Here is a plant survey showing
the results as well as the end results from such an endeavor (Tables I
and II).

In order to determine how well the operation is in compliance, one

could do an examination once in a while to show what results would be obtained by sampling at frequent intervals; thus one can determine whether or not the operation is in compliance. Table III shows this.

Here is the situation in which frozen beef pies were sampled at one-

TABLE III

FROZEN BEEF PIES[a]

Time sampled (A.M.)	Bacteria/gram (SPC)[b]	Time sampled (A.M.)	Bacteria/gram (SPC)
8:57	2,800	9:27	5,900
8:58	1,300	9:28	3,200
8:59	2,600	9:29	1,900
9:00	2,200	9:30	1,800
9:01	2,300	9:31	1,500
9:02	3,300	9:32	1,500
9:03	2,700	9:33	3,600
9:04	2,700	9:34	2,300
9:05	5,700	9:35	3,100
9:06	3,600	9:36	4,200
9:07	7,400	9:37	2,400
9:08	4,200	9:38	1,700
9:09	3,900	9:39	2,700
9:10	2,400	9:40	1,800
9:11	1,600	9:41	3,200
9:12	3,400	9:42	2,000
9:13	2,300	9:43	2,300
9:14	2,100	9:44	2,900
9:15	2,600	9:45	1,400
9:16	3,900	9:46	3,700
9:17	2,600	9:47	2,000
9:18	2,400	9:48	13,000
9:19	3,000	9:49	1,100
9:20	6,800	9:50	1,300
9:21	3,200	9:51	2,200
9:22	3,100	9:52	2,500
9:23	1,600	9:53	2,200
9:24	3,500	9:54	2,000
9:25	1,000	9:55	1,500
9:26	4,400	9:56	4,200

Average: 3,000
Range: 1,000–13,000

[a] Bacterial counts on this product when samples were taken from the production line each minute for one hour on same day reported in Table II.

[b] SPC is the count on TGE agar incubated at 35°C for 48 hours.

minute intervals, and we will report the total count for each minute for that hour. From examination of these results one would decide that the operation was under control. Unfortunately microbiological examinations are like post-mortems. We get the results when it is too late to do anything about them. With a check, such as this one, we can feel sure that plant clean up and the entire processing operation is being run carefully with the end results of low count products in mind. Good supervision considers all of the various ingredients under their control. It is important therefore to determine these bacteriological results. Table IV shows the weekly experience in producing diced chicken meat.

TABLE IV

CHICKEN MEAT—COOKED, DICED, FROZEN; SUMMARY OF BACTERIAL COUNTS ON WEEKLY BASIS

Week No.	Total count (SPC)			Coliform count			No. of samples
	High	Low	Average	High	Low	Average	
1	110,000	18,000	57,000	—	—	—	6
2	43,000	4,600	19,000	50	0[a]	5	15
3	28,000	6,700	17,000	30	0	10	4
4	30,000	4,700	13,000	20	0	2	8
5	44,000	7,200	16,000	10	0	2	8
6	17,000	2,700	11,000	60	0	24	7
7	41,000	4,600	16,000	600	0	120	12
8	52,000	8,800	29,000	80	50	65	4
9	94,000	9,900	35,000	400	0	100	20
10	59,000	9,200	26,000	90	0	17	16
11	76,000	6,900	31,000	160	0	25	15
12	47,000	1,000	16,000	100	0	18	15
13	100,000	2,900	17,000	20	0	4	37
14	89,000	3,000	12,000	100	0	8	56
15	120,000	3,700	31,000	90	0	9	24
16	17,000	11,000	13,000	0	0	0	4
17	11,000	2,300	7,800	60	0	15	14
18	61,000	1,000	13,000	40	0	2	23
19	53,000	1,200	16,000	900	0	73	20
20	28,000	500	11,000	230	0	39	24
21	18,000	600	4,800	30	0	10	7
Over-all average			20,000			30	

[a] 0 indicates no growth at 0.1 dilution.

What is the end result of these endeavors? We are presenting a summary from a larger survey of all products. This summary shows the market experience in the Delaware Valley for turkey pies, and these counts are low (Table V).

With respect to precooked frozen foods we would like to point out that these are no different than any other delicatessen item, except in one respect. Frozen foods are frozen for the maintenance of the original quality. They are frozen quickly so as to assure low microbial counts. It

TABLE V

PERCENTAGE OF THE TOTAL NUMBER OF ORGANISMS, COLIFORMS, AND STAPHYLOCOCCI PER GRAM OF RETAIL SAMPLES OF TURKEY PIES BELOW SPECIFIC LEVELS FOR EACH BRAND AND FOR ALL BRANDS

Brand	Percentage of samples with total numbers of organisms per gram below						
	1,000	5,000	10,000	50,000	100,000	500,000	1,000,000
A	4.17	12.50	29.17	54.17	70.83	83.33	100.00
B	29.17	79.17	87.50	100.00	100.00	100.00	100.00
C	58.33	91.67	100.00	100.00	100.00	100.00	100.00
D	100.00	100.00	100.00	100.00	100.00	100.00	100.00
E	25.00	75.00	91.67	100.00	100.00	100.00	100.00
F	0.00	12.50	45.83	91.67	95.83	95.83	95.83
G	8.33	16.67	16.67	16.67	25.00	37.50	41.67
H	12.50	62.50	79.17	100.00	100.00	100.00	100.00
J	25.00	75.00	95.83	100.00	100.00	100.00	100.00
K	0.00	58.33	79.17	95.83	100.00	100.00	100.00
L	91.67	95.83	95.83	100.00	100.00	100.00	100.00
M	0.00	37.50	45.83	66.67	79.17	83.33	87.50
N	95.83	100.00	100.00	100.00	100.00	100.00	100.00
All brands	34.62	62.82	74.36	86.54	90.06	92.31	94.23

Brand	Percentage of samples with coliforms per gram of sample below					
	10	50	100	500	1,000	5,000
A	54.17	66.67	70.83	83.33	87.50	87.50
B	100.00	100.00	100.00	100.00	100.00	100.00
C	95.83	95.83	100.00	100.00	100.00	100.00
D	95.83	95.83	100.00	100.00	100.00	100.00
E	100.00	100.00	100.00	100.00	100.00	100.00
F	75.00	79.17	83.33	95.83	95.83	95.83
G	45.83	62.50	66.67	83.33	83.33	100.00
H	100.00	100.00	100.00	100.00	100.00	100.00
J	95.83	100.00	100.00	100.00	100.00	100.00
K	91.67	100.00	100.00	100.00	100.00	100.00
L	100.00	100.00	100.00	100.00	100.00	100.00
M	79.17	87.50	87.50	100.00	100.00	100.00
N	100.00	100.00	100.00	100.00	100.00	100.00
All brands	87.18	91.35	92.95	97.12	97.44	98.72

TABLE V (*continued*)

Brand	Percentage of samples with staphylococci per gram of sample below						
	10	50	100	500	1,000	5,000	10,000
A	4.17	4.17	8.33	33.33	45.83	62.50	79.17
B	37.50	62.50	66.67	91.67	100.00	100.00	100.00
C	41.67	87.50	91.67	100.00	100.00	100.00	100.00
D	95.83	100.00	100.00	100.00	100.00	100.00	100.00
E	50.00	75.00	91.67	100.00	100.00	100.00	100.00
F	12.50	16.67	16.67	29.17	37.50	87.50	91.67
G	8.33	12.50	12.50	20.83	29.17	45.83	45.83
H	54.17	79.17	83.33	100.00	100.00	100.00	100.00
J	66.67	95.83	95.83	100.00	100.00	100.00	100.00
K	4.17	16.67	29.17	70.83	83.33	100.00	100.00
L	87.50	91.67	91.67	100.00	100.00	100.00	100.00
M	20.83	45.83	50.00	91.67	91.67	100.00	100.00
N	66.67	100.00	100.00	100.00	100.00	100.00	100.00
All brands	42.63	60.58	64.42	79.81	83.65	91.87	93.59

should be remembered that organisms of public health significance do not grow at temperatures below 5°C, frozen foods come from the frozen state up to the point of accidental defrost, then the psychrophiles will grow. These are organisms which produce changes in odor, flavor, and appearance. There has never been a well authenticated case of food illness due to commercially prepared precooked frozen foods. I would prefer to have frozen foods with contamination in normal proportions, so that the competing psychrophiles and mesophiles can successfully outgrow and thus repress the development of staphylococci. (See the paper by Dr. Peterson in this symposium.)

Discussion by C. T. Townsend

National Canners Association, Berkeley, California

I would like to enlarge on several points brought out in Dr. Gunderson's paper. He dwelt at some length on the bacterial counts of frozen foods under various conditions. These are closely related to general sanitation and product handling procedures. With food plants operating around the clock, it is becoming increasingly necessary that equipment be designed for ease of cleaning, and, if possible, for cleaning without dismantling. To a considerable extent, cleaning in the future is likely to be controlled automatically, like so many of our manufacturing processes. Research is needed on cleaning and sanitizing agents for this purpose, and there should be close working relations between the design engineer and the microbiologist.

For the sake of efficiency and of minimizing product damage, fruits and vegetables are increasingly being conveyed hydraulically through pumps, pipes, and flumes. It is necessary that these procedures be carefully studied so that bacterial contamination be kept to a minimum. Because of water shortages and waste disposal problems, water must be reused as many times as possible. This means recirculation, with some method for controlling makeup water automatically, based on the sanitary condition of the water. In handling fruits, the initial clean water may increase much more rapidly in bacterial count than water after use, when it contains considerable acid leached from the fruit. Perhaps the clean water could be acidified to inhibit bacterial growth. In the case of vegetables, rechlorination after each use is essential. This has worked out most successfully in the counterflow use of water in pea canning. Research is urgently needed on methods for reconditioning water in the food plant to reduce the volume consumed and the amount of liquid waste.

I stress water conservation and waste disposal because I believe these are two of the most serious problems facing industry today. Not only are our water resources becoming seriously depleted, but many municipal treatment plants are having great difficulty in handling the large volumes of high BOD waste waters from food plants during the summer season. Processors should be concerned with reducing the volume and pollution potential of this water as much as possible. The disposal of solid wastes is just as serious a problem. Land disposal is becoming more and more difficult, if not impossible in many places, because of the high price of land near centers of population, as well as because of odor and fly problems which are a nuisance to the residents of the area. Urgently needed is a method of treatment which can be used at the food plant to save hauling costs, such as continuous composting or anaerobic digestion. Agricultural wastes generally should be included in such a study, because waste dumps become breeding grounds for insects which can cause serious trouble in fruit-growing areas and create nuisance problems.

Dr. Gunderson mentioned the early work of the National Canners Association and others in developing basic methods and criteria for determining canned food processes. This work is continuing unabated, but what we need now are safe methods of canning which will not result in so much quality loss due to heat as is the case at present. There has already been much research in this field, such as radiation sterilization and the use of antibiotics, neither of which has so far shown much promise. Aseptic canning is a step in the right direction, but at present can only be applied to products which can be pumped through heat exchangers. Olin Ball's studies on short time-high temperature processing followed by cold storage of the canned product are also of interest. Perhaps one of the greatest hopes for the future lies in the fundamental studies on the mechanism of spore production and spore germination now being carried on in a number of laboratories, including our own. When these basic processes are understood, it may be possible to develop reliable methods of permanently inhibiting spore germination in foods, or of sensitizing the spores to heat or to some other sterilizing procedure.

At this point I should say that the National Institutes of Health are already cooperating in research programs on water conservation and bacterial spores. Their entry into the field of food preservation should be most helpful to the industry.

One of the stumbling blocks in the way of reducing the severity of various sterilizing procedures for low acid foods, such as heating and radiation, has been the concept of 12D for spores of *Clostridium botulinum*. For the last thirty-five years or so it has been assumed necessary for safety that a process for canned foods must be based on twelve times the death rate of *C. botulinum* spores in the particular food, or in other words, of reducing a million million spores to one spore. This is a fantastically large number of spores to kill, and there is evidence in some cases that it may be unnecessarily high. If evidence could be presented that this 12D value could safely be reduced, it would have a most beneficial effect in lowering process levels. It might even bring radiation sterilization back into the realm of possibilities.

There has been an immense development in holding fruits and certain vegetables in cold storage before processing. This smooths out the peaks in deliveries, and enables food plants to operate with a uniform flow of product. Spoilage is sometimes a serious problem, particularly if storage is prolonged, and it is largely due to species of fungi. Methods for delaying the onset of spoilage, by the application of fungicides or by some other treatment, would be of great value.

I would like to add one final thought. Too often the production and engineering departments of a food plant decide to introduce new processes or equipment without consulting the quality control department. Not only should quality control be consulted in decisions of this kind, but the men in charge of engineering and production should have some basic knowledge of bacteriological processes, so that they can avoid the errors in judgment which now occur. As production procedures become more complex and mechanized, production management needs a broader background in the food sciences.

The Role of Universities

in Food Microbiological Research

E. M. FOSTER

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin

On reflection one is bound to conclude, it seems to me, that universities have one unique and clearly defined role in food microbiological research —that is the training of students. In our position as advisors we can see that our graduate students take a proper selection of courses to fit them for a career in food microbiology, and we can give them research experience. In this way we can learn enough about the student to evaluate him for future employers. We can find out if he has ideas, if he is a self-starter, if he will work, if he is self-reliant, if he is persistent, if he is calm under pressure, and if he can get along with other people. One quality that I find difficult to evaluate, though, is leadership. Potential employers, quite justifiably, want to know if a man will make a good supervisor or group leader. Most graduate students work alone, however, and rarely have opportunity to demonstrate a capacity for leadership.

Is it essential that a graduate student work on a problem related to food if he is to prepare himself for a career in food research? I don't think so. In my opinion it is far more important that he have sound training in chemistry, physics, mathematics, biology, genetics, statistics, and other related sciences, in addition to microbiology, than it is for him to associate himself with a study that might be classified as food microbiology. The important thing is that he learn *how* to do research. However, I must admit that a man who works on a problem related to foods is more likely to go into food research and to be happy there than is one, for example, who studies the transfer of DNA by T_2 coliphage.

Other than training students, I fail to see very much that a university group can do in food microbiological research that any other well equipped and adequately staffed laboratory could not do if it were willing to spend the money. Economics being what they are, however, there are some types of investigations that are especially well suited to an academic environ-

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ment. University personnel can devote time, facilities, and talents to long range problems that may have no immediate practical importance, but which may produce information of great value to the food industry and the public. An example of this is the work on staphylococcal food poisoning in the laboratory of Dr. G. M. Dack at the University of Chicago. No one can deny that this work has had tremendous importance to the food industry. But it is inconceivable to me that any industrial laboratory ever could or would have been able to do what Dr. Dack's group has done.

Most universities try to provide a favorable environment for research. They are likely to have good libraries and a wide variety of specialized equipment. Usually they have large numbers of specialists in many scientific disciplines. This combination of facilities and talents can be very helpful. To illustrate, a few years ago one of our professors of veterinary science wanted to observe the localization of staphylococci in the udders of cows afflicted with mastitis. He conceived the idea of tagging the organisms in tissue sections with fluorescent antibodies, and he purchased the necessary equipment for the purpose.

One of my colleagues in bacteriology knew of this work and also knew about the problem of detecting staphylococci in dry milk. As we all know, there have been instances of suspected staphylococcal food poisoning in reconstituted dry milk from which the causal organisms could not be cultured, suggesting that they had been killed after the toxin was formed. It would be useful, therefore, if we had a positive method of identifying staphylococci even though they were dead.

Using established fluorescent antibody techniques, and the equipment in the Department of Veterinary Science, my colleague developed a method of detecting and counting staphylococci in films prepared from dry milk. This procedure may be very useful for investigating suspected sources of staphylococcal food poisoning.

The university food microbiologist—at least in my institution—has a free choice of problems and is under no pressure to produce immediately applicable results. In a practical sense this may be an inefficient system, but sometimes it produces information of real value. One of my associates, for example, is working on the mechanism of heat resistance of bacterial spores. Why? He just wants to know. He may find the answer next month or next year or 10 years from now, or he may never find it. In a university he can continue trying at whatever level of intensity he chooses for as long as he is interested in the problem. I believe all of us will agree that an explanation of the mechanism of heat resistance of spores would be significant to the food preservation industry.

But universities have certain disadvantages. Most of our research is done by graduate students who are working on advanced degrees. It takes

quite a while for a beginning student to understand his problem, to work out his methods, and to start producing results. Even then, his work is interrupted by classes and other distractions. Eventually he receives his degree and leaves. If the problem is a continuing one a new student must be broken in, with the inevitable delay. Work done in this way is bound to be slow and inefficient compared with that done by trained, full-time people.

Whereas, we have a wide range of choices in the types of problems we attack, many of us in universities still have little day-to-day flexibility in terms of what we do. Let us say, for example, that I go to a meeting in October and come back with an idea about something that ought to be done. Can I act at once? Hardly. All of my graduate students already are well along with problems that will be used for their theses. I can hardly interrupt their work and put one of them on a new project. It is unlikely that I can find another qualified student until the following July or September, and even then it may take several months actually to start doing experiments.

Meanwhile, my research director friend from a food company laboratory goes home from the same meeting with the same idea. The next day he calls in one of his microbiologists, discusses the problem with him, and tells him to drop everything else and get to work on it immediately. Within a week or two, perhaps, results are appearing. In a case such as this the university laboratory obviously is not in the race.

To make the most of university research facilities, we should use them for what they can do best. In my opinion, universities—at least those supported by public funds—should confine their major efforts to problems of broad, general interest that will benefit an entire industry. Sound research work, no matter how irrelevant to practical problems, should not be discouraged, and investigators should be free to follow up leads and ideas as they please. Not infrequently, the most unapplied work imaginable may turn up useful ideas.

Several years ago, for example, we became interested in the development of slime on vacuum-packaged cured meats. With some of these products, slime development severely limits shelf-life. We performed a typical storage trial, holding several products at refrigeration temperatures and following the development of the microbial flora until spoilage occurred. Examination of the organisms showed lactobacilli to be the spoilage agents, and a careful taxonomic study of representative isolates revealed two apparently new species. Nothing, it would appear, could be less important to the meat packing industry.

But in characterizing the organisms we observed that many of them produced copious amounts of a slimy, gummy dextran when they grew on

sucrose agar. Ordinary colonies of the organisms on glucose agar were only 1 to 2 millimeters in diameter, but the gummy colonies on sucrose were perhaps ¼ inch in diameter. To us it seemed likely that the organisms also produced dextran when they grew on the meat products. If so, the gum would add appreciably to the volume of slime, thus making spoilage apparent sooner to the naked eye.

Naturally, we tried the obvious experiment and learned that products containing sucrose developed visible slime at least a week sooner than did those containing glucose. With some products, an additional week of shelf-life would be highly significant.

In making the foregoing statements I do not wish to imply that universities should ignore the practical problems of industry; quite the contrary. Those of us in agricultural experiment stations obviously owe part of our existence to the food industry, and we must be constantly alert to ways we can help solve its problems. But I believe that universities should, in the main, devote their facilities and the talents of their personnel to broad problems of major importance, and not to the specific applied problems of an individual company. Again I should like to cite the response of Dr. Dack to the problem of botulism in cheese products several years ago. This study was undertaken at the request of the National Cheese Institute. Similarly, personnel at the Universities of Wisconsin and Minnesota have been conducting an extensive study on food poisoning staphylococci in dry milk at the request of the American Dry Milk Institute.

Through his free choice of problems and his outside contacts, the university food microbiologist has the opportunity to achieve a breadth of experience that is difficult to attain in, for example, an industrial laboratory. But unless he makes a concerted effort, a university professor can become isolated in his "ivory tower" and never find out what industry needs to know. In my opinion, industrial organizations could do much to help themselves if they would make greater effort to discuss their problems with university investigators. I certainly would welcome closer affiliation on questions that have a direct bearing on the needs of the food industry. It would help me to plan my research program more meaningfully and would stimulate work toward more useful goals.

Thus, I believe that universities can contribute most successfully to food microbiological research if they are apprised of the problems that need to be solved, if they are given adequate financial support, and if they are allowed ample latitude in the specific direction the work may take. Even then, there should be no special hurry for the results. The organization of the typical university laboratory simply isn't geared to

"crash programs." If answers are needed in a hurry, a university usually isn't the place to go for help.

Open Discussion

DR. SLANETZ: While our papers this morning have been somewhat varied and general in nature, they are now open for discussion. I hope each participant will feel free to ask questions or make comments during all our open discussion periods.

DR. LITSKY: I would like to try to strike a tone of warning. Most of our graduate students are going into more glamorous fields such as genetics, which means that the number of graduate students in microbiology is declining. Most of the older people in food microbiology have retired or are about to retire; unless we have a crash program in this area, we're going to lose a generation of food microbiologists.

DR. SLANETZ: Thank you for those comments, Dr. Litsky. From the reaction of the audience you apparently have considerable support. Is there anyone else who would like to make a comment at this time?

MR. GILBERTSON: I would like to comment, reemphasizing a point that was brought up by Dr. Buchbinder and referred to also by several of the other speakers, with reference to the question of the need for microbiological surveillance in connection with local food control. I think that it is evident that important changes are taking place in the food industry. Certain types of centralized kitchens, commissaries, and vending operations are being installed in a type of firm not represented by Dr. Gunderson and others. In these cases, there is an absence of any laboratory backup. In our national studies of this particular problem, we find that with a few exceptions like Dr. Buchbinder's, Dr. Schiffman's, and a half-dozen others, this particular control is almost absent in local programs, except in the case of water and milk. To cite an example, last week I was on the West Coast at a meeting where a comprehensive environmental study of a fast growing metropolitan industrial area is being done, and I asked whether any microbiological work in connection with their regular food sanitation program was carried out. The answer was, "only in connection with a specific outbreak." In other words, this was not a preventive program; it was what you might call a curative one. Here is an area in which there is a very big gap. The reason is probably largely methodology, and the question comes up as to why this kind of methodology has not moved along like some of the others. I don't know that I can answer that—maybe it is a question of which comes first, the egg or the salmonellae! Do we require the examination and then force the methodology in or do we get the methodology first and let this flow into the operational program?

I say from our experience in other fields, that if the methodology is available, then this will be adopted. I think this is the real challenge to the folks here and many others; that is, to develop the kind of methodology that can be useful, because for routine use the methodology has to be simple—it has to be cheap, it has to be reliable; this is not the kind of research perhaps that everybody likes to do but I can say it is very important area and is a real challenge.

DR. GUNDERSON: First of all, before you need the method you have to have a demand for the method, so there is no point in doing methodology for salmonellae in eggs until somebody finds that eggs have salmonellae.

But the other thing I wanted to point out is that, with the frozen food industry particularly, it is a matter of education. The National Association of Frozen Food Packers have put on seminars all across the country—and I have been to every one of them—trying to get the little frozen food packers in, because we don't want the little frozen food packer to get us into trouble. So we get them all together and we talk about employee training and sanitation, personal hygiene for the employees, nuts and bolts in frozen vegetables— things that can happen—and bacteriology. We encourage the establishment of bacteriological laboratories, and we are getting them into the smaller plants and getting them to recognize the time-temperature-quantity relationship. And the frozen food industry is just like anything else in the American tradition— you just start out. When Swanson started, they hauled their mistakes to the dump and they had land-fill methods (laughter), and they got rid of part of it that way. Back in 1945–1946, anyone who did "counts" would report a high count. Anybody who reports counts in 1963 or 1968 is going to see an improvement because as these people become aware of what their problems have been or are, they will attempt to remedy the situation. I always feel that a person will do something just because he ought to do it, and I question whether or not force is necessary to make people do things.

You know the pocketbook is very vulnerable. We buy vegetables on bacterial count. So it is money in their pockets if they have a low bacterial count and it is money in their pockets if something has a shelf life that it didn't have before. So self-interest is served by sanitation and you don't need laws to get that. It comes about automatically as soon as they find out it is going to hit them in the pocketbook.

DR. APPLEMAN: I agree with Dr. Gunderson's remarks in general. However, I have attended the seminars on the West Coast. The difficulty is frequently that we don't reach the right people. For example, at the seminar in Los Angeles, every progressive producer was at the meeting, but the small ones that we have to reach did not come.

DR. GUNDERSON: What was your objection?

DR. APPLEMAN: Well we are still picking up a hundred thousand staphylococci in meat pies and four million total count per gram, and these are the producers on the borderline. They are not the large producers. So as you say we have got to get them educated and we have to do it rapidly.

DR. HOBBS: I might suggest that perhaps sometimes we try to oversimplify, and this is a question I intended to put. Isn't it true that different foods require different methods of examination?

Problems in Foodborne Diseases

G. M. DACK

Food Research Institute, University of Chicago, Chicago, Illinois

Reporting of foodborne illnesses in the United States is not compulsory and, therefore, there is no information as to the true incidence of these illnesses. Table I summarizes the outbreaks and cases in the United States from 1957 to 1962 as related to food commodities involved.

An effort is being made by the Public Health Service to improve the reporting of short-term gastroenteric episodes (*1*). A study group was established to study this matter and has had several meetings. This group has made recommendations to the Conference of State Health Officers with a view to stimulating better reporting.

The Public Health Service is contemplating building an environmental health center near Washington which includes a unit on Milk and Food. This was recommended by the Paul Gross Committee on Environmental Health Problems (*2*) set up by the Surgeon General, U.S. Health Service in August 1961 for the purpose of developing long-range objectives for the environmental health problems. This report is concerned with the food protection aspect of environmental health and covers some of the material presented in the Gross Report. Since much of the information to be presented at this conference is categorized under specific agents causing illness, only some of the challenging, unsolved problems will be mentioned.

Of the microbial agents causing intoxications, botulinum toxin and staphylococcus enterotoxin are outstanding. With the new methods of processing foods the food technologist must always be aware of hazards, e.g., in the case of botulism, the use of the plastic wrap for food which is impermeable to oxygen may prolong the storage life of a product by preventing the growth of molds which normally limit its shelf-life, and the added storage time may permit the growth and toxin production of *C. botulinum*. An example for this is the outbreak of type E *C. botulinum* from smoked Ciscoes (Lake Superior fresh-water fish) (*3*). The work of Dr. Clarence Schmidt has indicated that *C. botulinum* type E will grow at common refrigeration temperatures and produce toxin (3.3°C for 6 weeks) (*4*).

TABLE I

Foodborne Illnesses Reported in the U. S. from 1957 to 1962[a]

Food	Botulism		Staphylococcus		Salmonella		Clostridium perfringens		Shigella		Trichinosis		Chemical		Other	
	O.[b]	Cases	O.	Cases	O.	Cases	O.	Cases	O.	Cases	O.	Cases	O.	Cases	O.	Cases
Meat	1	1	61	2386	8	1534	1	21	3	107	13	149	4	30	51	1628
Eggs	—	—	3	58	3	127	—	—	—	—	—	—	—	—	2	140
Poultry	2	3	28	1563	16	1633	2	13	2	200	—	—	—	—	39	2383
Fish	5	9	4	91	3	113	—	—	—	—	—	—	3	15	11	158
Dairy products	—	—	18	799	9	122	—	—	—	—	—	—	1	1	5	249
Bakery items	—	—	34	293	6	328	—	—	—	—	—	—	—	—	14	153
Miscellaneous	21	46	58	2678	24	791	6	266	7	476	—	—	14	245	119	3675
Total	29	59	206	7868	69	4648	9	300	12	783	13	149	22	291	241	8386

[a] Morbidity and Mortality Weekly Reports, Public Health Service, U. S. Department of Health, Education, and Welfare.

[b] Outbreaks.

In the case of staphylococcus enterotoxin it is important to consider the natural flora of the food which may compete with contaminating food-poisoning staphylococci (5). Certain food items are more selective for the growth of staphylococci than are others, i.e., the staphylococcus can grow in concentrations of sugar and salt which are inhibitory to many of the other food-poisoning organisms.

Concerning foodborne infections, there is evidence that salmonellosis is still a major problem. Processed eggs have come in for their share in the dissemination of *Salmonella* serotypes (6). The widespread incidence of unusual *Salmonella* serotypes that caused outbreaks of human illness, has pointed to foods in national distribution as the probable source (7, 8).

As the methodology for the isolation of *Salmonella* in foods has improved, together with the development of new processed foods, more salmonellae are being detected in a wide assortment of foods. Common sources of salmonellae are poultry products, high-protein animal feed, such as bone meal, feather meal, and fish meal (9, 10, 11, 12). The impact of salmonellae in these products is currently being studied.

In the case of microorganisms causing gastrointestinal disturbances without fever and leukocytosis, there are *Clostridium perfringens*, *Streptococcus faecalis*, and *Bacillus cereus*, all of which produce identical symptoms with similar incubation periods (from the time of the ingestion of the implicated food to the time of the onset of the illness). For these agents the mechanism involved in producing the illness is not understood and often variable results have been obtained in feeding cultures of experimentally contaminated foods to human volunteers. There is little doubt as to the role of these agents in foodborne illness, but it is important that more information be developed to explain the mode of action of these agents in causing illness.

The Japanese have described a *Pseudomonas enteritis* as giving rise to illnesses simulating those of salmonellosis. (See the paper by Kawabata and Sakaguchi in this symposium.)

No laboratory studies of enteric viruses have been made in foods. Epidemiological studies have indicated that enteric viruses may be spread by foods. Epidemic diarrhea has been reported in premature and older infants and has been traced to ECHO virus type 18 (13). Duncan and Hutchison (14) report an outbreak of gastrointestinal illness in twenty-one members of five families living in nearby houses on the same street. Three of the patients developed pharyngoconjunctival fever. The authors are careful, however, to state that the numerous isolations of adenovirus type 3 make it quite clear that the members of families A and B were infected with this virus when they had symptoms, but this does not necessarily prove that the virus caused the symptoms. Although another agent

could have been responsible, no other bacterial or viral cause was discovered. In Mr. A's case at least, it was shown by a rise in antibody titer that the acute stage of infection with adenovirus coincided with the acute symptoms of illness. If the adenoviruses had been of no etiological significance it would probably have been possible to isolate them from healthy people and from patients with other types of illness during the same period. Stool specimens from forty-four patients with various febrile and neurological conditions were examined, without adenoviruses being isolated. The authors considered, therefore, that adenovirus type 3 did in fact cause these illnesses.

ECHO virus type 11 has been found by Bergamini and Bonetti (*15*) in an acute gastroenteritis outbreak in May 1959 involving thirteen children. The syndrome was characterized by fever, vomiting and diarrhea with semiliquid stools attacking six sucklings, 8–11 months of age. Two deaths occurred (the first on day 4, the second on day 6); no samples for virological testing were taken from the first child. Examinations for *Salmonella, Shigella, Escherichia coli* (serotypes 25, 026, 027, 055, 075, 086, 0111, 0112) and enterotoxic staphylococci in the stools of thirteen sucklings and of seven adults of the ward, gave negative results. Neutralizing antibodies for the isolated strain and the ECHO virus type 11 parent strain were found in three patients tested. However, this virus was found in the stools of two healthy sucklings in contact with the patients.

Goldstein *et al.* (*16*) studied eighteen cases of poliomyelitis, an enteric virus, which occurred in a west coast (U.S.) Naval Training School. The epidemiological investigation pointed to food, probably milk. The authors also reported the literature listing a number of other outbreaks of poliomyelitis in which milk was suspected. It is important that information be developed to determine the significance of enteric viruses in various food commodities. Obviously, viruses, unlike many bacteria contaminating food, do not multiply in the food.

The virus of infectious hepatitis has a history of being spread by contaminated foods. Read *et al.* (*17*) reported a foodborne outbreak of infectious hepatitis in a fraternity house. Sixty men ate all or part of their meals at the house and among these twenty were affected. Of the remaining twenty-seven members who ate only an occasional meal at the house none became ill and no cases occurred in students outside of the fraternity. An outbreak in the army was reported by Kaufmann *et al.* (*18*). Roos (*19*) reported an outbreak in Sweden caused by the ingestion of raw oysters. Since raw oysters are not a commonly eaten food in Sweden many of the patients could state the exact time the oysters were eaten, thus establishing the exact incubation period.

In the United States, Mason and McLean (20) presented a paper entitled "Infectious Hepatitis Traced to the Consumption of Raw Oysters" at the American Public Health Association Conference, November 14, 1961 (Engineering and Sanitation, Epidemiology, Laboratory Sections). Seventy-seven cases of infectious hepatitis were reported during the first 3 months of 1961 in Mississippi and Alabama (21, 22). These cases were traced to the consumption of raw oysters obtained from the localized area at the mouth of the heavily contaminated Pascagoula River.

Outbreaks of infectious hepatitis were also traced to raw clams and on May 1, 1961 Dr. R. B. Kandle, State Commissioner of Health of New Jersey, closed the New Jersey areas of Raritan Bay to the taking of shellfish. Similar restrictions were placed on the New York areas of the Bay (23).

During a 25-day period from November 18 to December 13, 1961, twenty-two officers at a Naval facility in Florida developed signs and symptoms of hepatitis. Food histories were obtained on 21 of the 22 officers with hepatitis and also on 128 of the 138 officers who had eaten the meals but had not developed clinical hepatitis (24). It was suspected that potato salad was the food most likely involved in this outbreak of infectious hepatitis. One of the three salad handlers working in the kitchen had an illness in October of several weeks duration consistent with anicteric hepatitis. The evidence obtained suggested that the salad handler contaminated the potato salad which on October 26–27 was probably eaten by all 22 persons subsequently developing infectious hepatitis.

In view of the foodborne outbreaks of infectious hepatitis, a review of the agent and its properties may be summarized as follows.

Isolation of the virus. There are considerable differences of opinion among virologists concerning the viral agents which have been isolated from infectious hepatitis. Some of these authors have claimed to have reproduced infectious hepatitis in volunteers given the virus. Rightsel *et al.* (25) have isolated viruses. An A-1 virus reported by O'Malley *et al.* (26) is somewhat different from the others.

Resistance of the virus to chemical agents. Dr. Albert P. McKee (27) of the State University of Iowa in a personal communication stated that strains of the virus he was working with withstand 0.2% formaldehyde for 24 hours but not for 7 days. In the case of chlorine, in water which had previously been treated with a coagulant, settled and filtered containing the virus (28, 29), the virus withstands 1 ppm of residual chlorine for 30 minutes. However, the virus is inactivated when similar water is treated with 1.1 ppm residual chlorine. The virus was not inactivated with raw water with 15 ppm of chlorine after 30 minutes.

Resistance to physical agents. (a) Heat. Rightsel and his co-workers (*25*) heated serum samples from patients with infectious hepatitis at 60°C for 30 minutes as a routine isolation procedure. Davis (*30*) states that the virus he was working with withstands heating at 60°C for 40 minutes. In a personal communication, McKee (*27*) writes that his viruses withstand heating at 60°C for 2 hours.

Bolin *et al.* (*31*) in the case of the serum hepatitis virus observed that it was not inactivated by heating for 4–6 hours at 60°C, whereas the infectious hepatitis virus is inactivated at this temperature. The A-1 virus described by O'Malley *et al.* (*26*) is inactivated by heating at 56°C for 30 minutes.

(b) *Cold (freezing).* Viruses are active in materials frozen for 1 to 1½ years, but they become inactivated after being frozen 3 years at −10 to −20°C (*28*).

Carrier status. Stokes *et al.* (*32*) cite the case of one patient who developed symptoms on December 5, 1949. A fecal emulsion was prepared from this patient on March 15, 1951 and tested orally on four volunteers. One of these volunteers developed hepatitis and jaundice. Eichenwald and Mosley (*29*) stated that the period of viremia may persist from 1–5 years. Telephone calls have been received from food processors asking whether they could return food handlers, convalescing from infectious hepatitis, to work. Obviously, from the possibility of a prolonged carrier state it is not safe to put a patient in a position of handling food that is not cooked before eating unless this person can be proved to be free of virus.

From the foregoing it is obvious that the agent of infectious hepatitis may survive pasteurizing temperatures adequate to destroy the majority of pathogenic microorganisms. It is, therefore, important that the survival of the virus of infectious hepatitis in various types of food be established. Furthermore, it is important to settle the question whether the bacterial microbial standards for enteric pathogens are effective against enteric viruses.

Fungi. Although fungi have been connected with food spoilage, fungus-borne diseases have not been associated with foods. In the author's experience, however, some food plants have storage areas accessible to starlings, pigeons and sparrows where containers are kept. Foods which may be consumed without cooking may subsequently be placed in these containers. These storage areas contaminated with bird excreta containing spores of *Histoplasma capsulatum* may seed this fungus into foods placed in these containers. Since this disease is so prevalent in man, there is a possibility that some of the cases may be foodborne. The general subject of histoplasmosis is discussed by Furgolow *et al.* (*33*).

Summary

From the brief resume of some microbial agents associated with foodborne illness it is evident that much is known about the role of *Clostridium botulinum*, enterotoxigenic staphylococci, and salmonellae in foods, although even with these agents there are many gaps in our knowledge. With better reporting of foodborne illness and followup with epidemiological and laboratory investigations the proper role of the less recognized bacterial agents in foodborne illnesses should be established. The role of the enteric viruses as foodborne agents of disease awaits the development of laboratory procedure for isolating these viruses from food. The resistance of certain enteric viruses (infectious hepatitis) to heat and chemical agents raises the question whether the microbial standards for fecal contamination of food apply to enteric viruses. A possible foodborne spread of pathogenic fungi is suggested in the case of histoplasmosis.

REFERENCES

1. U.S. Department of Health, Education, and Welfare. The Public Health Conference on Records and Statistics. Study Group on Methods of Improving the Reporting of Short-term Gastroenteric Episodes. Doc. No. 530. 4–3/16/62 (1962).
2. U.S. Department of Health, Education, and Welfare. Public Health Service. Report of the Committee on Environmental Health Problems to the Surgeon General. Public Health Service Publication No. 908 (1962).
3. K. R. Lundeberg. Personal communication. 22 September (1960).
4. C. F. Schmidt, R. V. Lechowich, and J. F. Folinazzo. Growth and Toxin Production by Type E *Clostridium botulinum* below 40°F. Presented at the Twenty First Annual Meeting of the Institute of Food Technologists, May 8 (1961).
5. G. M. Dack and G. Lippitz. Fate of food poisoning microorganisms introduced into slurry of frozen pot pies. *Appl. Microbiol.* 10, 472–479 (1962).
6. F. S. Thatcher and J. Montford. Egg-products as a source of salmonellae in processed foods. *Can. J. Public Health,* 53, 61–69 (1962).
7. Morbidity and Mortality Weekly Report, Public Health Service, U.S. Department of Health, Education, and Welfare, Vol. 11, No. 16, April 27 (1962).
8. Morbidity and Mortality Weekly Report, Public Health Service, U.S. Department of Health, Education, and Welfare, Vol. 11, No. 23, June 15 (1962).
9. A. Clarenburg and E. H. Kampelmacher. Salmonella-culturen uit mens endier, rioolwater, dier-en vismeel en eiprodukten, geisoleerd in Nederland, 1946-1957. *Verslag. Mededel. Betreffende Volksgezondheid.* No. 2, pp. 369–375, (1959).
10. J. Müller. Bacteriological examination of imported meat-and-bone meal and the like. *Nord. Veterinarmed.* 4, 290-295 (1952).

11. J. Müller. Le problème des salmonelloses au Danemark. Office International des Epizooties (Epidemic Disease of Animals), Rapport à la XXVe Session, No. 458, pp. 1–13 (1957).

12. H. P. R. Seeliger. Food-borne infections and intoxications in Europe. *Bull. World Health Organ.* 22, 469–484 (1960).

13. H. F. Eichenwald, A. Ababio, A. M. Arky, and A. P. Hartman. Epidemic diarrhea in premature and older infants caused by ECHO Virus Type 18. *J. Am. Med. Assoc.* 166, 1563–1566 (1958).

14. I. B. R. Duncan and J. G. P. Hutchison. Type-3 adenovirus infection with gastrointestinal symptoms. *Lancet*, March 11, pp. 530–532 (1961).

15. F. Bergamini and F. Bonetti. Episodio epidemico di gastroenterite acuta da virus ECHO–11 in un brefotrofio. *Boll. Ist. Sieroterap. Milan.* 39 (11/12), 510–515 (1960).

16. D. M. Goldstein, W. McD. Hammon, and H. R. Viets. An outbreak of polioencephalitis among navy cadets, possibly food borne. *J. Am. Med. Assoc.* 131, 569–573 (1956).

17. M. R. Read, H. Bancroft, J. A. Doull, and R. F. Parker. Infectious hepatitis—presumedly food-borne outbreak. *Am. J. Public Health* 36, 367–370 (1946).

18. G. G. Kaufmann, V. M. Sborov, and W. P. Havens, Jr. Outbreak of infectious hepatitis—presumably food-borne. *J. Am. Med. Assoc.* 149, 993–994 (1952).

19. B. Roos. Hepatitis epidemic conveyed by oysters. *Svenska Lakartidn.* 53, 989–1003 (1956).

20. J. O. Mason and W. R. McLean. Infectious hepatitis traced to the consumption of raw oysters. Presented before the Engineering and Sanitation, Epidemiology, Laboratory Sections, The American Public Health Association Conference, November 14 (1961).

21. Morbidity and Mortality Weekly Report, Public Health Service, U.S. Department of Health, Education, and Welfare, Vol. 10, No. 6, February 17 (1961).

22. Morbidity and Mortality Weekly Report, Public Health Service, U.S. Department of Health, Education, and Welfare, Vol. 10, No. 13, April 7 (1961).

23. Morbidity and Mortality Weekly Report, Public Health Service, U.S. Department of Health, Education, and Welfare. Vol. 10, No. 17, May 5 (1961).

24. Morbidity and Mortality Weekly Report, Public Health Service, U.S. Department of Health, Education, and Welfare. Vol. 11, No. 8, March 2 (1962).

25. W. A. Rightsel, R. A. Keltsch, A. R. Taylor, J. D. Boggs, and I. W. McLean, Jr. Status report on tissue-culture cultivated hepatitis virus. I. Virology laboratory studies. II. Clinical trials. *J. Am. Med. Assoc.* 177, 671–682 (1961).

26. J. P. O'Malley, H. M. Meyer, Jr., and J. E. Smadel. Antibody in hepatitis patients against a newly isolated virus. *Proc. Soc. Exptl. Biol. Med.* 108, 200–205 (1961).

27. A. P. McKee. Personal communication, 28 December (1961).

28. W. P. Havens, Jr. Infectious hepatitis. *Medicine* 27, 279–326 (1948).

29. H. F. Eichenwald and J. W. Mosley. Viral hepatitis. Clinical and public health aspects. Public Health Service Publication No. 435 Reprinted (1960).
30. E. V. Davis. Isolation of viruses from children with infectious hepatitis. *Science* 133, 2059–2061 (1961).
31. V. S. Bolin, J. B. Alsever, J. B. Barger, and T. B. Jarvis. Studies on serum and infectious hepatitis viruses of man. I. Preliminary report on the isolation of serum and infectious hepatitis viruses from man in tissue culture. *Transfusion* 1, 360–366 (1961).
32. J. Stokes, Jr., J. E. Berk, L. L. Malamut, M. E. Drake, J. A. Barondess, W. J. Bashe, I. J. Wolman, J. D. Farquhar, B. Bevan, R. J. Drummond, W. d'A. Maycock, R. B. Capps, and A. M. Bennett. The carrier state in viral hepatitis. *J. Am. Med. Assoc.* 154, 1059–1064 (1954).
33. M. L. Furgolow, F. E. Tosh, H. W. Larsh, H. J. Lynch, Jr., and G. Shaw. The emerging pattern of urban histoplasmosis. Studies on an epidemic in Mexico, Missouri. *New Eng. J. Med.* 264, 1226–1230 (1961).

The Nature and Detection

of Staphylococcal Enterotoxin

E. P. CASMAN

Division of Microbiology, Food and Drug Administration,
U. S. Department of Health, Education, and Welfare, Washington, D. C.

In 1947 in the Division of Microbiology of the Food and Drug Administration, studies were begun for the development of practical procedures for the detection of staphylococcal enterotoxin in food, and for the demonstration of enterotoxigenicity of staphylococci. The available procedures for the detection of this toxin depended on the use of monkeys or cats. The tests were difficult to perform, of variable reliability, and not sufficiently sensitive. Their use had resulted in contradictory reports in almost all aspects of the problem of staphylococcal food poisoning. The very sensitive and specific immunological procedures which had been so successfully applied in the study of other bacterial toxins appeared to hold some promise in spite of conflicting reports as to the antigenicity of staphylococcal enterotoxin and the absence of studies confirming the work of Dolman and Wilson (*1*) who in 1938 demonstrated a specific flocculation reaction which appeared to involve enterotoxin and its homologous antibody.

In order to attain the goals indicated above by the use of serological procedures, it was necessary to demonstrate the antigenicity of enterotoxin, to determine the number of antigenic types of enterotoxin that might be involved, to detect and make available for use the specific anti-enterotoxins, and to develop methods for the production of enterotoxin and for its serological detection in bacterial cultures and foods.

In 1958 (*2*) and in 1960 (*3*) we reported the results of our studies in which we demonstrated: (1) the antigenicity of enterotoxin, by conferring to cats a passive immunity using antiserum produced in rabbits; (2) the occurrence of two serologic types of heat-resistant enterotoxin of which only one appeared to be associated with food poisoning; (3) the production by certain staphylococci of a heat-labile emetic substance, the

activity of which was lost on dialysis; and (4) the application of the Ouchterlony gel-diffusion procedure for the detection of enterotoxigenicity. There remained the objectives of developing better methods for the detection of enterotoxigenicity and the serological detection of enterotoxin in foods.

Since only one type of enterotoxin appeared to be associated with food poisoning, we have directed our attention to the study of this enterotoxin. It was designated by us in 1960 (*3*) by the letter "F" (food poisoning), but is now designated as enterotoxin A (*4*) to facilitate the orderly naming of enterotoxins as they become serological entities.

In 1958 (*2*), we described a medium which we had used for more than 10 years for the production of staphylococcal enterotoxin. This medium was completely dialysable and free from ammonium-sulfate-precipitable substances. In developing it we had used the production of alpha-hemolysin rather than enterotoxin as an index of productivity because of the technical difficulties and variations inherent in the available biological tests for enterotoxin. The medium was considered adequate for the production of enterotoxin since it was found to equal Dolman and Wilson's soft agar medium (*5*) in this respect. It consisted of an acid hydrolysate of casein with phosphate, sodium acetate, tryptophan, cystine, salts of iron and magnesium, and growth-accessory factors.

Our development of a serological test for enterotoxin offered the opportunity to select or develop a better medium for the production of enterotoxin. For this purpose we used Wadsworth's miniaturization of the gel double diffusion test (*6*) as described in detail by Crowle (*7*). Measurement of enterotoxin production consisted of the determination of the highest dilution of toxin giving a zone of precipitation. To produce a line of precipitation in the gel-diffusion test, a concentration of at least 2.5 to 5.0 cat-vomiting doses of enterotoxin per milliliter was required. Since this degree of toxicity was not obtained with most strains cultured in the fluid medium described above, it was necessary to concentrate these toxins by ammonium sulfate precipitation or by other procedures before testing. The enterotoxigenicity of strains was routinely determined by the slide gel-diffusion test after treating the toxins produced in this medium with ammonium sulfate to 85% saturation, collecting the precipitate on a millipore membrane and dissolving the precipitate in one hundredth the original volume of the toxin.

Our development of a culture technique by which growth and toxin production were confined to the interior of a cellophane tube lying on fluid medium (*8*) made possible the study of modifications of our original medium and the testing of other media without regard to the presence of ammonium-sulfate-precipitable substances. By employing this procedure,

a survey of available media and modifications of our original medium was made. With certain strains it became evident that concentration techniques could be dispensed with when the acid hydrolysate of casein in our medium was replaced with enzymatic digests of certain proteins or when brain heart infusion broth was used as the medium.

Further study of the media for the production of enterotoxin A was therefore carried out with strain 246-3A in fluid media without employing concentration techniques. Commercial brain heart infusion broth was used as a standard for comparison and as a pattern for the preparation of other media. Twenty-five milliliter quantities in 8 ounce hexagonal Pyrex nursing bottles with narrow necks were inoculated and aerated by rotation in a horizontal position at a rate of 20 revolutions per minute for 18 to 24 hours.

Under these conditions, the productivity of brain heart infusion broth was found to be affected by the pH to which the medium was adjusted before use. Adjustment of this medium to an initial pH of 5.5 gave maximum growth and enterotoxin production.

Attempts were made to find a suitable medium less complex than brain heart infusion. These involved replacement of the peptone and infusions with 2% of a pancreatic digest of lactalbumin (Edamin S of Sheffield Farms), which had been found best for the production of enterotoxin A in the cellophane tube screening procedure. The Edamin S was enriched with thiamine, nicotinic acid, and calcium pantothenate, and the effect of varying the pH, glucose, and phosphate concentrations on enterotoxin production was determined.

The effect on growth and toxin production by strain 246-3A on adding 0.2% glucose and varying concentrations of NaH_2PO_4 to the Edamin S base is shown in Table I.

It is apparent from Table I that in the presence or absence of 0.2% glucose, growth and toxin production are functions of the initial pH of the medium and of its phosphate content. In order to avoid the precipitation obtained on autoclaving the medium at the higher pHs and phosphate concentrations, the following formula was selected for further study: 2.0% Edamin S, 0.2% glucose, 0.4% NaH_2PO_4, 0.000004% thiamine hydrochloride, 0.00012% nicotinic acid, 0.00005% calcium pantothenate, and pH 5.8. It was found that the production of enterotoxin by strain 246-3A in the latter medium was equal to that in brain heart infusion broth of pH 5.5. It consistently produced approximately 20 cat-vomiting doses per milliliter. However, when the two media were compared by employing 27 additional strains, 14 of the strains produced more enterotoxin in the brain heart infusion broth.

The latter medium at pH 5.5 was then used to test all of our strains for

TABLE I

EFFECT OF GLUCOSE AND NaH$_2$PO$_4$ IN EDAMIN S BASE[a] ON PRODUCTION OF
ENTEROTOXIN BY 246-3A AT pH 5.5 AND pH 5.9

Broth pH	Culture pH	% glucose	% NaH$_2$PO$_4$	Plate count billions/ml	Enterotoxin (reciprocal of titer)
5.5	8.3	0	0.0	3	2
5.5	8.2	0	0.2	12	2
5.5	8.0	0	0.4	27	2
5.5	7.5	0	0.6	28	4
5.9	7.9	0.2	0.0	7	2
5.9	8.25	0.2	0.2	21	2
5.9	7.8	0.2	0.4	31	4
5.7[b]	7.05	0.2	0.6	27	4

[a] 2% Edamin S, 0.000004% thiamine hydrochloride, 0.00012% nicotinic acid, 0.00005% calcium pantothenate.

[b] Precipitation and lowering of pH on autoclaving.

their ability to produce enterotoxin A. All but 4 of 49 strains gave positive results. The exceptions were strains which appeared not able to alkalinize the medium. The pHs after overnight incubation and appreciable growth were slightly lower than the initial pH 5.5. To demonstrate enterotoxicity in these cultures by means of the gel-diffusion test, it was necessary to concentrate them to from ¼ to ½ their original volumes.

The four exceptions were then grown for 48 hours on the surface of 25 milliliter quantities of the brain heart infusion of pH 5.5 made semisolid by the addition of 0.6 to 0.7% agar. When this was done, alkalinization and positive tests for enterotoxin were obtained with the unconcentrated toxins. It was further found that with this simple aeration procedure, the initial pH of the medium could be lowered to 5.3 to give a slightly higher yield of enterotoxin and positive results with all of our strains. Five of seven lots of one brand of commercially prepared brain heart infusion broth and two of three lots of another brand were found to be satisfactory for the production of enterotoxin A.

Although brain heart infusion is not the best medium for the production of enterotoxin B (formerly type "E"), we have demonstrated that it can be produced in this medium by all but 1 of 14 strains. The one exception produced trace amounts of this enterotoxin so that regardless of the medium employed for its production, considerable concentration was necessary before it could be detected by the gel-diffusion test.

Having developed a practical procedure for the detection of staphylococcal enterotoxicity, our efforts are now directed toward the attainment

of the ultimate goal of our study—the demonstration of small amounts of enterotoxin in foods. Preliminary attempts to apply the very sensitive technique of hemagglutination inhibition for this purpose revealed the need for antigenically pure enterotoxin for the sensitization of the erythrocytes. We are engaged now in purification studies which we hope will make possible the successful conclusion of this long-range program.

REFERENCES

1. C. E. Dolman and R. J. Wilson. Experiments with staphylococcal enterotoxin. *J. Immunol.* 35, 13–30 (1938).
2. E. P. Casman. Serologic studies of staphylococcal enterotoxin. Public Health Rep. 73, 599–609 (1958).
3. E. P. Casman. Further serological studies of staphylococcal enterotoxin. *J. Bacteriol.* 79, 849–856 (1960).
4. E. P. Casman, M. S. Bergdoll, and J. Robinson. Designation of staphylococcal enterotoxins. *J. Bacteriol.* 85, 715–716 (1963).
5. C. E. Dolman and R. J. Wilson. The kitten test for staphylococcus enterotoxin. *Can. J. Public Health* 31, 68–71 (1940).
6. C. A. Wadsworth. Slide microtechnique for the analysis of immune precipitates in gel. *Intern. Arch. Allergy Appl. Immunol.* 10, 355–360 (1957).
7. A. J. Crowle. A simplified micro double-diffusion agar precipitin technique. *J. Lab. Clin. Med.* 52, 784–787 (1958).
8. E. P. Casman and R. W. Bennett. Simplified procedure for producing small amounts of potent staphylococcal enterotoxin. *Bacteriol. Proc. Soc. Am. Bacteriologists* (1961).

Discussion by M. S. Bergdoll

Food Research Institute, University of Chicago, Chicago, Illinois

Dr. Casman has discussed the production of small volumes of potent solutions of enterotoxin for use in the detection of enterotoxin-producing strains. Since our work on enterotoxin production has been primarily concerned with large quantities for purification studies we can add very little to his thorough discussion of that phase of the problem. Large scale production has, of necessity, been limited to deep culture aeration usually employing a simple medium such as enzyme-hydrolyzed casein supplemented with niacin and thiamin. The simpler the medium the easier is the recovery of the enterotoxin from the bacterial culture supernatants. Methods that are suitable for production of enterotoxin by one strain may not be suitable for another strain or even a variant of the same strain.

Production of enterotoxin is very important; however, there are other aspects of the enterotoxin problem which we believe pertinent to any dis-

cussion of this food-poisoning agent. Some of these Dr. Casman mentioned briefly, others were not included in his remarks.

Early in our studies we suspected the existence of more than one immunologically distinct type of enterotoxin (1). This was confirmed when one of the enterotoxins, which is now labeled enterotoxin B, was purified and specific antibodies to it prepared. What is now called enterotoxin A did not react with this specific B antiserum (2). This was confirmed independently by Dr. Casman by the use of specific antibodies to A and B prepared by serum adsorption techniques (3). His studies indicated that only enterotoxin A might be involved in food poisoning outbreaks since all the strains he tested that were isolated from foods involved in food poisoning cases appeared to produce only enterotoxin A.

In 1961 Dr. Kikuo Fujiwara, Chiba University, Japan, reported the isolation of five strains of staphylococci from foods involved in food poisoning outbreaks that produced enterotoxin B (4). When Dr. Casman had these strains phage typed the results indicated a single source and it was later learned that the foods from which they were isolated came from the same food store over a period of several months. These strains also produced enterotoxin A. Although preliminary attempts to produce enterotoxin B in foods experimentally under conditions similar to those that might prevail in food poisoning cases have been with one or two exceptions unsuccessful, the possibility that types other than A are involved in food poisoning cannot be dismissed.

It is not known how many types of enterotoxin there are and it will not be known until specific antisera can be prepared to other types. At the present time we are beginning work on what we believe to be a third type.

What methods are available for assaying enterotoxin? Dr. Casman uses intravenous injection of cats and Dr. Thatcher and co-workers, Dept. of National Health and Welfare, Ottawa, Canada, use intraperitoneal injection of cats. These methods, while used successfully by these investigators, require careful interpretation of the results because of the possible danger of interference by other toxic materials produced by the staphylococci. To avoid this problem we use the intragastric injection of monkeys. This is a costly method, but we believe it to be a worthwhile investment.

The progress toward development of a more practical assay technique has been slow. Dr. Casman is able to detect enterotoxins A and B by the use of specific serums developed by adsorption techniques. The preparation of specific antiserum for A has not been duplicated by others, hence, this method is not yet in general use.

In March, 1962, at the Fourteenth Research Conference of the American Meat Institute Foundation, we reported on the use of the simple gel-diffusion technique for quantitative assay of enterotoxin B (5). This

method requires the use of purified enterotoxin as a standard and specific antiserum prepared by use of the purified enterotoxin. This method is not yet available for enterotoxin A because the purification of A is not yet complete. However, we have tentatively identified the specific antibody for A and we believe that this method should be available within the next year.

Other investigators have attempted to develop practical assay techniques for enterotoxin. In 1960, Miss Del Valle of the University of Wisconsin wrote a thesis on the use of nematodes for the detection of enterotoxin, but it was later discovered that the action observed was non-specific (6). Dr. Milone of the University of Michigan presented a paper on the use of tissue cultures for the detection of enterotoxin (7). Dr. Raj of the University of Washington presented a paper on the detection of enterotoxin by the use of tropical fish (8). In both of these instances, the use of purified enterotoxin indicated the observed reaction was due to a substance other than enterotoxin. Dr. Kienitz, University of Münster, Germany, reported recently on the use of chick embryos for assaying enterotoxin (9). His results indicated that enterotoxin had a lethal effect, but this action was not specific since beta-lysin had the same effect. Dr. Hopper, Indiana University Medical School, presented a paper on the detection of enterotoxin in foods by a flotation method employing specific enterotoxin antibodies (10). This method shows promise, but is dependent on the availability of specific antisera.

Since the title of Dr. Casman's presentation includes the nature of enterotoxin, some comments are in order. Only enterotoxin B has been studied because this is the only one that has been purified (11). Enterotoxin B is a heat-coagulable, water-soluble protein with a molecular weight of approximately 23,000 and an isoelectric point at pH 8.5 (12). The approximate amino acid composition shows a relatively high lysine, aspartic acid, and tyrosine content. Its composition appears to be confined to amino acids.

Dr. Casman has referred to enterotoxins A and B as heat stable. This depends on one's viewpoint and the method used in assaying. Enterotoxin solutions that have been boiled for 30 minutes are toxic for cats by the intravenous or intraperitoneal routes, but are nontoxic for monkeys by the intragastric route. The fact that enterotoxin B is heat coagulable complicates the picture, but if coagulation can be prevented by using very dilute solutions, boiling for 30 minutes does not seem to affect the toxicity in cats. The heating of the enterotoxin apparently changes it in some way to make it vulnerable to the proteolytic enzymes of the digestive tract of the monkey. Unheated enterotoxin appears to be quite resistant to the proteolytic enzymes.

Dr. Thatcher *et al.* (*13, 14*) have examined enterotoxin B with infrared spectrophotometry. Preliminary examination indicated the relationship of one peak to enterotoxin, but sufficient studies have not been done to confirm this.

One other phase of the enterotoxin problem that should be mentioned is the work by Dr. Sugiyama of the Food Research Institute, University of Chicago, on the pharmacological action of enterotoxin. Dr. Sugiyama has investigated many activities related to crude enterotoxin preparations, some of which proved to be unrelated to enterotoxin when tests were made with purified toxin. One of the most significant studies showed that bilateral destruction of the area postrema on the floor of the fourth ventricle (chemoreceptor trigger zone) makes rhesus monkeys completely refractory to the emetic action of staphylococcal enterotoxins A and B (*15*). Vagotomy at the level of the diaphragm gave protection in all animals. A species difference between cats and rhesus monkeys in the importance of the area postrema region for enterotoxin-stimulated vomiting is indicated by results reported by Dr. Wesley Clark (*16*) from use of purified enterotoxin in cats.

It is apparent that we know much more about enterotoxin now than we did 10 years ago and I am sure the investigations being carried out in laboratories in all parts of the world will yield much additional valuable information.

REFERENCES

1. M. J. Surgalla, M. S. Bergdoll, and G. M. Dack. *J. Lab. Clin. Med.* **41**, 782 (1953).
2. M. S. Bergdoll, M. J. Surgalla, and G. M. Dack. *J. Immunol.* **83**, 334 (1959).
3. E. P. Casman. *J. Bacteriol.* **79**, 849 (1960).
4. K. Fujiwara. *Ann. Rep. Inst. Food Microbiol.* (Chiba University, Japan) **14**, 1 (1961).
5. M. S. Bergdoll *Proc. Res. Conf. Res. Advisory Council Am. Meat Inst. Found. Univ. Chicago.* **14**, 47 (1962).
6. M. R. Del Valle. M.S. Thesis, Univ. of Wisconsin (1960).
7. A. N. Milone. *Am. Public Health Assoc.*, 89th Annual Meeting, Detroit, Michigan, Nov. 16, 1961.
8. H. Raj. *Bacteriol. Proc. Abstr.*, 62nd Ann. Meeting, p. 65 (1962).
9. M. Kienitz. *Zentr. Bakteriol. Parasitenk. Abt. I. Orig.* **184**, 87 (1962).
10. S. H. Hopper. Private communication.
11. M. S. Bergdoll, H. Sugiyama, and G. M. Dack. *Arch. Biochem. Biophys.* **85**, 62 (1959).
12. H. E. Hibnick and M. S. Bergdoll. *Arch. Biochem. Biophys.* **85**, 70 (1959).
13. L. Levi, B. H. Matheson, and F. S. Thatcher. *Science* **123**, 64 (1956).
14. F. S. Thatcher. Private communication.

15. H. Sugiyama, K. L. Chow, and L. R. Dragstedt, II. *Proc. Soc. Exptl. Biol. Med.* 108, 92 (1961).

16. W. Clark. Ph.D. Thesis, University of Utah (1962).

Open Discussion

DR. THATCHER: I would like to suggest that we review these papers in the order presented by the respective speakers. Would anyone care to comment on food as a vehicle for virus infection? If not, may I point to what seems highly significant to me, and that is the growing recommendation for the application of irradiation using Cobalt 60 or other sources for pasteurization of foods. We should bear in mind that most of the indicator organisms, in fact most bacteria other than spore forms would be destroyed at levels considerably lower than those required to destroy viruses. That would mean a food apparently safe on bacteriological examination may not necessarily be safe from a point of view of virus involvement.

We should also remember that the irradiated viruses have the opportunity for reactivation if one molecule comes in contact with another molecule. Further, these viruses may undergo what we could roughly call a "sexual" fusion and the irradiation process may result in a phase during which they can undergo this fusion which is followed by the usual genetic segregation for a period much longer than would be normal. Hence, we have to bear in mind the potentiality of, shall we say, breeding new strains of virus.

FROM THE FLOOR: I would like to return to the problem of infectious hepatitis. Dr. Dack implied that there might be some question of safety in allowing food handlers to return back to the food handling line after having infectious hepatitis because of the long period in which the virus is shed. At a recent meeting I made a similar comment or inference and there were in the room about 40 epidemiologists from California who took issue with the suggestion made that it would not be safe to allow food handlers to return back to the line following infection with infectious hepatitis. They made a point that this is not done with food handlers who have had other viral infections and that with proper sanitation, washing of their hands, and so on, that they did not see any problem. So, apparently there seems to be some difference of opinion on the hazards involved in allowing food handlers who have had infectious hepatitis to return back to the food handling line.

DR. THATCHER: Thank you. Would you care to comment on that, Doctor Dack?

DR. DACK: The only comment I can make is that we don't know, any of us, what we are talking about unless we further study this problem.

We actually had an outbreak of hepatitis that we know was probably due to potato salad. We have had several cases that we know are due to raw oysters, not only in this country but in Sweden. We have cases that we know are due to clams, and until we get some factual data, nobody can answer these questions and that's the only reason I brought it up.

I think we in the food field are way behind medical virologists and others in evaluating the significance this might have. But certainly, as far as I am concerned, I wouldn't want to eat a product that wasn't properly cooked, that

had been handled by a person coming back from a case of infectious hepatitis. The evidence with some of the viruses that have been isolated is that they are extremely resistant to heat and they are resistant to some chemical agents, but until we are able to develop a methodology for their detection in food, I think this is still going to be an open question.

DR. THATCHER: Thank you, Dr. Dack.

Returning now to fungi, I wonder if our British colleagues would like to talk about the recent clear-cut demonstration of a toxin formed by a fungus and specifically found in peanuts. Doctor Ingram, would you say a word on that.

DR. INGRAM: It poses a question that I can't answer. The discovery of the toxin arose from the fact that a large number of poultry died of a mysterious disease involving severe liver damage. Dr. Roberts discovered this occurred by the feeding of peanuts infected with *Aspergillus flavus*. It turns out that only particular strains of the fungus are capable of forming this toxin. There is work going on at the moment to try to purify and identify the substance. It is perfectly clear it is a specific chemical. Its presence can be demonstrated by ultraviolet fluorescence. This is not an uncommon fungus. It is usual indeed in some tropical countries in many common food products. But this is a warning to us that we really don't know much about the toxic products of fungi and particularly how dangerous they might be. When this situation was described at the microbiological congress in Montreal, the speaker was mentioning that there are enormous growths of fungi on, for example, prepared hay, so cows and animals consuming this material would in fact be consuming material composed of fungus, and he was raising in general terms the question whether there might be toxins in this material.

While I am on my feet, I have one other word. I would like to make a suggestion which I have made before. I have been very uncomfortable, rather in the same way as Dr. Thatcher, in connection with irradiation, and particularly so where antibiotics are used in the preparation of meat from fowls which are apt to carry pathogenic fungi.

, DR. THATCHER: Thank you, Doctor Ingram. Any other comments about the fungi? I might add that our organization has recently intercepted a shipment of peanuts with *Aspergillus flavus*. Specimens were toxic to test animals. The specific toxin was demonstrated chemically.

DR. MRAK: I would like to ask Dr. Ingram if anything is being done to control this where the peanuts are grown, or does it occur in storage, or do we know much about it?

DR. INGRAM: This is not clear.

DR. MRAK: Is someone working on this?

DR. INGRAM: So far as I am aware, not specifically.

DR. MILNER: First of all, I think it should be pointed out that this is not something new. If you want to find a history on mycotoxicoses, veterinarians have known about this in animal feeds for a long time. I think the factor that precipitated it into the human nutrition area was the publication last December that there was a relationship to cancer. Work is going on in the United Kingdom where they are devoting tremendous resources to this at the present time. They are well on the way to isolating the toxin and they have also developed a fluorescence test by ultraviolet irradiation, for the specific compounds. They have even determined by infrared techniques some of the active

molecular arrangements in this compound. The Veterinary Institute at Wey-bridge has been active, particularly from the pathological point of view.

In the United States, a number of groups have been working on this. At MIT, the food technology group is well advanced in their studies. From an international aspect, there is more going on than we expected, particularly from the veterinary pathology aspect. I recommend to you a recent review in Advances in Veterinary Science on mycotoxicoses, published in 1962. You will find there a surprisingly large amount of literature.

DR. MRAK: The thing I am interested in was not brought out this morning: do we know the moisture content at which this fungus grows? Is it related to cultural practices and does it come from the soil? Is there anything we can tell the farmers to do or what not to do? These are things I can't get any information on.

DR. MILNER: The present information indicates it is largely a fungal storage problem.

DR. MRAK: Is anybody working in this area?

DR. MILNER: Yes. The Tropical Products Institute is well advanced on this. Doctor Ingram has indicated correctly that we are dealing with a specific strain of *Aspergillus flavus*. This recalls some work I was connected with a number of years ago, which indicated one could isolate a toxic fungus from moldy soybeans. When this organism was placed on sound soybeans it almost completely destroyed their viability. This has been known for some time but it was the inference of cancer which has recently made the subject quite popular. One of the plant problem pathologists of the Tropical Products group has now been looking at this in Brazil, West Africa, and East Africa, areas of major production of peanuts, and he is virtually convinced from his studies that it is not a question simply of contamination by soil microorganisms since the condition does not exist in peanuts that are allowed to dry in the normal fashion without breaking and crushing. It occurs only when the peanuts are crushed by handling or being walked upon; in other words the shell of the nut apparently carries a certain amount of protection to the immature damp peanut. As soon as one crushes the nut, allowing the entry of fungi, the problem begins, just as is the case in storage of grain.

DR. THATCHER: That then brings us to the many questions that Dr. Casman has raised. Does anyone wish to comment or ask questions on the staphylococcal enterotoxins?

DR. HURST: I wanted to ask two things about the staphylococcal enterotoxins. First of all, whether it has been considered that possibly in the purification of these toxins, the toxin becomes departiculated?

DR. THATCHER: I know this is a problem that Dr. Casman, Dr. Dack, and myself have pondered over. Dr. Casman, will you answer that.

DR. CASMAN: In our studies, the purified material was not as heat resistant as the unpurified enterotoxin A and B.

DR. THATCHER: May I comment on our own experience? We believe we have purified an enterotoxin which is distinguishable from the other two. It is distinctively different in terms of its action in tissue-culture. The first two, A and B toxins, are not pathogenic on tissue using human heart fibroblasts but this third one is toxic. Whether this is involved in human poisoning, I am afraid is still a bit problematical. The strain was isolated from what was first reported to us as food poisoning, but then the health officer decided that this

was an arsenic poisoning case. However, there were 30 million staphylococci per milliliter in the milk that caused this outbreak.

DR. HURST: I'd like to make my second point if I may. Working with entero-toxin in animals which were free of *E. coli* but otherwise were normal, showed that sensitivity in an animal depends upon the intestinal flora. We are con-tinuing with a comparison of the sensitivity of cats and monkeys.

DR. CHRISTIAN: I would like to ask a question about staphylococcal entero-toxin because we are all aware that the staphylococcus is somewhat unique in its remarkable ability to grow in very concentrated environments. It has a very low water requirement and as such we might possibly look for it in solid products when concerned with public health aspects. I would like to ask, therefore, whether the ability of the staphylococci to produce toxins has been investigated under conditions of growth, and whether it is in effect as danger-ous as we sometimes imagine. I raise this question because of the remark made by Dr. Casman relating to the pH of 5.5. We have found that the staphylo-coccus in normal rich bacteriological medium has a very wide pH range, but when we get to concentrations toward its growth limits in terms of salt, somewhere around 3.5 Molar, we find its pH range between 5.0 and 6.0.

DR. SMITH: I would like to make one brief suggestion to people who are concerned with the heat stability of the enterotoxin. There are a number of compounds as far as I known that exhibit what is known as anomalous heat inactivation. They are inactivated by heating to 60 or 70°C for a few minutes and can be activated on heating at 100°C. Some of the staphylococcal toxins may be of this nature, and if you are trying to work out the heat resistance of a substance that shows anomalous heat resistance, you are in a good deal of trouble until you find this out.

DR. INGRAM: I would just like to remind everyone that there hasn't been an answer to Dr. Christian's question, and I think you might possibly have some information. There must be some sort of information on it.

DR. THATCHER: We have positively determined production of two toxins by our specific strains in bacon, both aerobically and anaerobically. The salt level is that of Canadian market bacon. I think Dr. Silliker would know what the salt concentration of our Canadian-type bacon is.

DR. SILLIKER: It would be 3 or 4% in brine.

DR. THATCHER: We have made some studies on the production of other toxins. Roughly at about the concentration of 5%, the alpha and beta toxins fail to appear.

DR. SILLIKER: I can't give a direct answer to Dr. Christian's question except to say that I have encountered some meat products which characteristically have high brine or high salt concentrations and therefore are selective for staphylococci and micrococci. We have isolated fairly large numbers of coagu-lase positive staphylococci from them but these same products have no history of causing staphylococcal food poisoning, so there is an implication at least that perhaps toxin formation doesn't occur.

DR. THATCHER: We have some further evidence on that. We established that bacon which contained toxin after being cooked to a degree of crispness, appeared to be toxin-free. A semi-crisp bacon still contained an amount of toxin.

DR. CASMAN: There have been reports of outbreaks of staphylococcal food

poisoning or what appeared to be staphylococcal food poisoning from a meat product packed in brine.

DR. THATCHER: What is the salt content of the product?

DR. CASMAN: It is packed in a brine, but I don't know the exact concentration.

DR. NIVEN: I am perhaps being a little presumptuous but I wonder if the group knows the origin or terminology of the toxins that you have heard designated as A and B? A group at the American Society for Microbiology meeting in Kansas City this year agreed to this terminology. They discarded the enterotoxin 1 and 2 designations because of the possible confusion with phage types. I think it is a tribute to these investigators to agree at an early date.

DR. THATCHER: I might comment that I am quite convinced that our studies have been very much aided and stimulated by my opportunity to discuss freely with Dr. Dack, Dr. Bergdoll, Dr. Slocum, and Dr. Casman over the years. Whether they learned anything from me I doubt, but I certainly want to voice that appreciation.

Now, gentlemen, let us continue with our program. It is now my privilege to call on Dr. Kawabata who is the chief of the Division of Fish Poisoning and Botulism, National Institute of Health, Shinagawa-ku, Tokyo, Japan, who will discuss "Studies on *Clostridium botulinum* Type E Toxin" and *"Pseudomonas Food Poisoning."*

Halophilic Bacteria

as a Cause of Food Poisoning

T. Kawabata and G. Sakaguchi

Department of Food Control, National Institute of Health,
Shinagawa-ku, Tokyo, Japan

Incidence of Food Poisoning in Japan and Its Characteristics

There have been annually 2000 outbreaks of food poisoning with about 20,000–30,000 cases, of which about 50 to 60% implicated fish or fish products. Foodstuffs implicated in food poisoning in the period of 1958–1960 are shown in Table I. Causative agents were not identified in more than 80% of the outbreaks. Bacterial food poisoning occupied about 50–60% of the diagnosed outbreaks; a large part of the remainder was due to poisoning with naturally occurring poison, and those caused by chemicals were small in number. It has been presumed that the majority of the undiagnosed cases were of bacterial origin, judging from the features of the outbreaks. Unavailability of specimens for laboratory examinations must have been the main reason for the failure of diagnoses. Another possibility is that the cases were due to unknown microorganisms.

Recently, attention has been paid to so-called "pathogenic halophilic bacteria" as a possible cause of so-far undiagnosed food poisoning.

Historical Review of the Pathogenic Halophiles (1)

An outbreak of gastroenteritis took place at a hospital in Yokohama on August 20, 1955 affecting 120 persons who had eaten salted cucumbers. Examination did not disclose any bacteria known to cause food poisoning, while unfamiliar bacteria were recovered in high ratio on the agar plates containing 5% NaCl. Positive agglutination reactions between the serum specimens from the patients and the heat-killed organisms were observed. Further, it was demonstrated that the bacteria produced symptoms when fed to human volunteers.

In 1959, outbreaks of food poisoning with symptoms of acute gastro-

TABLE I

OUTBREAKS OF FOOD POISONING IN RESPONSE TO A VARIETY OF FOODSTUFFS IN THE PERIOD 1958–1960[a]

Incriminated foodstuffs	1958			1959			1960		
	Outbreaks	Patients	Deaths	Outbreaks	Patients	Deaths	Outbreaks	Patients	Deaths
Fish and shellfish, total	778	7280	217	1142	12460	190	850	11320	147
Shellfish	70	1201	3	130	1242	15	90	1086	8
Globefish	187	289	176	132	211	118	108	155	95
Others	521	5790	38	880	11007	57	652	10081	44
Fish products, total	225	4923	23	234	2967	27	177	4317	12
Fish cakes	123	3043	11	133	1979	12	88	3255	6
Others	102	1830	12	101	988	15	89	1062	6
Meat and meat products	49	1444	2	45	3537	2	24	226	0
Egg and egg products	12	285	1	14	206	0	24	605	0
Milk and dairy products	12	541	0	12	486	1	12	905	0
Cereals	81	1279	4	81	2075	7	66	1968	7
Vegetables and beans	160	2256	24	200	2806	17	129	3472	7
Miscellaneous	240	5129	29	227	7640	17	204	6060	5
Unidentified	354	7919	34	513	7722	57	391	8084	37
Total	1911	31056	332	2468	39899	318	1887	37253	218

[a] Figures cited were based on the data collected by the Food Sanitation Section, Ministry of Health and Welfare.

enteritis from eating raw horse-mackerel and other fish took place in Shizuoka Prefecture.

Halophilic bacteria having similar properties were occasionally isolated from feces of the patients.

Clinical Features

Symptoms noted were abdominal pain at the upper part, watery diarrhea for ten times or more, and vomiting, with or without fever (maximum of 38°C) or headache. Dryness of skin from repeated diarrhea, collapse, and lowered blood pressure were occasionally observed in severe cases. The patients usually recovered in 4 to 5 days. No infection from man to man has been reported. The mortality rate is very low. The post-mortem examination of the victims in the Osaka outbreak (*2*) showed slight ulceration of the jejunum and ileum, catarrhal gastritis, loss of blood plasma, and congestion of some of the organs.

Epidemiology (*3*)

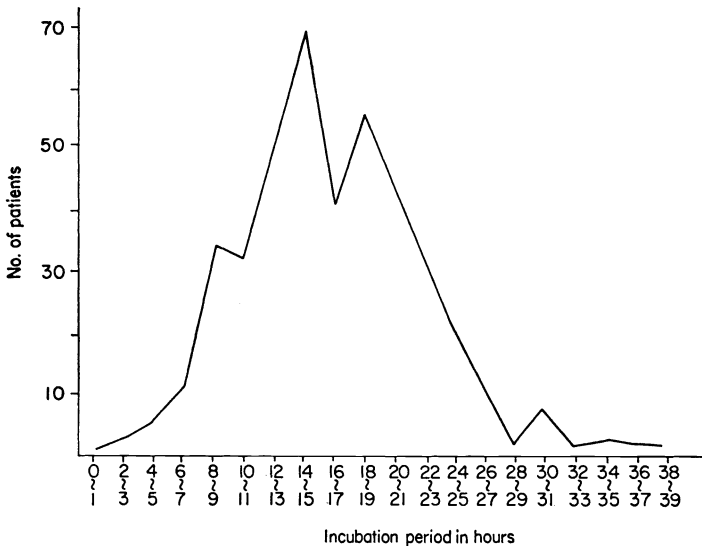(1) Implicated foodstuffs: This kind of food poisoning has principally

FIG. 1. Distribution of incubation periods observed in the food poisoning caused by so-called "pathogenic halophilic bacteria" in 1960. Figures were based on 28 outbreaks of food poisoning with 437 patients. The average incubation period was 17 hours.

been due to consumption of raw horse-mackerel, squid, and other fish. Contamination of cooked food is also implicated in some outbreaks.

(2) Incubation period: The majority of 437 cases in 28 outbreaks in 1960 showed the incubation periods of 14–20 hours as shown in Figure 1.

(3) Morbidity: No difference in morbidity can be seen between sexes. A larger number of cases was counted in adults than in children, which may be due to the difference in consumption of raw fish.

(4) Seasonal variation: The outbreaks of poisoning took place mainly from June to October and especially in September.

Characteristics of the Pathogenic Halophiles

(1) The organisms are facultative anaerobic, gram-negative, pleomorphic rods with a single flagella. The optimal temperature for growth is 37°C. They grow vigorously at the presence of 2–3% NaCl with a generation time of 10–15 minutes.

(2) General biological characteristics are shown in Table II.

TABLE II

BIOLOGICAL PROPERTIES OF SO-CALLED PATHOGENIC HALOPHILIC BACTERIA

Gram staining	−	Citrate	+
Mortility	+	Liquefaction of gelatine	+
Pigment	−	Coagulation and liquefaction of milk	+
Fluorescence	−	Reduction of methylene blue	+
Gas production	−	Ammonia formation from peptone	+
H_2S production	−	Cytochrome oxidase test	+
Urea utilization	−	Voges-Proskauer test (V. P. test)	+ or −
Catalase	+	Methyl red reaction 37° C	+
Indole production	+	22° C	−
Nitrate to nitrite	+		
Hemolysis	+		

Optimum temperature for growth	37° C
Optimum growing pH	7.5–8.0
Optimum NaCl concentration for growth	2–3%

(3) The carbohydrates fermented by the organisms with formation of acids are glucose, fructose, galactose, mannose, maltose, starch, dextrin, glycogen, and mannitol.

(4) Carboyhdrates irregularly fermented are arabinose, sucrose, glycerine, dulcitol, and sorbitol.

(5) Most of the organisms from the feces of patients fail to ferment sucrose, while those from sea water or fish do ferment it (Table II) (4). Sucrose-negative organisms do not produce acetylmethylcarbinol, while

TABLE III

SUCROSE FERMENTATION BY SO-CALLED PATHOGENIC HALOPHILES FOUND IN THE
FECES OF PATIENTS, SEA WATER, OR FISH SAMPLES[a]

| | Sucrose fermentation | | |
Origin	+	−	Total
Fish or sea water	292	72	364
	(80.2%)	(19.8%)	
Patients	61	1,077	1,138
	(5.4%)	(94.6%)	
Total	353	1,147	1,502

[a] All the strains tested were presented to the Pathogenic Halophilic Bacteria Center
of the National Institute of Health, Tokyo, in 1960.

90% of sucrose-fermenters produce it. From these properties, the follow-
ing four biotypes have been classified (3).

Biotype 1. Sucrose nonfermentative, V.P. test negative.
Biotype 2. Sucrose nonfermentative, V.P. test positive
Biotype 3. Sucrose fermentative,   V.P. test positive
Biotype 4. Sucrose fermentative,   V.P. test negative

The question whether biotypes 2, 3, and 4 are responsible for human
food poisoning or should be excluded from "pathogenic halophiles" re-
mains to be solved.

(6) Serological properties: Takigawa (5) set up 26 serotypes with the
serums immunized against living organisms. More recently, using agglu-
tination techniques, 12 serotypes have been established (4) (Table IV).
The commonly responsible O-serum types have been O-2, O-3, O-4, O-5, and
O-9 (Table V) (4).

Taxonomical Position

Takigawa (1) named the prototype of this group *Pseudomonas enteritis*.
In this group he included *Pasteurella parahemolytica*, isolated by Fujino
(2) from "shirasu-boshi," (boiled semidried sardine larva), which was
responsible for the Osaka outbreak in 1955. Miyamoto (6) claimed
Aeromonas as the classification of this group of organisms from their
fermentative properties. Recently, Aiso *et al.* (7) and other workers (8)
examined the sensitivity of these organisms to the vibrio static agent
(2,4-diamino-6,7-diisopropyl pteridine). Definite genus name has not been
given as yet.

TABLE IV

COMPARISON OF SEROTYPES PROPOSED BY TAKIGAWA (5) AND THOSE RECENTLY PROPOSED[a]

O-group	Takigawa's group
1	1, 14
2	2, 20
3	3, 21
4	4, 6, 17, 19, 22, 26
5	5, 13, 18
6	8
7	11, 12[b]
8	25
9	—[c]
10[d]	7[b]
11[d]	9[b], 23[b]
12[d]	24[b]

[a] Shokuhin Eisei Kenkyu 12: 75–93 (1962).
[b] Sucrose fermenter
[c] No corresponding serotype existed
[d] Tentative O-groups.

Distribution in Nature

From the beginning of investigation (9), these organisms have been suspected of being marine bacteria from their halophilic property. The distribution of these organisms were studied by Miyamoto (10, 11, 12), Shimizu (13), Wagatsuma (14), and Horie (15). Although it has suggested that these organisms may not be pelagic but rather coastal, the point will have to be investigated further. A survey for these organisms carried out at the Tokyo Central Fish Market in 1961 detected in fish several typical cultures with O-agglutinogen identical to known types (16). The Ministry of Health and Welfare is planning a nationwide survey this summer.

Problems in the Future

A new type of bacteria, so-called pathogenic halophilic bacteria, suspected to cause food poisoning was described. It has been only a short period since the investigation started and there are still many problems to be solved. Some of the important problems are: (1) Pathogenicity of the organisms is still obscure due to the unavailability of susceptible experimental animals. No infection of experimental animals has been successful, except a single positive feeding test to a monkey by Aiso et al. (13). (2) Distribution and the route of contamination by these organisms.

TABLE V

SEROTYPES OF SO-CALLED PATHOGENIC HALOPHILIC BACTERIA COLLECTED IN 1961[a][b]

| O-group | Sucrose non-fermentative | | Sucrose fermentative | Total no. of strains |
	No. of strains (%)	No. of incidents (%)		
1	8 (0.7)	6 (2.0)		8
2	150 (13.9)	37 (12.4)	2 (0.9)	152
3	181 (16.8)	46 (15.4)	5 (2.3)	186
4	351 (32.7)	81 (27.2)	17 (7.7)	368
5	111 (10.3)	45 (15.1)	18 (8.2)	129
6	1 (0.1)	1 (0.3)	—	1
7	20 (2.8)	8 (2.6)	8 (3.6)	28
8	51 (4.7)	23 (7.7)	7 (3.2)	58
9	202 (18.8)	39 (13.1)	5 (2.3)	207
10[c]	—	—	3 (1.4)	3
11[c]	—	—	1 (0.5)	1
12[c]	—	—	30 (13.6)	30
Unknown	12 (1.1)	8 (2.6)	107 (48.8)	119
Unidentified	3 (0.2)	3 (1.0)	—	3
	1,073	297	220	1,293

[a] Shokuhin Eisei Kenkyu 12: 75–93 (1962).

[b] Organisms tested were presented to the Pathogenic Halophilic Bacteria Center of the National Institute of Health, Tokyo, in 1961.

[c] Tentative.

(3) The role in human food poisoning development of many heterologous microorganisms having similar properties detectable in sea water or fish body. Method suitable for differentiation should be established. (4) Predilection for pathogenic halophiles by health officers. Precaution should be taken to rule out any other causative agents in examining food poisoning. Especially, the test for *Clostridium perfringens* should be performed, since the syndrome produced by *C. perfringens* and that by so-called pathogenic halophiles are similar. (5) Prevention of fish poisoning presumably due to pathogenic halophiles. This is an important problem for the Japanese whose major protein source is fish.

REFERENCES

1. I. Takigawa. Studies on pathogenic halophilic bacteria. *Yokohama Med. Bull.* 2, 313–322 (1958).
2. T. Fujino. On the bacteriological examination of "shirasu" food poisoning. *Med. J. Osaka Univ.*, 4, 299–304 (1953).
3. Examination guide for the food poisoning caused by pathogenic halophilic

bacteria. Revised Ed. Issued by the Ministry of Health and Welfare (1962).

4. Report on the research for so-called "pathogenic halophilic bacteria" in 1961, by the Special Research Committee for the Investigation of "Pathogenic Halophilic Bacteria" of the Ministry of Health and Welfare. *Shokuhin Eisei Kenkyu* (text in Japanese) 12, 75–83 (1962).

5. I. Takigawa. On the serotypes of pathogenic halophilic bacteria, *Pseudononas enteritis*. A paper presented at the 3rd Annual Meeting of *Japan Assoc. Infect. Diseases*, March 6 (1959).

6. Y. Miyamoto. Food poisoning caused by pathogenic halophilic bacteria. *J. Food Hyg. Soc. Japan* (text in Japanese) 2, 29–39 (1961).

7. K. Aiso, K. Fujiwara, U. Shimizu, H. Kato, and K. Tatsumi. Taxonomical studies on pathogenic halophilic bacteria. A paper presented at the 15th Meeting of Kanto Branch, *Japan Soc. Bacteriol.* June 16 (1962).

8. R. Sakazaki, Y. Murata, and H. Fukumi. Studies on the so-called pathogenic halophilic bacteria, I. A paper presented at the 15th Meeting of Kanto Branch, *Japan Soc. Bacteriol.* June 16 (1962).

9. I. Takigawa and T. Fujisawa. An outbreak of food poisoning presumably caused by a marine bacteria. *Shokuhin Eisei Kenkyu* (text in Japanese) 6, 15–19 (1956).

10. Y. Miyamoto, K. Nakamura, K. Takizawa, and T. Kodama. Carangina fish poisoning, I. *Japan J. Public Health* (text in Japanese) 7, 587–592 (1960).

11. Y. Miyamoto, K. Nakamura, K. Takizawa, and T. Kodama. Mackerel (carangidae) poisoning, II. *Japan J. Public Health* (text in Japanese) 8, 673–678 (1961).

12. Y. Miyamoto, K. Nakamura, K. Takizawa, and T. Kodama. Poisoning caused by mackerel (carangidae), III. *Japan J. Public Health* (text in Japanese) 8, 703–707 (1961).

13. U. Shimizu and H. Matsuno. Distribution of pathogenic halophilic bacteria in the coastal sea water and fish caught in Chiba Prefecture. A paper presented at the 14th Meeting of Kanto Branch, *Japan Soc. Bacteriol.* June 23 (1961).

14. S. Wagatsuma. Epidemiology on the pathogenic halophilic bacteria. *Modern Media* (text in Japanese) 8, 236–240 (1962).

15. S. Horie and K. Saheki. Personal communication (1961).

16. Y. Mochinaga. Personal communication (1961).

The Problem of Type E Botulism in Japan

T. KAWABATA AND G. SAKAGUCHI

Department of Food Control, National Institute of Health,
Shinagawa-ku, Tokyo, Japan

Incidence of Botulism in Japan

No outbreak of botulism had been reported in Japan before May 1951, when a large-scale outbreak of food poisoning involving 14 cases and 4 deaths occurred at Iwanai, Hokkaido Island, from eating herring-"izushi." It was found that the outbreak was due to type E botulinus toxin (1). Features of this and other outbreaks which took place during the period from 1951 to 1960 are summarized in Table I.

All of the outbreaks in Japan implicated type E toxin and the responsible food was "izushi" with a single exception. "Izushi" is a preserved food popularly made at home in the northern part of Japan. Raw fish, vegetables, cooked rice, malted rice ("koji"), and a small amount of salt and vinegar are packed tightly in a wooden tub with a lid. It is kept for 3 weeks or longer to bring about lactic fermentation.

Why "Izushi" Is Exclusively Implicated in Type E Botulism in Japan

"Izushi" provides an excellent anaerobic condition in the tub. The natural habitat of *Clostridium botulinum* type E is regarded as marine rather than terrestrial, and fish serves as a vehicle for type E spores (2). Although the heat resistance of type E spores is much lower than that of type A or B spores, the fish in "izushi" never has a chance to be cooked in the processes of preparation or before serving. The occasional outbreaks of type E botulism in the northern part of Japan may be attributable to the side dissemination of type E spores in this area (1, 2) and the traditional predilection for "izushi" by the people in this area.

It has been pointed out by many workers that lethality of mice for the

TABLE I

OUTBREAKS OF BOTULISM IN JAPAN IN THE PERIOD 1951–1960

No.	Date	Place	Incriminated foodstuffs	Patients	Deaths	Mortality rate (%)
1	May 1951	Iwanai, Hokkaido Is.	Herring "izushi"	14	4	29
2	Nov. 1952	Okoppe, Hokkaido Is.	Flat-fish "izushi"	4	0	0
3	Dec. 1952	Memanbetsu, Hokkaido Is.	Herring "izushi"	5	2	40
4	Oct. 1953	Saroma, Hokkaido Is.	Flat-fish "izushi"	5	1	20
5	Oct. 1953	Tenno-machi, Akita	"Kawa-dai izushi"	4	2	50
6	Aug. 1954	Abashiri, Hokkaido Is.	Flat-fish "izushi"	5	1	20
7	Dec. 1954	Onbetsu, Hokkaido Is.	"Hata-hata izushi"	2	1	50
8	May 1955	Futto-mura, Akita	Flat-fish "izushi"	6	5	83
9	Jul. 1955	Kitami, Hokkaido Is.	Salmon eggs	2	1	50
10	Sep. 1955	Aomori, Aomori Pref.	Saury "izushi"	3	3	100
11	Oct. 1955	Otaru, Hokkaido Is.	Mackerel "izushi"	5	1	20
12	Oct. 1955	Kamiyama, Hokkaido Is.	Saury "izushi"	3	3	100
13	Jul. 1956	Takahata, Yamagata	Canned mackerel	3	3	100

14	Sep. 1956	Kushiro, Hokkaido Is.	Trout "izushi"	12	3	25
15	Oct. 1956	Zenikamesawa, Hokkaido Is.	"Hata-hata izushi"	11	4	36
16	Oct. 1956	Wakkanai, Hokkaido Is.	Flat-fish "izushi"	5	2	40
17	Oct. 1956	Takaiyama, Hokkaido Is.	Horse-mackerel "izushi"	4	3	75
18	Oct. 1956	Aomori, Aomori Pref.	Flat-fish "izushi"	1	0	0
19	Oct. 1956	Nozawa-mura, Aomori Pref.	Horse-mackerel "izushi"	2	1	50
20	Mar. 1957	Bekkai, Hokkaido Is.	"Hata-hata izushi"	4	0	0
21	Sep. 1957	Wakkanai, Hokkaido Is.	Flat-fish "izushi"	6	4	67
22	Oct. 1957	Kotohama, Akita Pref.	"Itoyo izushi"	9	0	0
23	Nov. 1957	Esashi, Hokkaido Is.	Flat-fish "izushi"	1	1	100
24	Nov. 1957	Mashike, Hokkaido Is.	"Hata-hata izushi"	35	9	26
25	Jun. 1958	Funagawa, Akita Pref.	Sardine "izushi"	5	1	20
26	Sep. 1958	Akita, Akita Pref.	"Goze izushi"	3	1	33
27	Oct. 1959	Oami, Akita Pref.	Carp "izushi"	2	2	100
28	Nov. 1959	Kochi-mura, Akita	"Hata-hata izushi"	1	0	0
29	Jan. 1960	Sapporo, Hokkaido Is.	"Hata-hata izushi"	4	0	0
			Total	166	58	35(%)

cultures of *Clostridium botulinum* type E is generally very low as com-
pared with those of the other types. The high incidence and mortality rate
of type E botulism in this country with such low toxigenic type E organ-
isms were well explained by a phenomenon of "activation" of type E
toxin by contaminating putrefactive organisms in "izushi" (*3*).

Activation of Type E Botulinus Toxin

Sakaguchi and Tohyama (*3*) postulated that "activation," a phenome-
non by which parenteral lethality of a type E culture is enhanced 100
times or more by a proteolytic enzyme, may play an important role in
pathogenesis of human type E botulism. The precursor of type E toxin
or protoxin was characterized by Sakaguchi *et al.* (*4*). Duff *et al.* (*5*)
reported activation of type E toxin by trypsin. Dolman (*2*) ascribed an
unusually highly potent type E toxin to activation by the proteolytic
enzyme of a proteolytic mutant strain.

The fact that trypsin can activate type E toxin implies that activation
may take place not only before consumption of food but also after oral
ingestion. The *in vivo* activation was demonstrated in the stomach con-
tents of human beings (*2*) and also in mice (*5*). Activation requires an
optimal pH range of 5.5–5.6 (*3, 5*), which is significantly lower than the
optimal pH for trypsin activity. It has been shown that neither chymo-
trypsin, papain, nor pepsin can activate type E toxin. Trypsin is an effec-
tive activator of type E precursor and it acts also as a destructor of
botulinus toxin (*6*). Although trypsin has been shown to destroy type E
toxin very rapidly at pH 7.5, there was no indication of destruction by
trypsin at pH 6.0 in a few hours (*7*).

Control of Type E Botulism

No doubt general principles for controlling food poisoning, especially
low temperature control measures from initial catch of fish to final con-
sumption, should be applicable to type E, "izushi"-borne, botulism. How-
ever, it is anticipated that complete eradication of botulism caused by
eating "izushi" made of raw fish and eaten raw would not be accomplished
unless the habit of eating "izushi" is completely inhibited by providing
an alternate method for preserving fish for food in the winter season in
the northern part of Japan.

Prevention of growth and toxin formation by *Clostridium botulinum* in
fish by use of antibiotics was tested by Boyd *et al.* (*8*) and Sakaguchi
et al. (*9*). It was found by the latter authors that 0.2 ppm of oxytetracy-
cline inhibited germination and outgrowth of type E spores *in vitro* and
treatment of fresh flounders by dipping in CTC or OTC solution inhibited

formation of toxin and the precursor by the injected intramuscularly spores. Whether delicacy of "izushi" is brought about of the fish treated with tetracycline will have to be examined before the method is recommended.

According to Iida of Hokkaido Institute of Public Health (10), a type E antitoxic horse serum made available through the courtesy of Dr. C. E. Dolman of University of British Columbia showed remarkable therapeutic effect when injected into patients manifesting even considerably severe symptoms. In Japan, production of type E antitoxic horse immune serum has been carried out since 1961. Highly purified type E toxin, obtained through activation by trypsin of the precursor extract of type E cells followed by repeated salting out with ammonium sulfate, was used as the immunogen (11). The serum prepared was already administered to the patients of an outbreak which occurred in June, 1962 and its effectiveness was proven. Further investigation on the effectiveness is expected to be carried out with experimental animals.

Conclusion

The history of botulism is very long and research in botulism has been done for many years. During the early 1920's, extensive investigation was carried out to solve the problems of the canning industry in the United States. During World War II, much attention was given toward purification of the toxins and large-scale manufacturing methods of toxoids. There are still many problems left from the standpoints of microbiology, chemistry, and physiology. No satisfactory chemical basis has been given for the specificity and extraordinary toxicity of the botulinus toxins.

In the work done, attention has been principally paid to type A toxin by the workers in the United States for the reason that this type of botulism has been predominant in that country and that *Clostridium botulinum* type A produces large amounts of toxin in culture media. In Japan, however, type E outbreaks have been exclusive. The incidence and mortality rates are high in Japan. Unfortunately, *Clostridium botulinum* type E is a poor producer of toxin and more research is needed to bring the level of information about type E toxin to that of type A toxin.

Studies on botulism in Japan have been carried out since 1951. One of the important contributions is the finding of enhancement of lethal toxicity of type E toxin by certain proteolytic enzymes, which is now known as "activation." The mechanisms involved in "activation" are of great interest and importance, and deserve to be under such intensive investigation in Japan. In the pathogenesis of type E botulism, the fate of botulinus toxin in food-stuffs before and after consumption is also an important problem to be studied. To accomplish this task, such fundamental prob-

lems as mechanisms of formation of toxin, influence of digestive enzymes upon the precursor and the toxin, absorption of toxin from the intestinal wall etc., should first be investigated.

REFERENCES

1. Y. Nakamura, H. Iida, K. Saeki, K. Kanzawa, and T. Karashimada. Type E botulism in Hokkaido, Japan. *Japan J. Med. Sci. Biol.* 9, 45–58 (1956).
2. C. E. Dolman. Type E (fish-borne) botulism, *Japan J. Med. Sci. Biol.* 10, 383–395 (1957).
3. G. Sakaguchi and Y. Tohyama. Studies on the toxin production of *Clostridium botulinum* type E. I & II. *Japan J. Med. Sci. Biol.* 8, 247–262 (1955).
4. G. Sakaguchi, and S. Sakaguchi. Studies on toxin production of *Clostridium botulinum* type E. III Characterization of toxin precursor. *J. Bacteriol.* 78, 1–9 (1959).
5. J. T. Duff, G. G. Wright, and A. Yarinsky. Activation of *Clostridium botulinum* type E toxin by trypsin. *J. Bacteriol.* 72, 455–460 (1956).
6. E. A. Meyer and C. Lamanna. Activity of type A botulinal toxin and hemagglutinin exposed to proteolytic enzyme. *J. Bacteriol.* 78, 175–180 (1959).
7. G. Sakaguchi and S. Sakaguchi. Unpublished.
8. J. W. Boyd, H. M. Bluhn, C. R. Muirhead, and H. L. A. Tarr. Use of antibiotic for the preservation of fish and sea foods. *Am. J. Public Health* 46, 1531–1539 (1956).
9. G. Sakaguchi, S. Sakaguchi, T. Kawabata, Y. Nakamura, T. Akano, and K. Shiromizu. Influence of oxytetracycline upon the toxin production of type E. *Cl. botulinum. Japan J. Med. Sci. Biol.* 13, 13–22 (1960).
10. H. Iida. Personal communication (1962).
11. G. Sakaguchi and S. Sakaguchi. A simple method for purification of type E botulinal toxin from the precursor extract of the bacterial cells. *Japan J. Med. Sci. Biol.* 14, 243–248 (1961).

Clostridium perfringens Food Poisoning

Louis DS. Smith

Montana State College, Bozeman, Montana

Food poisoning caused by *Clostridium perfringens* was first described by Klein in 1895, (1) and again very clearly by McClung in 1945, but it did not really attract the attention that it deserved until the report in 1953 of Dr. Hobbs and her colleagues (2). They described, in an already classical piece of work, a number of outbreaks occurring in Great Britain, the symptoms of the patients, the types of food involved, and the characteristics of the bacteria responsible.

Today we know that this type of food poisoning is caused by the ingestion of several hundred million living cells of *C. perfringens* (3); that there is an incubation period of 8 to 20 hours; that the symptoms are those of an acute abdominal upset characterized by nausea, marked cramps, and diarrhea; and that vomiting and fever are uncommon but sometimes occur. Fatalities are rarely encountered and no immunity seems to result.

Although strains producing heat-resistant spores and not producing the hemolytic theta toxin are often involved, this is by no means always the case. Angelotti and his co-workers (4) have shown that many strains involved in outbreaks of food poisoning in the United States did not produce heat-resistant spores but did produce theta toxin. It appears very likely that the heat-resistance of the spores of a strain involved in any particular outbreak may be related to whether the food was contaminated before or after cooking. If contamination occurred before cooking, the spores would almost necessarily be heat resistant. If contamination occurred after cooking the spores would probably not be heat resistant, for heat-susceptible strains greatly outnumber heat-resistant strains and there would be no heating to eliminate them.

It is not certain whether any of the known toxins of *C. perfringens* are involved in food poisoning. The hemolytic theta toxin is not concerned, but as the late Dr. MacLennan pointed out, the lecithin-splitting alpha toxins may be involved. Indeed, the work of Nygren (9) in Sweden indi-

cates that phosphoryl choline, one of the split products of lecithin, may be the chemical agent responsible for *Bacillus cereus* food poisoning, and also that the phosphoryl choline is produced by the action of *B. cereus* lecithinase upon lecithin in the food. If this were the case, we would expect that very similar types of food poisoning would be brought about by *Bacillus cereus, Clostridium perfringens*, and the *Pseudomonas* species, for all of these produce phosphoryl choline as the result of the splitting of lecithin by their lecithinolytic enzymes. We would also expect, if this hypothesis were true, that some item of food containing an appreciable concentration of lecithin would be involved in every attack of this type of food poisoning.

So far as the factors influencing the growth of *C. perfringens* in food are concerned, our information is less detailed. There does not seem to be any way to keep this organism out of food, for it is widespread in nature. Its numbers in soil are considerable, but vary a good deal from one specimen of soil to another, as the data in Table I show. These data were obtained by the Most Probable Number method and represent a minimum rather than a true estimate of the number of *C. perfringens* cells per gram.

TABLE I

Clostridium perfringens IN SOIL[a]

Soil	C. perfringens per gram
Clay loam	1,000
Black loam	56,700
Sandy garden loam	1,700
Clay garden soil	110
Swamp muck	1,500
Sandy soil	1,200
Soil under turf	1,200

[a] From Smith and Gardner (6).

It is also found in spices and condiments as well as other types of food. We investigated, as part of a search for *C. perfringens* in foodstuffs, several different kinds of pepper, as an example of a widely used condiment that could possibly serve to contaminate food otherwise free of *C. perfringens*. Table II shows the data from this investigation.

It is evident even from this small amount of information on the occurrence of *C. perfringens* that we cannot, as a practical measure, keep *C. perfringens* out of our food. Further evidence, if any is needed, is available from the report of Elizabeth McKillop (5), who studied foods in a

TABLE II

Clostridium perfringens IN PEPPER

Type	C. perfringens per gram
Whole peppercorns (ground)	6
Cayenne pepper	2
White pepper	4
Black pepper, pulverized	4
Chili pepper	12
Paprika	4
Whole red pepper (ground)	0

hospital kitchen in Great Britain. Table III shows the occurrence of *C. perfringens* in some of the items of uncooked food that she examined. (The material in Tables III and IV was selected from that listed in her paper and does not represent the entire array of foods that she investigated.) It can be seen that many items of uncooked food were

TABLE III

Clostridium perfringens IN UNCOOKED FOOD[a]

Food	Number of samples	Number with C. perfringens	Percent with C. perfringens
Fish	18	11	61
Steak and mince	10	6	60
Tripe	6	2	33
Sausage	38	38	100
Chicken	7	7	100
Liver	2	1	50

[a] From McKillop (5).

TABLE IV

Clostridium perfringens IN COOKED FOOD[a]

Food	Number of samples	Number with C. perfringens	Percent with C. perfringens
Fish	6	1	17
Steak and mince	15	2	13
Tripe	6	1	17
Sausage	25	5	20
Chicken	46	24	52
Cold roast meats	15	6	40

[a] From McKillop (5).

contaminated with *C. perfringens*, especially sausage and chicken. Cooking, however, apparently served to reduce the numbers of *C. perfringens*, as the data in Table IV show. Here the numbers are much lower and the ratio of items from which this organism was isolated was reduced from about two-thirds of the uncooked foods to about one-fourth of the cooked foods.

Obviously, the mere presence of *C. perfringens* in food is not enough to induce food poisoning if the critical dose is several hundred million organisms, or five to ten million organisms per gram. Mere contamination cannot possibly account for such numbers, so it is obvious that we must be concerned with the growth of *C. perfringens* in food. This line of reasoning led us to consider some of the factors that influence the growth of this organism; the amino acid requirements; the growth factor requirements; the temperature; and the hydrogen ion concentration. All of these must be suitable or the organism cannot multiply.

The data in Table V indicate the amino acids required by *C. per-*

TABLE V

Amino Acids Required by *Clostridium perfringens*

	From Boyd et al. (7)	From Fuchs & Bonde (8)		From Boyd et al. (7)	From Fuchs & Bonde (8)
Arginine	+	+	Histidine	+	+
Leucine	+	+	Cystine	+	+
Isoleucine	+	+	Glutamic acid	+	+
Methionine	+	+	Serine	+	−
Phenylalanine	+	+	Tyrosine	+	+
Threonine	+	+	Alanine	−	+
Valine	+	+	Aspartic acid	−	+
Tryptophane	+	+			

fringens. Although there may be some variation from strain to strain with regard to serine, alanine, and aspartic acid, it is evident the thirteen or fourteen amino acids are required. The growth factors required are shown in Table VI. Here the requirement is not so complex, but five or six growth factors, including biotin, pantothenate, pyridoxal, nicotinamide, and adenine are necessary.

We used five strains of *C. perfringens* to study the effect of temperature on the rapidity of growth. None of these strains would grow appreciably at 15°C, nor did any of them grow at 55°C, but they did grow well over a range of 30 degrees, from 20 to 50°C. Figure 1 shows the relation between

TABLE VI

GROWTH FACTORS REQUIRED BY *Clostridium perfringens*

	From Boyd, et al. (7)	From Fuchs & Bonde (8)
Biotin	+	+
Pantothenate	+	+
Pyridoxal	+	+
Riboflavine	+	−
Adenine	+	+
Uracil	+	−
Nicotinamide	−	+
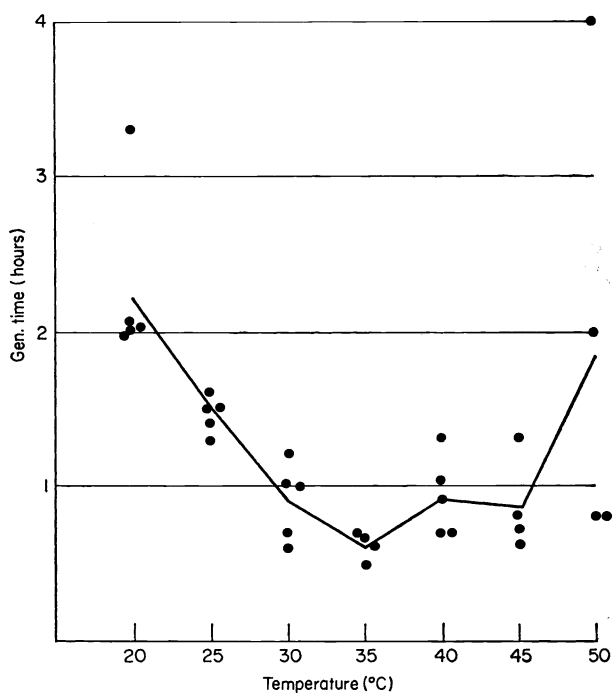
FIG. 1. Growth of *C. perfringens* at different temperatures.

generation time—the time required for the number of bacteria per unit volume to double—and the temperature. Values for individual strains are represented by dots. The mean value for the four strains is represented by the line. Growth is maximal over a range of about fifteen degrees—from 30° to about 45°C. Above this temperature, the generation time increased sharply for two strains but remained about the same for two strains.

I would like to emphasize, however, that even these two strains were unable to grow appreciably at 55°C.

We also studied the effect of pH on generation time. Figure 2 shows the
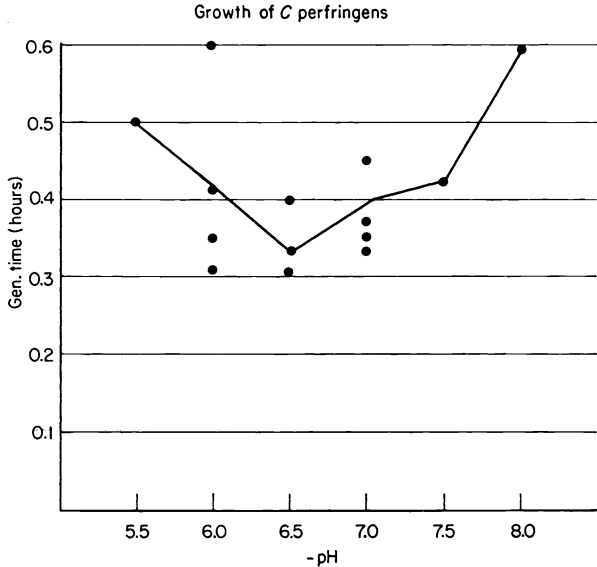
FIG. 2.   Growth of *C. perfringens* at different pH.

relation between pH and generation time in hours for four of the strains used before. No growth was evident at pH 5, nor at pH 8.5. From pH 5.5 to pH 8.0, however, fairly rapid growth occurred. As in the preceding figure, points represent observations made on individual strains and the line represents mean values. It can be seen that the rate of growth varied but little from pH 6.0 to pH 7.5, with no sharp optimum.

The shortest generation times obtained in these trials, which were all carried out in the usual bacteriological media, were in the neighborhood of $\frac{3}{10}$ hour or about 18 minutes. We were also interested in how rapidly *C. perfringens* could grow in food. Four kinds of commercially canned soup were inoculated with *C. perfringens* and incubated at 45°C to determine generation time. Table VII illustrates the results. Almost as rapid growth occurred in these foods, for the generation times ranged from $\frac{4}{10}$ hour, or 24 minutes, for chicken-rice soup through 25 minutes for beef broth and 29 minutes for beef-noodle soup, to 32 minutes for turkey-noodle soup.

In summary, we may expect to find *C. perfringens* in almost all food. However, we may expect *C. perfringens* food poisoning only from certain

TABLE VII

GROWTH OF *Clostridium perfringens* IN FOOD

Food	Generation time (hours)
Chicken rice soup	0.40
Beef noodle soup	0.48
Turkey noodle soup	0.53
Beef broth	0.42

dishes—those neutral in pH, containing an ample supply of amino acids and growth factors, that have been kept for some hours between 20° and 50°C.

Avoidance of *C. perfringens* food poisoning obviously can be attained by not exposing food to these conditions, or possibly by heating such items of food to boiling immediately before serving to reduce the number of vegetative cells below the apparently critical level of several million per gram.

REFERENCES

1. E. Klein. *Zbl. Bakt.* (1, Abt. Orig.), 18, 737 (1895).
2. B. C. Hobbs, M. E. Smith, C. L. Oakley, G. H. Warrack, and J. C. Cruick-shank. *J. Hyg.* 51, 75 (1953).
3. F. E. Dische and S. D. Elek. *Lancet* 2, 71 (1957).
4. H. E. Hall and R. A. Angelotti. *Bacteriol. Proc.* 22, (1962).
5. E. J. McKillop. *J. Hyg.* 57, 30 (1959).
6. L. DS. Smith and M. V. Gardner. *J. Bacteriol.* 58, 407 (1949).
7. M. J. Boyd, M. A. Logan, and A. A. Tytell. *J. Biol. Chem.* 174, 1027 (1948).
8. A. R. Fuchs and G. J. Bonde. *J. Gen. Microbiol.* 16, 317 (1957).
9. B. Nygren. *Acta Pathol. Microbiol. Scand. Suppl.* 144, 297 (1961).

The Role of Salmonellae

in Foodborne Diseases

E. H. KAMPELMACHER

National Institute of Public Health, Utrecht, Netherlands

It is general knowledge today that the vast majority of cases of human food poisoning are caused by types of the genus *Salmonella* and by staphylococci. The latter pathogens seem to be of great importance in the United States, while in postwar Europe it is chiefly *Salmonella* infections that constitute a major public health problem. The reasons for these differences, which in my opinion are mainly to be ascribed to eating habits and mode of storage, will be discussed later.

It would be possible to give a general impression of the role played by *Salmonella* germs in foodborne infections, in which respect we would chiefly deal with *Salmonella* germs in poultry and poultry products, meat and meat products, and possible secondary contamination of other foodstuffs. In the presence of specialists in this field, however it seems more appropriate to give an account of the current situation in my country. First, because we had the opportunity in recent years to discover a causal relation between the occurrence of salmonellosis and certain foodstuffs, and to make an exhaustive investigation into this relation. Second, because many of the generally important factors in this infection are bound to crop up in such a specific discussion.

In the past 10 years, human *Salmonella* infections have shown a distinct increase in the Netherlands. This increase, demonstrated in Figure 1 both as to number of infections and as to number of types, is determined by two factors, to wit: the absolute and the relative increase.

As to the latter, an explanation may lie in the fact that this disease is notifiable, that the interest has increased, as has the amount of research, and particularly that, unlike other countries, the Netherlands National *Salmonella* Centre receives for typing virtually all *Salmonella* strains isolated from man and animals, from foodstuffs and animal feeds
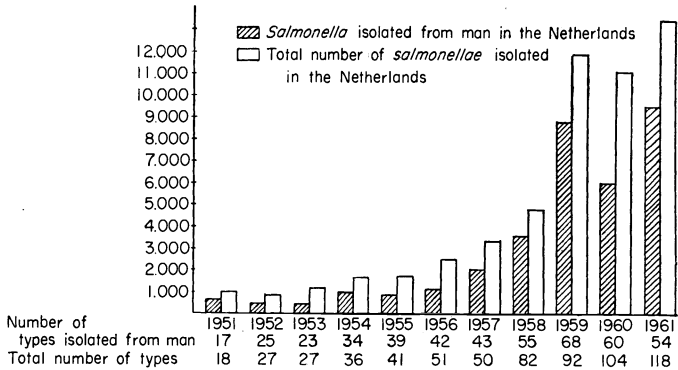
FIG. 1. A comparison of types of *Salmonella* isolated from man and other organisms in the Netherlands during the period 1951–1961.

from sewage and surface water. This ensures adequate registration. The importance of the factor of absolute increase is shown by the pronounced increase in the annual number of cases of illness in the past few years; other factors than merely those of increased investigation and good registration are responsible for this. In Table I, 2 years show peaks in the ascending general line; these years are 1959 and 1961. The high values in these years are determined by an epidemic during the summer months of 1959, and another epidemic during the autumn months of 1961. In both seasons, outdoor temperatures were above normal for some considerable time; in 1960, however, outdoor temperatures remained below the normal average during both the summer and the autumn months. The fact that a large number of cases of infection was nevertheless registered in 1960, indicates that high outdoor temperatures may be one of the factors in the etiology of the infections but that other factors also play an important role. A number of these factors will be discussed later. Figure 1 also shows, in addition to the increase in the number of infections, an increase in the annual number of *Salmonella* types found in the Netherlands. Yet Table I shows that, in the past few years, about 80% of all human infections have been caused by only six types, slightly different each year; *Salmonella typhimurium* invariably ranks first among these types. Some years show a distinct predominance of certain types, which caused specific epidemics. For example, *S. reading* in 1953, and *S. stanley* and *S. panama* in 1961.

Figures 2a, b, and c present a survey of the *Salmonella* collections during the years 1959, 1960 and 1961; only the most important types are listed.

For many years, food poisoning in the Netherlands has been submitted to epidemiological investigation so far as possible; whenever food rem-

TABLE I

INCIDENCE OF A NUMBER OF COMMON *Salmonella* TYPES IN MAN IN THE NETHERLANDS, ARRANGED ACCORDING TO THE PERCENTAGE
OF TOTAL NUMBER OF STRAINS TYPED DURING THE PERIOD 1951–1961

Salmonella type	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961
S. typhimurium	23	12	20	33	36	48	53	62	56	55	53
S. paratyphi B	20	20	14	9	14	11	14	6	5	5	1
S. bareilly	14	37	18	21	5	15	4	4	2	2	1
S. heidelberg	—	0.2	—	4	3	1	2	4	5	6	4
S. bredeney	0.3	0.4	0.2	2	4	1	1	0.9	6	6	3
S. newport	0.4	0.2	0.2	0.9	0.4	2	4	6	5	2	1
S. dublin	1	3	8	3	4	2	1	0.9	1	0.9	0.4
S. typhi	3	8	8	2	8	3	2	1	0.9	1	0.9
S. muenchen	4	0.9	0.4	4	1	1	1	1	1	0.8	1
S. bovis morbificans	20	2	—	4	1	0.3	4	1	2	2	2
S. enteritidis	0.9	2	9	1	4	3	1	0.7	0.6	0.2	1
S. senftenberg	0.1	4	0.8	0.8	1	0.3	0.1	0.2	0.03	0.03	—
S. stanley	9	0.9	—	0.1	1	0.2	0.1	3	0.2	0.08	15

1959

☐	S. TYPHIMURIUM	56 %
☐	S. BREDENEY	6 %
▤	S. NEWPORT	5 %
☐	S. HEIDELBERG	5 %
◺	S PARATYPHI B	5 %
▩	S. BOVIS MORBIFICANS	3 %
☐	REST	20 %

A

1960

☐	S. TYPHIMURIUM	54 %
▦	S. HEIDELBERG	7 %
▨	S. BREDENEY	7 %
▨	S. PARATYPHI B	5 %
▥	S. NEWPORT	3 %
▩	S BOVIS MORBIFICANS	2 %
▨	REST	22 %

B

1961

☐	S. TYPHIMURIUM	53 %
☐	S. STANLEY	15 %
☐	S. PANAMA	8 %
☐	S. HEIDELBERG	4 %
☐	S. BREDENEY	3 %
☐	S. BOVIS MORBIFICANS	2 %
☐	REST	15 %
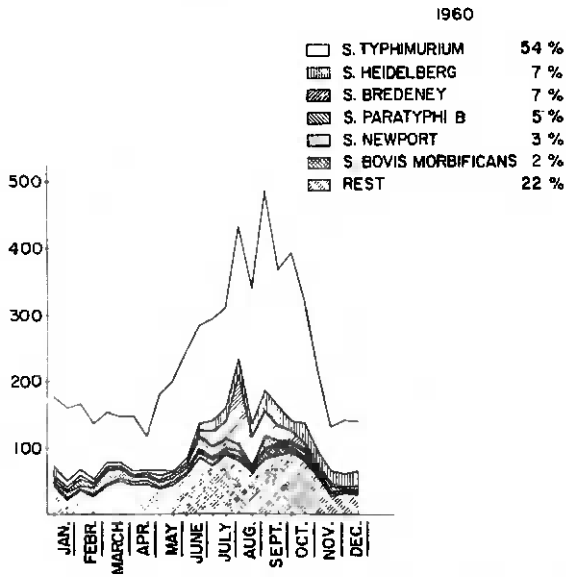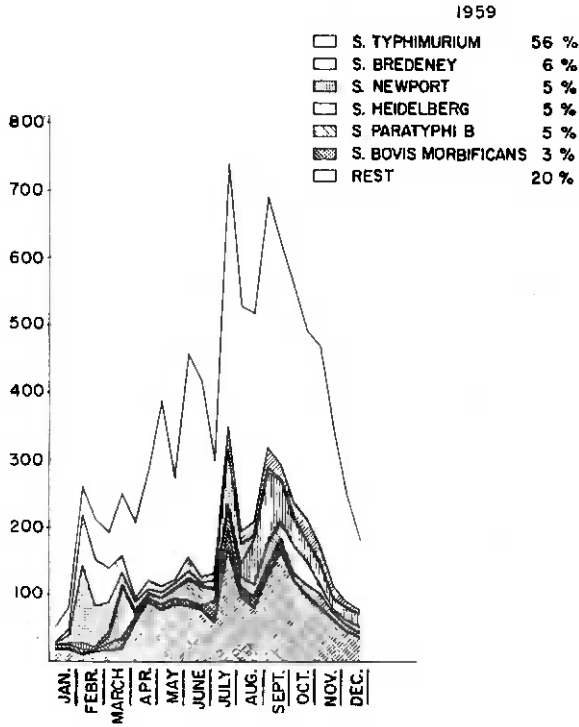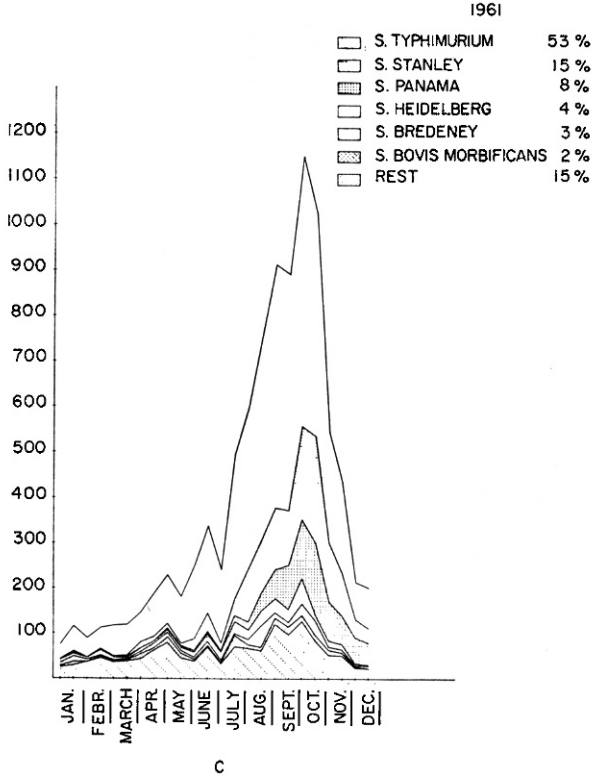
FIG. 2. A survey of the *Salmonella* collections for the years 1959, 1960, and 1961.

nants were available, bacteriological investigations were also carried out. In many cases these regular investigations demonstrated a causel relation between human disease and foodstuffs of animal origin. It need hardly be pointed out that, in this respect, it is not only primary contamination of foodstuffs but, in some cases, also secondary contamination by man that must be taken into account.

On the occasion of the 1959 summer epidemic, exhaustive investigations were carried out for the first time, after it was established that a very large number of cases of human infection could be explained by consumption of meat and meat products prepared from pork. Both this first investigation and those made in subsequent years chiefly pointed in the direction of minced meat. Food habits in the Netherlands show that minced meat is often only lightly fried, that is, insufficiently heated for consumption.

Systematic investigation revealed that 25% of normal slaughterhouse

pigs (that is, the animals showing no anomaly when presented for slaughter) harbor *Salmonella* germs in the mesenteric and/or portal lymph glands and/or in the feces. In view of this large percentage of infections it is not surprising that many animals are also contaminated on the outside during the slaughtering process. To reduce this contamination, experiments were carried out with chemicals in the scalding water, disinfectant sprays in scraping machines, with scalding ovens, and by flaming the animals at the end of the slaughterhouse line. Part of these experiments, and the results, were described by Kampelmacher *et al.* (*1*).

These experiments can be summarized as greatly reducing the percentage of infection without, however, giving the guarantee of slaughterhouse animals free of *Salmonella*. This publication also mentions the many complications attending decontamination, for example in the scalding oven.

Apart from these experiments, some measures were taken to promote the detection of *Salmonella* germs and prevent their dissemination.

In emergency slaughtering, extensive *Salmonella* testing (accumulation in selective media, isolation on selective plates) was made mandatory. After it was discovered that a fairly large percentage of slaughtering knives were contaminated with *Salmonella* germs, incision of the mesenteric lymph glands in the normal inspection of slaughterhouse pigs was made no longer mandatory; also, melting-out of mesenteriums was prescribed.

Further investigation showed that all these measures can have only a limited effect, for it was found that normal slaughterhouse animals were suffering not only from external but unfortunately also from relatively severe internal infection, i.e., infection of meat and/or organs. Large-scale investigations not yet completed indicate that a given percentage of

TABLE II

INCIDENCE OF *Salmonella* ORGANISMS IN SLAUGHTERHOUSE PIGS

Source	Slaughter-house I	Slaughter-house II	Slaughter-house III	Total
Diaphragm Pillar	4 (2%)	4 (2%)	25 (12.5%)	35 (5.5%)
Spleen	2 (1%)	4 (2%)	14 (7%)	20 (3.3%)
Liver	9 (4.5%)	1 (0.5%)	13 (6.5%)	23 (3.7%)
Bile	19 (9.5%)	13 (6.5%)	27 (13.5%)	59 (10%)
Mesenteric lymph nodes	16 (8%)	36 (18%)	38 (19%)	90 (15%)
Portal lymph nodes	9 (5.4%)	21 (10.5%)	19 (9.5%)	49 (8%)
Feces	10 (5%)	26 (13%)	30 (15%)	66 (11%)

normal slaughterhouse pigs harbor *Salmonella* germs in meat and/or
organs. Table II presents data on 600 pigs examined in three different
slaughterhouses.

Initially, pillars of the diaphragm were examined as representative
of a sample of meat. In view of the large local lymph vessels and the
possibility of infection in the internal muscular layers, these pillars were
found less suitable for the purpose. Subsequent examinations of muscle
fragments from the foreleg revealed a lower percentage of infection
(0.5–1.5%).

These results are a serious threat to one of the maxims of meat inspec-
tion, that normal healthy animals harbor no pathogens in the spleen
and/or meat. Although these animals were certainly not suffering from
septicemia in the proper sense, and although only small numbers of germs
were found in meat and/or organs, these animals nevertheless constitute a
serious problem of meat inspection.

All investigations, which have so far included some thousands of pigs,
revealed *S. typhimurium* in 30–35% of isolations from feces, glands, meat,
and organs; this type, it has been pointed out, is of great importance in
human infection. In addition a large number of other types were found,
including some that are regularly isolated from man in the Netherlands.
In connection with these findings it is of interest to consider a number of
questions of general importance.

The question can be raised as to how it is possible that pigs are so
severely infected with *Salmonella* germs. In the Netherlands, by far the
majority of foodstuffs must be imported. In the past few years there have
been innumerable publications on *Salmonella* isolation not only from
animal- and fish-meal, but also from vegetable products. Without empha-
sizing any one particular foodstuff, and without reaching any conclusion
as to the causes of *Salmonella* infection of foodstuffs, it can be stated that
pigs infect themselves by consuming feeds containing *Salmonella*. These
feeds are infected, either primarily or secondarily, for example by rodents.
The exact importance of the latter factor cannot be estimated but it is not
improbable that, on many farms, vicious circles of infection are main-
tained by rodents. Sterilization of feed constituents or feeds, for example
in pellet form, can perhaps be expected to provide a solution of this
problem. At the present time, experiments on these lines are in progress.

It is an interesting question whether such an extensive infection is
specific for one particular country. Although insufficient evidence is avail-
able on this question, I believe that the percentage of infection may also
be high in many countries. For the United States, this suggestion has
been confirmed particularly by the investigations of Galton. The fact

that, in the United States, human *Salmonella* infections related to the consumption of meat and meat products are less frequent, might be explained by the factor of nutritional and storage methods. In the Netherlands today, about 20% of households possess a refrigerator; only a proportion of the consumers, therefore, can store meat and meat products at sufficiently low temperatures during the summer months. In the United States refrigerators are found in 98% of households and, apart from this, there is a perfect chain of refrigeration between slaughterhouse and housewife. In our country, moreover, minced meat is consumed in large quantities, often insufficiently heated or entirely uncooked. This minced meat partly consists of pork.

Another factor to be considered, and one which impedes country-to-country comparison, is the registration of illness, including the notification laws. A striking feature is also that the various laboratory staffs employ such widely different methods of investigation.

As to the last factor, for the past 10 years there have been standardized methods, initially developed chiefly for isolation of salmonellae from human feces. In recent years it has become necessary to examine foodstuffs and animal feeds of a widely varied nature, such as meat, sausages, egg products, dry meals, and other products. Factors of great importance in this respect are the method of sample taking, size of sample relative to the enrichment medium, selective media, and incubation periods. It may well be said that, in an era of motorcars, aircraft, and even rockets, we are still using horse-and-buggy methods in *Salmonella* investigation. To find the former "vehicles" and thus to renovate *Salmonella* investigation, is an urgent problem of our days—a problem which must be solved if future country-to-country comparison of epidemiological findings is to be possible. The necessity of standardization, say of nutrient media, is illustrated by the fact that media of the same name are often prepared by different methods and with different substances. A few years ago in our laboratory, we found that brilliant green-phenol red agar prepared with different brands of brilliant green, yields very different results. One particular brand gives very large red stained *Salmonella* colonies, with good inhibition of the accompanying flora; with other brands, the colonies are pinpoint-sized and difficult to recognize or even overgrown by the accompanying flora.

By giving only this one example, I should like to emphasize how necessary it is to come to an international standardization of *Salmonella* investigation, which will only be possible when suitable methods are developed for the investigation of various foodstuffs and feeds.

Another factor—and it will be obvious that only random factors can be

Examination during life

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII		Pig no.
	fish meal →					← animal meal					Number of examinations										+*) time of slaughter			
	S.cubana										S.cubana, S.new brunswick				S.cubana	+*)							A	
											S.new brunswick					+							B	17
									S.cubana						S.cubana	S.bovis m.	S.cubana	+					C	
											S.new brunswick		S.bovis m.			+							D	
																	S.senften-berg	S.simsbury	S.cubana	+			A	
				S.bovis m.					S.cubana	S.cubana							S.cubana	+					B	19
																S.cubana		+					C	
																		+					D	
				S.cubana														+		+			A	
																							B	20
													S.bovis m.	S.cubana	S.cubana			+					C	
																							D	
												S.cubana	S.cubana		+								A	
											S.new brunswick			S.cubana	+								B	21
														S.cubana	S.bovis m.	S.cubana		+					C	
											S.new brunswick								S.simsbury		+		D	
	S.bredeney			S.cubana							S.cubana						S.simsbury, S.senften-berg			+			A	
				S.cubana																+			B	22
								S.cubana								S.cubana			S.simsbury	+			C	
							S.cubana										S.alachua		S.simsbury	+			D	
											S.cubana, S.new brunswick		S.cubana	S.cubana	S.bovis m.		S.cubana	S.simsbury	S.cubana		S.simsbury	+	A	
		S.cubana									S.cubana				S.cubana	S.cubana			S.simsbury			+	B	23
															S.bovis m.			S.cubana				+	C	
											S.new brunswick											+	D	
	S.cubana								S.cubana		S.new brunswick, S.cubana					S.cubana			S.cubana			+	A	
	S.bovis m.										S.new brunswick							+					B	24
	S.cubana															S.cubana			S.cubana		S.taksony	+	C	
																							D	
	S.cubana												S.cubana			+			S.cubana				A	
													S.bovis m.				S.cubana		S.cubana			+	B	25
																S.taksony	S.taksony, S.senftenb	+					C	
																		S.simsbury					D	

Type and Localization of Salmonella in Living and Dead Slaughterhouse Pigs

Post mortem examination

Meat	Dia-phram	Spleen	Liver	Gall bladder	Portal lymph nodes	I	II	III	IV	V	VI	ddI	ddII	Rectum	Fig. no.
			S. cubana								S. cubana	S. cubana / S. bovis m. }	S. cubana	S. cubana / S.t.m.	A } 17
										S. london	S. cubana	S. cubana	S. cubana		B
															C
												S. cubana	S. cubana		D
										S. montevideo					A } 19
											S. cubana				B
									S. bredeny						C
											S. cubana				D
										S. bovis m.	S. cubana			S. simsbury	A } 20
											S. minnesota				B
						S. bovis m.	S. bovis m.	S. bovis m.							C
										S. cubana	S. cubana / S. bovis m. }	S. cubana		S. cubana	D
			S. cubana					S. bredeney / S.t.m.	S.t.m. / S. bredeney }	S.t.m. / S. bredeney }	S. bovis m.	S. cubana		S. cubana	A } 21
								S. bovis m / S. minnesota	S.t.m.	S. bovis m. / S. minnesota }	S. bovis m.	S.t.m.			B
												S. cubana			C
											S. cubana	S. cubana			D
								S. worthington	S. bovis m. / S. bredeney }	S. bovis m. / S. bredeny }	S. bovis m.				A } 22
											S. minnesota				B
															C
														S. simsbury	D
										S. t.m.	S. cubana	S. cubana }	S. taksony } S. cubana	S. cubana	A } 23
															B
										S.worthington	S. simsbury	S.meleagridis	S. simsbury		C
											S. cubana	S. cubana			D
									S. t.m.	S. t.m. / S. bovis m. / S. minnesota }	S. bovis m.	S. cubana / S. simsbury } S. cubana	S. cubana	S. cubana	A } 24
S. simsbury			S. simsbury							S.t.m.	S. t.m.			S. cubana	B
										S. t.m.	S.t.m.			S. bovis m.	C
			S.worthington	S.worthington	S.worthington	S.worthington	S.worthington	S.worthington	S.worthington	S.worthington	S.worthington	S.worthington		S.worthington	D
			S. bovis m. / S. minnesota }	S. bovis m.	S.t.m. / S. bovis m. }	S. bovis m.						S. cubana	S. cubana	S. cubana	A } 25
			S.worthington	S. bovis m.	S. t.m. / S. heidelberg	S.t.m. } S.t.m.								S. bovis m.	B
											S. minnesota	S. simsbury	S. alachua	S. cubana	C
									S. minnesota	S. minnesota	S. cubana			S. cubana / S. simsbury }	D

presented during a short paper such as this—is the frequency of examination of animals which are clinically healthy germ carriers and a potential danger to the consumer of products subsequently obtained from them. Table III reviews the results of feces examination over a protracted period of life in pigs, and examinations of feces, glands, meat, and organs after slaughtering. These data clearly show that examination of one or two fecal samples, e.g., by means of swabs, supplies only a very fractional picture of the true incident of *Salmonella* germs.

I have dwelt on one particular epidemiological situation in which large-scale human *Salmonella* infections can occur, and on which some information is at this time available in the Netherlands. I hope that this example and the problems related to it will provide material for further discussion.

REFERENCE

1. E. H. Kampelmacher, Guinee, Van Keulen, and Hostra. *Zentr. Veterinarmed.* 8, 1025–1042 (1961).

Discussion by M. J. Foter

Robert A. Taft Sanitary Engineering Center,
U.S. Public Health Service, Cincinnati, Ohio

Dr. Kawabata referred to the fact that filtrates of pure cultures of *Clostridium botulinum* type E have characteristically shown a low intraperitoneal toxicity, but a high oral toxicity. The fatality rate in type E outbreaks has usually been high. These observations leave open the possibility that type E toxin is produced in precursor form different from that responsible for poisoning.

The work of Duff *et al.*, which was cited by the authors, showed that potentiation, or "activation," of the toxin could be accomplished by trypsin. Sakaguchi and Tohyama in their first and second papers, which were referenced by the authors and became available about the same time as Duff's paper, showed that concomitant proteolytic *Bacillus* and *Clostridia* were capable of potentiating type E protoxin in "izushi."

Subsequent work by Bonventre and Kempe (*1, 2*) indicated that types A and B toxin are also produced as precursors, but are self-activated by proteolytic enzymes produced by the toxic anaerobes themselves. Dolman (*3*) showed that a self-activating mechanism is also indicated for a proteolytic type E mutant strain.

In type E botulism, activation by concomitant bacteria in food products seems to be significant in producing a higher level of toxin than simple

activation by the digestive tract. This may be due to suboptimal pH conditions for activation or toxin destruction.

Attention might be called to the possibility of heating fish to destroy type E spores before the fish are used in modified "izushi." The spores are relatively heat labile, and treatment at about 80°C might be investigated as a possible method for treatment of fish. The use of approved preservatives in fish treatment might also interfere with lactic acid fermentation in the manufacture of "izushi."

The conclusions and suggestions of the authors are generally based on accepted findings and general experience in the field of botulism research. Japanese data on botulism are a worthwhile addition to the literature.

Dr. Kawabata presented an excellent description of a group of organisms referred to as the "pathogenic halophilic bacteria," which have been incriminated in several food-poisoning outbreaks in Japan. The disease apparently is restricted geographically to Japan and results from the ingestion of raw fish and raw fish products. The authors claim that to date these organisms have not been placed in a genus. Based on the report of Aiso, cited by the authors, and the open discussion at the conference, we might question whether the halophilic bacteria described are members of the genus Pseudomonas.

Dr. Smith presented a concise and lucid review of the role of *Clostridium perfringens* in food poisoning. The usefulness of the paper would be enhanced by the inclusion of the methodology and materials used. Most significant in the paper is the summary in which the author delineates some of the conditions necessary for the development of food poisoning. Whether there are other factors in addition to those listed is not known, and evidence is not presented to rule out other factors. For instance, does the degree of anaerobiosis influence growth in foods, or associative growth of other organisms? Is *C. perfringens* food poisoning the result more often of pre-cooking or post-cooking contamination?

Though it was probably meant to be understood, the author does not specifically state that all *C. perfringens* outbreaks studied to date have been caused by type A strains. It is most interesting that the author reported the shortest generation time at 35°C; whereas McClung and others have reported minimal generation time at or near 46°C.

The author indicates that the mere presence of *C. perfringens* in food is not sufficient to induce food poisoning, but that the critical dose is in the range from 5 to 10 million to several hundred million organisms per gram. In view of the significance of the critical dose, substantiating these estimates by reference would be helpful to the reader.

Dr. Kampelmacher indicates that the Netherlands has an active investi-

gative program on foodborne disease well under way. Others in the field of food microbiology will, I am sure, agree with the author that the problems associated with salmonellosis center around unified methods of detection and the control of animal infection, since the majority of outbreaks appear to be associated with meats of various kinds.

The author expressed most of the problems associated with salmonellosis of foodborne origin; however, it would have been helpful to interested public health and regulatory agencies if the author would have been more specific in his presentation of data. His tables do not specify whether the numbers given are cases or outbreaks. Also, where reference is made to the tables in the text, the item referenced may be absent. The usefulness of the paper would be enhanced too if the methodology and materials were included or cited by reference.

Some interesting differences in cooking and refrigeration practices in various countries are discussed, and studies on slaughtered pigs add weight to the contention that something needs to be done about animal infection.

On the whole, the paper serves as a reminder that salmonellosis is a world problem, as great in the Netherlands as in this country and others.

REFERENCES

1. P. F. Bonventre. "The Physiological Basis of Toxigenicity of *Clostridium botulinum* Types A and B." Thesis Univ. of Michigan, Ann Arbor, Michigan (1957).
2. P. F. Bonventre and L. L. Kempe. Physiology of toxin production by *Clostridium botulinum*, Types A and B. IV. Activation of toxin. *J. Bacteriol.* 79, 24–32 (1960).
3. C. E. Dolman, M. Tomisch, C. C. R. Campbell, and W. B. Laing. Fish eggs as the cause of human botulism. Two outbreaks in British Columbia due to types E and B botulinus toxin. *J. Infect. Diseases* 106, 5–19 (1960).

Open Discussion

DR. THATCHER: After these very stimulating papers, I feel sure there will be much comment from the floor. We will discuss the papers in order. First, are there any comments on pathogenic pseudomonas food poisoning?

DR. MOSSEL: If the halotolerant fermentative rods described by Doctor Kawabata will also occur in our area of the world, we shall have to devise methods of controlling them, because Buttiaux demonstrated large numbers of similar organisms (*Aeromonas* spp. and *V. costicolus*, resp.) in foods such as fermented sausage and meat curing brines and probably nothing will prevent Doctor Kawabata's organisms from taking the place of the former.

DR. PEDERSON: I would like to raise a question about the preparation of this fish preserve made with raw fish, vegetables, cooked rice, malted rice, and a small amount of salt and vinegar packed tightly in a wooden tub with a lid.

Is there any standardization of packaging this product or any attempt being made to standardize the product?

DR. KAWABATA: No, we don't have any standardization. This "izushi" is made at home.

DR. PEDERSON: Are you doing any experimental work to develop a method of preparing this food?

DR. KAWABATA: We are trying to.

DR. HANSEN: I should like to ask whether any information is available as to the temperature range for growth of this pathogenic halophilic organism, since it was stated in your paper that optimal temperature for growth was about 37°C. What would perhaps be more interesting is the lower limits.

DR. KAWABATA: As far as I know the minimum temperature for growth was 20°C.

DR. EL-BISIS: I would like to ask Dr. Kawabata whether there is any information on the pattern of this. Does the growth occur in the first stages of fermentation or where? How do they relate to each other?

DR. KAWABATA: I am sorry. I don't have any idea but lack of fermentation will not prevent formation of toxins.

DR. EL-BISI: But lactic acid concentration is associated with the organisms responsible?

DR. KAWABATA: Yes.

DR. EL-BISI: And with the pseudomonas activity?

DR. KAWABATA: Yes.

DR. WERTMAN: I would like to ask if the agar contains any proteolytic enzymes, and if this has any bearing on the activation of the toxin in the product?

DR. KAWABATA: I am sorry, we have not made this study, but it might. I can't say for certain.

DR. THATCHER: Shall we proceed now to type E botulism?

FROM THE FLOOR: One observation that Dolman made quite recently was that type E botulism is frequently involved in fish and fish products. Nevertheless, he could not say if it was a marine or terrestrial organism. We find it in aqueous flow into muddy regions. I might add that, since the report on this to which Dr. Slocum referred, some people in our fisheries department have examined fresh caught fish, allowed them to spoil, and have indeed recovered *C. botulinum* type E and lethal doses of type E toxin. These fish were from inland waters.

DR. SLOCUM: I think a great many of the outbreaks of type E botulism have been associated with gross spoilage of products. Doctor Dolman made a point in Montreal that many of the products prepared from fish eggs and from sea mammals were just so repulsive it was hard to see how anyone could possibly eat them. But one of our major concerns about this product in connection with smoked fish is the fact that the strains involved are not proteolytic in themselves and we found clear evidence that toxin is produced in the absence of any demonstrable contamination.

DR. THATCHER: Any other comments on Type E botulism?

DR. MOSSEL: I understand that "izushi" is made in primitive homes, is this right?

DR. THATCHER: Yes.

DR. MOSSEL: So if suggestions were made for preventing this, I don't think it would be applicable at all. There are probably thousands of these people making this "izushi" in the northern islands.

DR. THATCHER: Type E botulism in Canada would be in the same category. There are preparations made by Indians and Eskimos under the crudest possible conditions and the food when finally consumed is considered a delicacy. On the other hand, Doctor Slocum's point is very important. When you change the environment, specifically on the smoked fish, the common spoilage organisms may not develop while stored at low temperatures, but the development of Type E toxin may occur. We found fish that were lethal to mice and a group of volunteers examined them thoroughly and they were of the opinion that those people who liked smoked fish would have eaten them. They contained a lethal level of type E toxin.

DR. INGRAM: One good way of removing this would be the irradiation pasteurization of fish.

DR. THATCHER: A very potent observation. Let us proceed now to a discussion of *Clostridium perfringens* food poisoning.

DR. ELLIOTT: I would like to ask Doctor Smith if he knows of anyone who has determined the minimum growth temperature of *Clostridium perfringens*?

DR. SMITH: I really couldn't answer that. Time is involved with this and probably different media. In one day's incubation we found no growth of any of five strains at 15°C.

In regard to the points that Doctor Foter raised, any medium that is at all viscous does not contain enough oxygen to inhibit growth. Second, other organisms sometimes did markedly interfere with the growth.

DR. FOTER: How about the lactics?

DR. SMITH: I don't know. I avoided commenting on the point that all outbreaks reported to date are type A only, because I did not want to discuss the type of outbreak which has been reported in Germany and which I suspect occurs in the United States. I also cannot effectively explain the reason for the optimum temperature of 35°C for our strains as compared to the optimum temperature of 46°C reported by McClung. All I can say is that this is what our strains did. And finally, with regard to whether contamination is pre-cooking or post-cooking, I can refer you to two people who know much more about it than I, namely, Dr. Hobbs and Dr. Angelotti. Doctor Hobbs, would you care to comment on whether it is pre-cooking or post-cooking?

DR. HOBBS: In England we rarely find *Clostridium welchii* outbreaks due to strains other than those producing heat-resistant spores. Heat resistance is established by isolating *C. welchii* from the stools of patients after steaming a small portion of the stool in broth for 1 hour before anaerobic incubation overnight followed by plating on to horse blood agar incubated anaerobically. This technique precludes the finding of β-hemolytic heat-sensitive strains in stool samples. Strains from the suspected meat dishes must be isolated directly without heat treatment as the spores have all germinated after cooking and they are rarely if ever found at this stage. It is valuable to obtain anaerobic and aerobic surface plate counts on blood agar because high anaerobic counts are suggestive of *C. welchii* food poisoning.

In order to identify heat-resistant strains of *C. welchii* it is necessary to examine the colonial appearance and hemolysis on horse blood agar. If a pour

plate medium is used, such as sodium sulphite agar giving black colonies for all *C. welchii strains*, there is no distinction between the classical type A and food poisoning strains and there is a danger that outbreaks may be wrongly ascribed to *C. welchii* merely because classical type A, β-hemolytic and heat-sensitive, strains have been isolated. Although in some instances this organism has been proved to cause food poisoning in meats contaminated after cooking the great majority of outbreaks have undoubtedly arisen from the germination of spores, originally present on the raw meats, which have survived the cooking and developed into thriving cultures on the cooked meat.

All strains from food and feces are serologically typed to confirm that one predominant serotype is responsible for the outbreak. It is essential that serological typing methods should be internationally applicable, and we hope that, in future, comparisons of serological types will be carried out internationally so that the media and methods used may be comparable.

DR. THATCHER: In order, Doctor Niven and Doctor Ingram indicated they wished to comment.

DR. NIVEN: I should like to ask if we take precautions against botulism episodes, need we be concerned about perfringens outbreaks?

DR. THATCHER: Would your response have anything to do with that, Doctor Ingram?

DR. INGRAM: Well, perfringens is not a very strict analogy. I wanted to add an observation which I think is relative to the question of post-cooking contamination. Working with one heat resistant *C. perfringens* strain which was provided by Doctor Hobbs, and I emphasize it is only one, we find the spores are almost 100% activated by heat. This indicates that if the spore from nature finds its way onto the product, the chances of its germinating are small unless it is cooked with the material and this would serve to say it is pre-cooking contamination. Why do we have so much trouble with cooked meats and comparatively so little with fresh?

DR. THATCHER: Thank you, Doctor Ingram. There was a suggestion put forward of the toxins possibly being altered. Would your experience have any relationship with that, Doctor Hobbs?

DR. HOBBS: I do not know. We have tried without success, to demonstrate toxin in filtrates of *C. welchii* meat cultures.

Volunteers have become ill after eating bacterial cultures only and not after drinking bacteria-free filtrates or supernatants.

It is possible that the toxic filtrates are inactivated before reaching the lower intestine and that it is necessary for the whole organism to pass through the stomach region. Whatever the reason, so far we have been unable to demonstrate the action of a specific toxin but only of a massive culture of the active organism.

DR. SMITH: I might say with regard to the specific toxin that what effect we did have in laboratory animals occurred only when this was administered with the living organism. Another point is that if this were the case, we would expect that we would take measurable amounts because the secretions of the small intestines are quite markedly inhibitory to the lecithin splitting activity of these toxins.

DR. MOSSELL: I have always felt, that the rather infrequent occurrence of *C. perfringens* food poisoning is possibly due to the fact that most frequently

a special thermoresistant type of the organism is involved, as suggested by Doctor Hobbs.

Strains of *C. perfringens* of lower thermoresistance (the ubiquitous ones) will either be eliminated from foods by cooking etc., or survive together with other organisms. The latter will lead to foods containing mixed cultures which will virtually always be spoiled before dangerous levels of *C. perfringens* have been reached. Thermoresistant spores of *C. perfringens* may survive in pure culture however and, when not particularly biochemically active, grow out to infectious levels without this being indicated by an off-taste.

DR. SMITH: I see the point. I might also add that I see no reason why it should require a heat resistant spore to colonize in the intestine. I don't know if any of you are familiar with sour dough bread. We ingest hundreds and probably thousands of millions of organisms per slice. If there were heat resistant spores there we might expect trouble. We don't have it as far as I know.

DR. THATCHER: We still haven't touched on the salmonellae. Are there any comments on salmonellae?

DR. NIVEN: I would like to say that we have been working with salmonellae which is a problem with meat animals and our results are at variance with yours, Doctor Kampelmacher, in that hogs at farm level have a very strikingly low incidence of salmonellae in the intestines, at least in our survey which has been reasonably distinct. In spite of the fact that the hogs have been fed food that probably contain low population levels of salmonellae, when they are transported direct to slaughter, the incidence of salmonellae in the feces or the colon contents and the mesenteric lymph nodes remains strikingly low at the time of slaughter. However, if these hogs are transported through the conventional channels, that is through the auctions, holding bins and the like, then the incidence goes up to just about the degree that you recorded. We feel this is a source of contamination of swine. The only thing that we have to do is to change our marketing system for hogs, but I don't know just how to accomplish this.

FROM THE FLOOR: May I briefly interject here, on the pigs where you got low incidence, were these grain fed pigs or were these swill fed pigs?

DR. NIVEN: These were typical hogs on typical midwestern farms.

FROM THE FLOOR: That is a pretty good grade of pig. In northern Ohio we still raise them on corn and they are treated like children.

DR. NIVEN: I might qualify my statement. We have only worked with young pigs, and the situation might be different there.

DR. KAMPELMACHER: I would like Mr. Chairman, to make some comments to supplement my previous brief statements. I totally agree with Doctor Niven that transportation is very important cause for salmonella enrichment. We have done an investigation with pigs which are slaughtered on the farm. A very small number of pigs are slaughtered for the farmers themselves without transportation and then the number or percentage of salmonellae infected animals decreased to about 3 per cent. So transport is a very important problem. We have made a detailed investigation of short and long distance transport. We transported pigs for half an hour and we transported them for 6 hours. There seems to be some effect of stress not only on salmonellae in the feces but also on their passage through the gut barrier. We don't know the

reason for this, but I may suggest, and we are working on this, that antibiotic feeding has something to do with it. There is another comment I would like to make in answer to a point raised by Doctor Foter. You will find the composition of our media in the paper that was published in English earlier.

MRS. GALTON: One of the first questions I planned to ask you, Dr. Kampelmacher, was the methodology you used for the benefit of the group, although I saw your technique when I visited your laboratory in February, and, as you say, it is described in your paper. I agree that this is very important, but, as Dr. Niven mentioned, results in this country do not agree entirely with some of your findings. In our early work in Florida in 1952, we found a very low percentage of infected animals on the farm, ranging from 7 to 10%. At the slaughterhouses, the percentage ranged from 30 to 70%. We found this high degree of contamination on materials throughout the environment of the plant and on the finished carcasses, so it is not surprising that 30 to 40% of the sausage samples purchased on the retail market in some areas, have been found to be contaminated with Salmonellae.

I would also like to make the following comments. Some of you, I know, are aware of our interest at the Communicable Disease Center in salmonella surveillance. We recently established a Salmonella Surveillance Unit with the cooperation of the State health departments. Reports of their salmonella isolations are sent to this unit weekly. The data are summarized in a surveillance report which is distributed monthly to health authorities and others concerned. We hope in this way to increase interest in the problem and to learn more about the true picture of the situation in this country. It is quite evident that we have only limited knowledge of the prevalence of salmonellae in our human population. We know that salmonellosis is widespread in animals, that animal feeds are frequently contaminated, and that transmission to man appears to be increasing.

DR. THATCHER: Thank you. I would like to interject, that whenever a discussion has to be cut off because of lack of time, that this is the mark of first class papers. I would like to thank all those who presented papers and thank those who have discussed them so enthusiastically.

Total Counts as Indexes of Food Quality

J. H. SILLIKER

Department of Pathology, St. James Hospital, Chicago Heights, Illinois

Total counts have been used to access sanitary quality, organoleptic acceptability, safety, and utility of various food products. The applicability of this tool for any of these purposes is influenced by the chemical and physical characteristics of the food, the manner in which it is merchandized, and the use to which it is put by the consumer. The multiplicity of factors involved makes generalizations on the value of total counts as indexes of food quality virtually impossible.

Sanitary Quality

Experience has taught that total counts are the most useful means of evaluating the sanitary quality of milk, whereas the detection of specific indicators of fecal contamination is used in bacteriological control of water. We deal here with differing raw materials, processing methods, and merchandizing conditions. These differences are reflected in fundamental differences in the philosophy of control measures designed for the evaluation of sanitary quality. Yet, few would argue that these divergent control procedures have not been important factors in the protection of public health.

With respect to other food products, the value of total counts as an index of sanitary quality is vague. If one studies standards promulgated by regulatory agencies and by processors for the purpose of evaluating sanitary quality, he finds that usually both total counts and the detection of specific microbial groups are involved in the control program [see Elliott and Michener (1)]. One would hope that this duality is for more complete evaluation of sanitary quality, but it seems more probable that in many cases, multiple analyses are done because of uncertainty as to whether the total count or the presence of specific organisms is of greater significance in the determination of sanitary quality.

Total counts most effectively evaluate the sanitary quality of foods

which do not support microbial growth. This resistance to attack may be due to physical state (dried), the presence of inhibitory chemicals (acids, salt, etc.), or to the manner in which the product is merchandized (frozen). Microbiologically such products are shelf stable, and within certain ill-defined limits, the total count becomes a measure of the sanitary control exercised in production [see Thatcher (2); Dack et al. (3)]. The obvious limitation of this evaluation is the fact that the total count measures only living organisms, and processing procedures may destroy microorganisms in a high count raw material or at a terminal stage in production. Further, prolonged storage in the frozen or dried state results in diminution of bacterial numbers. A number of workers have contended that direct microscopic counts give a better picture of sanitary quality than do viable count determinations (4, 5). With retorted products, total bacteria counts are valueless; aside from stability tests, direct microscopic examination constitutes the only means of evaluating sanitation.

Perishable foods present quite different problems, since by definition these products will spoil as a natural consequence of not being consumed. This spoilage almost always is attended by extensive microbial growth. Thus, the total count on products such as meat, fish, eggs, and poultry, represents composite reflections of raw materials, processing procedures, and handling conditions. The point at which the sample is taken determines whether its total count can reasonably be used as an index of sanitary quality.

The highest order of sanitation may be practiced by the packer in the production of ground meat, for example. Yet, if it is stored too long by the packer, the retailer, or the housewife, spoilage will occur. The total count on such meat will describe microbial quality, but it does not reflect sanitary quality.

On the other hand, the food processor is in a position to use total counts as a means of evaluating the sanitary quality of his raw materials, his processing methods, and the finished product, because he can interpret analytical results in terms of a known history.

Thatcher (6) has strongly indicated "control at the source" as the best means of assuring sanitary quality. It is apparent that only the samples "selected at the source" are reliable subjects for evaluation of sanitary quality on perishable foods. In view of this contention, one might ask why total counts have proved successful as a means of evaluating the sanitary quality of milk, a perishable product. The answer lies in the fact that we are dealing with a food which is processed and merchandized under conditions which are known within reasonably narrow limits. Further, those evaluating milk are mindful of its perishable nature and thus recognize

the importance of interpreting results in terms of the age and storage history of the product. At this time, no other perishable food is so well controlled that an analogous evaluation of sanitary quality is feasible.

Organoleptic Quality

Microbial growth leads to the development of undesirable organoleptic changes in numerous foods. For example, the spoilage of fresh meat is associated with the growth of gram-negative aerobic organisms. Their development is attended by changes in flavor, appearance, and odor. The rate of organoleptic degradation is influenced by a number of factors, particularly initial bacteria load and storage temperature. Regardless of the time at which recognizable organoleptic changes occur, they are associated with the presence of large numbers of microorganisms. Similar relationships can be seen in other perishable foods, e.g., eggs, fish, and poultry.

The fact that this relationship exists tends to negate the importance of total counts in evaluating quality. In most instances, experience has taught that organoleptic evaluations suffices, and bacteriological analyses only confirm the expected.

Total counts may be used as indicators of incipient spoilage. Foods may support the development of relatively large numbers of spoilage organisms without showing signs of organoleptic degradation. Bacteriological examination of such foods yields useful information to the investigator whose knowledge of the particular food allows him to associate bacteriological condition with possible shelf life, but in this application, the total count is not being used to assess existing organoleptic quality.

By definition, the total count is an attempt to estimate total numbers of microorganisms without reference to specific types. Yet, different organisms may affect organoleptic acceptance in quantitatively different ways. For example, Peterson and Gunderson (7) have shown that relatively small numbers of a psychrophilic pseudomonad bring about the development of off odors and flavors in defrosted chicken pies. This development occurred prior to the growth of large numbers of psychrophilic bacteria and was associated with the action of proteolytic and saccharolytic enzymes produced by the organism. Thus, this organism is quantitatively more important to organoleptic quality than metabolically less active bacteria present in the product.

Shank (8) has recently shown that the flavor of sliced cooked ham can be directly related to the development of microorganisms during storage. But the data summarized in Table I show that this relationship holds only on nonvacuum packaged product. The same meat, packaged in vacuum,

TABLE I[a]

THE RELATIONSHIP BETWEEN TOTAL COUNT AND FLAVOR OF SLICED, COOKED HAM[b]

Days Storage (40°F)	Package	Total count	Flavor score[c]
0	Vacuum	730	9.5
	Non-vacuum	1,400	9.5
7	Vacuum	140,000	8.5
	Non-vacuum	500,000	6.5
14	Vacuum	94,000,000	8.0
	Non-vacuum	27,000,000	6.5
21	Vacuum	460,000,000	7.6
	Non-vacuum	110,000,000	3.8
28	Vacuum	300,000,000	5.9
	Non-vacuum	183,000,000	1.4

[a] From Shank (8).

[b] Average score of a 5 man panel.

[c] 10 = Excellent, 7 = Borderline, 1 = Repulsive.

supports an even larger microbial population. But here the total count is not a reliable index of flavor. Studies have shown that the off-flavors developing under aerobic conditions are associated with the growth of yeasts and that these organisms do not develop in the vacuum packaged product. The dominant flora of vacuum packaged ham proved to consist of lactic acid bacteria which did not adversely affect flavor.

Silliker and Shank (9) and more recently Ordal (10) have found that a similar situation exists with respect to vacuum packaged fresh meats. Large populations of lactic acid organisms develop without detrimental effects of organoleptic quality. *Pseudomonas-Achromobacter* organisms, inhibited by efficient vacuumization of the package, rapidly bring about organoleptic degradation of nonvacuum packaged fresh meat.

Table II (9) shows that white bread develops large microbial populations during storage but that this development is not necessarily related to changes in the organoleptic quality. Factors other than microbial growth were responsible for degradation in flavor. On the other hand, it is well known that specific organisms can bring about undesirable changes in bread flavor and texture.

There are many foods wherein the growth of bacteria is necessary to the development of characteristic odors, flavors, and textures. Here, fermented sausage, sauerkraut, buttermilk, and various cheeses might be mentioned. In these instances, a high total bacterial is an index of quality; conversely, the absence of large numbers of microorganisms is associated with the absence of characteristic organoleptic attributes. Obviously, total

TABLE II[a]

THE RELATIONSHIP BETWEEN TOTAL COUNT AND THE ORGANOLEPTIC QUALITY OF
WHITE BREAD

Sample	Days of storage	Total count	Flavor[b,c]	Odor[b,c]
A	0	<100	4.2	4.4
B	0	<100	3.5	4.1
C	0	<100	3.3	3.5
A	2	800	3.3	3.0
B	2	<100	4.0	3.8
C	2	<100	2.3	1.8
A	7	100,000	2.5	2.3
B	7	1,100,000	3.8	3.5
C	7	<100	2.5	2.5

[a] From Silliker and Shank (9).
[b] Average results of an expert panel.
[c] 5 = Excellent, 1 = Repulsive.

counts cannot be used as a measure of sanitary quality in these products, either.

It is apparent that the value of total counts for grading of organoleptic quality, just as their use in determination of sanitary quality, is determined by the characteristics of the particular food product under consideration, and generalizations with respect to the relationships between total numbers and organoleptic quality are not possible.

Safety

Total bacteria counts are poor indexes of safety if we imply that such determinations can be used to predict the probability that a given food may contain pathogenic organisms or toxins. Perishable foods undergo spoilage with prolonged storage or mishandling; this spoilage is generally accompanied by the development of a microbial flora that is characteristic of the particular food. Since spoilage of perishable foods is seldom caused by the development of pathogenic organisms, the determination of total counts gives useful information with regard to microbial quality but does not permit speculation with regard to the probability that such food may constitute a health hazard. Indeed, the characteristic spoilage patterns constitute a major safeguard to the consumer. Any changes directed toward increased stability must take into account the effects these changes may have on the spoilage patterns which the consumer has associated with danger.

The existence of low total counts is no assurance of freedom from

pathogens. Hobbs *et al.* (*11*) found Salmonella on meats with extremely low counts and with no other organisms of fecal origin. Thatcher (*6*) cites egg yolk with low levels of bacteria as the cause of outbreaks of salmonellosis in babies. He also describes staphylococcus food poisoning traced to dried milk containing no viable staphylococci. *Staphylococcus* growth occurred in the milk before drying, and the resulting enterotoxin survived processing.

Of great concern are food ingredients which are introduced into finished products without cooking of either the ingredient or the product into which it is mixed. Here reference might be made to flour, seasonings, eggs, and flavoring components of various types. With foods that are not further cooked, pathogenic organisms in such ingredients are carried directly into the finished product. Control over the components of such foods demands that great care be exercised by the processor in his raw material selection. Experience has taught that bacteriological control over such food components requires direct analysis of each individual ingredient for the presence of potential food poisoning organisms. For example, the ingredient may show an extremely low total bacteria count, as well as minimum coliform contamination; yet, small numbers of salmonellae may be detected. Only painstaking analysis of such ingredients makes exclusion of salmonellae-containing raw materials possible. Raw material inspection programs based solely on total or coliform counts are not satisfactory.

While it is apparent that total bacteria counts are not a reliable guide to safety, nevertheless, a product showing excessive bacteria contamination may reasonably be assumed to be a potential public health hazard in the absence of demonstration of potential pathogens. Evaluation must be in terms of a knowledge of the specific product with reference to the microbiological problems it presents and the use to which it is to be placed.

Utility

Utility is here used with reference to the question of whether or not a particular food ingredient constitutes a satisfactory raw material for the product into which it is to be incorporated. This quality attribute may or may not be related to sanitary or organoleptic quality or to safety.

For example, let us assume a processor must meet a standard of 10,000 bacteria per gram on a finished product. If an uncooked ingredient is added at a level of 1% to this product, then the total count of that ingredient must not exceed 1,000,000 per gram, or he will be unable to meet the standard regardless of the microbial quality of all other components of this food. In this instance, the utility of the raw material, in terms of

meeting the standard, can be accessed in terms of a total bacteria count. This is, of course, the simplest possible example. Others are far more complex in their solution.

In the preparation of shelf-stable canned cured meats, a heat process sufficient to destroy *Clostridium botulinum* cannot be employed. The stability of such products, as well as their safety, is attributable to the presence of residual nitrite combined with the effect of heat injury to the surviving spores. The heat injured spores do not germinate and grow in the nitrite environment. The stability of this system is dependent upon minimal numbers of contaminating spores in the raw material. If this minimal level is exceeded, then the inhibitory system fails and spoilage occurs (*12*). Table III illustrates this situation. In experimental packs

TABLE III[a]

THE EFFECT OF THE INOCULUM SIZE ON PUTREFACTIVE SPOILAGE OF SHELF STABLE CURED MEAT

Experiment[b]	P. A. Inoculum	Cans packed	Putrid spoilage (%)
1	Indigenous[c]	96	0
2	2.0/gram	100	0
3	15,000/gram	80	66

[a] From Silliker *et al.* (*12*).
[b] All products contained 3.5% brine, 78 ppm NaNO₂, processed to Fo = 0.1.
[c] Indigenous spore level fresh meat trimmings = <1.0/gram.

with normal levels of putrefactive anaerobic spores in the raw emulsion, a stable product is obtained. If moderate numbers of these organisms are introduced into the product, extensive putrefaction occurs. While a total bacteria count might evaluate the organoleptic or even the sanitary quality of the meat trimmings used to prepare such products, such a count would be of little value in predicting the spore load in the product. Indeed, the putrefactive anaerobes would not be enumerated in a total bacteria count; but the significant point is that very small numbers of these organisms, relative to total numbers, determine stability.

Total bacteria counts can be used effectively in accessing the quality of a raw material being used in the production of a perishable product, such as domestic sausage. Table IV presents data on the destruction of bacteria in a sausage emulsion as a function of the final internal temperature reached during heat processing. Obviously, the higher the final temperature, the greater the destruction. If a processor has determined that a given bacteriological level in his finished product produces satisfactory stability, it is possible for him to predict, at any given processing tempera-

TABLE IV

DESTRUCTION OF BACTERIA IN A SAUSAGE EMULSION AS A FUNCTION OF FINAL
INTERNAL TEMPERATURE

Max. internal	%	Raw Material/limit/10,000
temp.	survival[a]	standard
155°F	0.4–2.0	500,000
165°F	0.2–0.9	1,100,000
175°F	0.1–0.2	5,000,000

[a] 95% confidence intervals.

ture, the quality of the raw material necessary to produce sausage within this standard.

This reasoning requires a knowledge of the nature of the contaminating bacteria. In the cases of meat trimmings, the major flora consists of heat sensitive organisms. If, however, a certain raw material contributes a completely heat resistant flora in terms of the process being used, then that raw material becomes the single important determinant of the bacteriological condition of the finished product. For example, many food products contain black pepper, a spice which is characteristically contaminated with aerobic spore-forming bacteria. In processes such as those

TABLE V

BACTERIOLOGY OF BLACK PEPPER AND ITS EFFECT ON THE BACTERIAL COUNTS OF
SAUSAGE

Year	Number of samples	Total counts/gram
1947	128	13,100,000
1959	18	20,000–94,000,000
	13	18,000–30,000,000

EFFECT OF ADDED PEPPER ON SPORE COUNT OF SAUSAGE[a]

Percent pepper	Spores/gram
0.1	10,000
0.2	20,000
0.3	30,000
0.4	40,000
0.5	50,000

[a] Assuming spore count of 10,000,000/gram.

used in sausage manufacture, these organisms are not destroyed, and the bacteriological content of the added pepper determines the count of the finished product, independent of the processing temperature and the bacteriological quality of other ingredients. Control over the finished product involves control over the spore load in this raw material (see Table V).

Even when severe heat processing is involved, the food processor must exercise control over the utility of his raw materials. Sometime ago, we became concerned with the production of a canned meat product containing cereal. Experience had taught that a meat emulsion receiving a process equivalent to an Fo of 5 would be completely stable. With the introduction of cereal, the problem of heat resistant thermophilic anaerobic bacteria presented itself. The spores of these organisms would survive a process which was adequate for uncured meat alone.

On a theoretical basis, it could be calculated that a given process would destroy a definite number of the thermophilic anaerobic spores. With a more severe process, a correspondingly greater number of spores could be handled. Representative data are summarized in Table VI (*13*). The

TABLE VI[a]

CALCULATED STABILITY OF MEAT-CEREAL PRODUCT AS A FUNCTION OF THERMOPHILIC ANAEROBIC SPORE CONTAMINATION

PROCESS	SPORES/CAN	% SPOILAGE
Fo-8	20	15
	10	7.5
	5	3.75
	2[b]	1.5
	1	0.75

[a] From Greenberg and Silliker (*13*).

[b] Commercial experience—product containing 2 spores per can showed spoilage rate of 1.5% when processed at Fo-8.

expected spoilage for a given process could be calculated from the degree of contamination of the cereal. Experimental work showed that this calculation was reasonably valid. The solution to the problem became one of selecting a single raw material—cereal—with sufficiently low thermophilic anaerobic spore contamination to allow the production of a uniformly stable product. In this instance, the stability of the finished product could be met only through the application of bacteriological standards to a single raw ingredient. The total numbers of bacteria in this ingredient were not of great importance. Rather, the presence or absence of a particular type of organism determined quality and indeed, this organism would not have been even counted in the determination of total bacteria count.

Conclusions

Total counts are of limited value in the evaluation of food quality. Just as with most analytical tools, the value of any particular determination must be assessed and interpreted in terms of the particular situation presented.

Since, by definition, perishable foods spoil if not consumed, total counts are not effective measures of either sanitary quality or safety of such products. While relationships exist between total numbers and organoleptic quality, bacteriological analysis is usually applied as a means of confirming the results of organoleptic evaluation.

In its application to shelf stable foods, the total count is subject to severe limitations for quality evaluation. While large numbers of viable organisms may give indication of poor sanitary quality, low total counts do not necessarily carry the opposite implication. Further, there is considerable evidence to indicate that total numbers of microorganisms cannot be used as a measure of safety.

The utility of food ingredients as raw materials for other products presents problems that are peculiar to each situation. In some cases, the total count is a direct measure of utility. But more often, it is necessary to assay the raw ingredient for the presence of specific organisms or groups known to be of significance in the performance of the final product.

REFERENCES

1. P. R. Elliott and H. D. Michener. Microbiological Process Report. Microbiological standards and handling codes for chilled and frozen foods. *Appl. Microbiol.* 9, 452–68 (1961).
2. F. S. Thatcher. Microbiological standards for foods: Their function and limitations. *J. Appl. Bacteriol.* 18, 449–461 (1955).
3. G. M. Dack, E. Wheaton, M. N. Mickelson, and M. N. Schuler. Public health significance of microorganisms in frozen pot pies. *Quick Frozen Foods* 22, 44–45, 160, 162 (1960).
4. D. A. A. Mossel. Microbiologische kwaliteitseisen voor voedings-middelen in het bijzonder voor export-artikelen. *Conserva* 1, 271–279 (1953).
5. B. E. Proctor and J. T. R. Nickerson. Improving frozen foods through microbiological control. *Food Ind.* 20, 682–683 (1948).
6. F. S. Thatcher. Microbiological standards for foods. II. What may microbiological standards mean? *Food Technol.* 12, 117–122 (1958).
7. A. C. Peterson and M. F. Gunderson. Role of psychrophilic bacteria in frozen food spoilage. *Food Technol.* 14, 413–417 (1960).
8. J. L. Shank. Unpublished data (1962).
9. J. H. Silliker and J. L. Shank. Unpublished data (1958).
10. Z. J. Ordal. Anaerobic packaging of fresh meat. *Proc. 14th Res. Conf. Amer. Meat Inst. Found. Circ.* 70, 39–45 (1962).

11. B. C. Hobbs and J. G. Wilson. Contamination of wholesale meat supplies with salmonellae and heat-resistant *Clostridium welchii. Monthly Bull. Ministry Health Lab. Serv. Gt. Brit.* 18, 198–206 (1959).

12. J. H. Silliker, R. A. Greenberg, and W. R. Schack. Effect of individual curing ingredients on the shelf stability of canned comminuted meats. *Food Technol.* 12, 551–553 (1958).

13. R. A. Greenberg, J. H. Silliker, and W. R. Schack. Unpublished data (1958).

Microbial Indexes of Food Quality:

The Coliform Group

FRANK R. PEABODY

Michigan State University, East Lansing, Michigan

The use of the coliform group of bacteria as indicators of fecal pollution of drinking water supplies has been a standard practice for nearly 60 years (*1*). Because of the direct correlation with the source of contamination, coliform organisms have given an apparently satisfactory measurement of the sanitary quality and presence of pathogenic bacteria. For many years the dairy industry has used this group as part of the standard analysis for determining the acceptability of a milk supply (*2*). In this case these organisms are more likely to be of animal origin and are less an indication of the presence of human pathogens and more a measurement of the general sanitary conditions of handling and processing. Because of the larger numbers of organisms present and the better nutritive quality of the sample as a substrate, more toxic selective media and plate counting methods are frequently used.

Now the coliform group is being used as a measurement of quality in food products in general and for frozen foods in particular. Are we justified in transferring this test which has been useful in the water and dairy fields to foods? Does this group of organisms represent insanitary conditions when found in a food product? Does it indicate any hazards of pathogenic bacteria? Do our present methods recover all of the viable organisms in a food sample? What interference may there be between selective and indicator systems in the medium and the food material which must be added to it—especially in samples with low bacterial population? How can the recovery rates be improved? These are some of the questions that must be answered before the coliform group may be established on a firm basis as an indicator of food quality.

In studying the problem of growth of the coliform group, the profound effect of environment on bacterial growth is obvious. An illustration of

the effect of improved nutritional factors is the work of Darby and Mallmann (*3*) who demonstrated the advantage of lauryl tryptose broth over standard lactose broth for the primary isolation of the coliform group. Of particular interest here is the fact that this difference was most pronounced on those samples containing organisms which had been weakened or attenuated by one means or another. The effect of pH has also been widely studied. A recent example is the study of Aea and Bushnell (*4*) on a frozen fruit juice with a pH of about 3.0. Coliform organisms were never found although many other micro-organisms were isolated from the samples. They point out that the coliform group would be a poor indicator of sanitary quality in this particular food. Many other factors have been similarly studied.

However, there may be some effects of the environment that are more subtle than those cited. The drop-plate surface agar technic was recently reported for isolating coliform organisms from high-population samples (*5*). When samples of water up to 0.1 ml in volume are placed on the dried agar surfaces, the water spreads out evenly and disappears rapidly leaving each organism or clump of cells in a tiny droplet culture on the surface. In effect, this is creating a "restricted environment" for the particular cell so that it can more readily initiate cell division and growth. Higher counts were obtained by this method than with the standard tube dilution method for the initial isolation of coliform organisms. Of greater significance was the fact that this increase was greater with samples from a "clean" stream than when testing heavily contaminated waters or sewage effluent.

These observations seem to correlate with the work of Wright et al. (*6*) who observed that single cell isolation of the hairy root and crown gall organisms would grow about 90% of the time when planted into tiny hanging drops as compared to less than one per cent of the time when inoculated into 10 ml of the same broth.

At an earlier session of this symposium, Mallmann reported on the use of a "shake tube" method in which even greater increases in coliform indices have been noted—especially on those samples in which the organisms have remained for a long period of time or for some other reason have become weakened.

All of these reports indicate that the application of the coliform index as a measurement of the quality of food products must be approached with caution and a full understanding of the limitations of our methodology for recovering viable organisms from the food products.

For the past year we have been working on the use of coliforms as indicators of quality in precooked frozen foods. Some of our experiences

TABLE I

COMPARISON OF FOUR MEDIA FOR THE ISOLATION OF COLIFORM BACTERIA FROM
PRECOOKED, FROZEN FOODS (ORGANISMS/GRAM)

Sample No.	Lauryl tryptose broth	Violet red bile agar	Deoxycholate agar	Lauryl tryptose bile agar
1	7.	360	380	250
	7.	280	190	160
2	0.5	31	2	37
	7.	49	3	27
4	4.8	44	34	49
5	4.8	0	0	0
	2.6	0	0	0
8	10.8	6	11	4
	4.8	11	2	2
9	7.	14	5	6
	2.6	7	3	5
10	2.6	0	11	16
11	0.1	0	0	2
	0.2	0	0	0
12	1.	47	41	49
	0.7	0	1	2
13	4.6	200	210	160
	15.8	270	240	140
A–1	110.	200	120	160
	7.	73	91	86
A–2	10.	65	57	53
	48.	160	180	200
A–3	2.6	4	4	48
	2.6	6	8	0
A–4	3.	70	6	230
	0.3	72	34	500
A–5	7.	52	40	860
A–6	1.5	58	44	2800
A–17	4.8	15	88	120
	4.8	57	110	140
A–18	4.8	6	250	50
	4.8	6	7	6
A–19	4.8	150	110	250
	4.8	79	80	120
A–22	70.	40	160	210
	48.	15	92	150
Arith Mean	11.7	68	73	191[a]

[a] 117 if the 2800 sample is omitted.

will illustrate a few of the problems that may be encountered. For discussion of some of these difficulties, data on a series of frozen meat, poultry, and fish pies are given in Table I. This includes 20 different samples and in most cases the evaluation on both halves of the pair is given. (There were a few cases where the data were not complete for all media on the other half.) The media used included: lauryl tryptose broth (Difco), violet red bile agar (Difco), deoxycholate agar (Difco), and an experimental agar designated as lauryl tryptose bile agar. The latter medium is constituted by adding 0.15% bile salts #3 (Difco) and 1.5% agar to the lauryl tryptose broth. All counts are expressed in terms of organisms per gram of food product.

A major problem arises in simply trying to determine which of the methods might be the most satisfactory. Comparing samples 1, 2, and A-22 shows that with different samples each one of the plating media can be shown to be superior. Even if we assume the lauryl tryptose to be a "base line" there are problems as shown by samples A-17, A-18, and A-19 where the coliform index on lauryl tryptose broth happened to be 4.8 on all six trials. The coliform count on violet red bile agar ranged from 6 to 150, on the deoxycholate agar from 7 to 250, and on the lauryl tryptose bile agar from 6 to 250. What conclusions can be drawn from data such as these? Also to be noted are marked discrepancies between the two halves of the same pie—both consistent and erratic—as seen in samples 12 and A-18.

One thing that seemed very consistent was the lower lauryl tryptose broth results in comparison with the plating media. Counts were made of typical colonies as generally described and directed. However, attempts to confirm these colonies usually resulted in less than 10% showing the ability to ferment lactose when transferred into lauryl tryptose broth. Studies to determine the cause of this low confirmation rate are in progress. Whether they represent "false positives" on the plates or true coliforms that have been altered so that they will appear typical on the plate and yet cannot ferment lactose in the tubes is yet to be determined.

More information on the behavior of the coliform organisms during and after the freezing process seems to be essential to working out a better understanding of this whole situation. Two approaches to this are presently underway: inoculation of autoclaved pies with a laboratory culture of a coliform organism and subsequent freezing and sampling of the pies; and freezing of laboratory cultures in media, such as brain heart infusion or in buffers. This permits the use of tryptone glucose extract agar as a base with which to compare the inhibitory media used for the regular isolation of the coliform group. In general, it was found that there were

greater reductions on the selective media after freezing than were found with the non-selective, non-toxic media. This suggests that there is some damage to the cell during the freezing process which renders it less capable of growing in the presence of the inhibitory agents of the selective media, but might be recovered under the proper environmental conditions. The different standards for direct microscopic and plate counts established in "Recommended Methods for the Microbiological Examination of Foods" (7) recognizes that there are many cells present in a frozen food product which do not grow on media used for the total bacterial count. Is it possible that some of these cells are viable but have been affected by the freezing so that they do not respond under the conditions of the test?

The principals of the restricted environment were again applied to increase the recovery rates of frozen cells. In this instance, activated charcoal was added to the lauryl tryptose broth with the idea that the small particles would form a focal point to which the bacterial cell would adhere and thus create a "restricted environment" within the larger environment of the tube of broth. The surface plating method could also be applied when the counts of the sample were high enough to make this possible. In Figure 1, is presented a comparison of the recoveries on lauryl
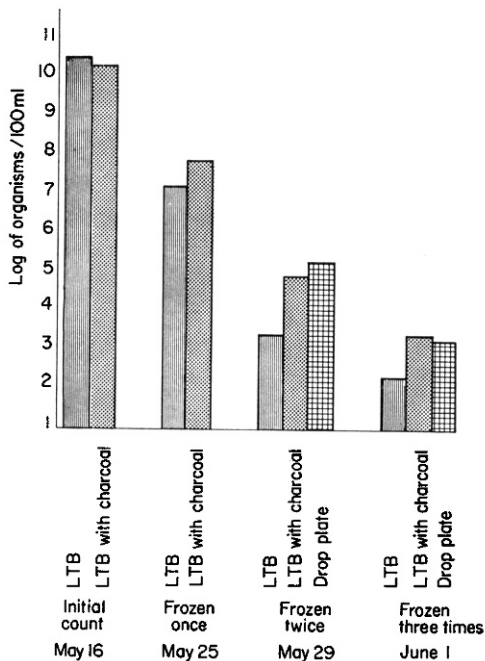
FIG. 1. A comparison of different techniques for the recovery of *Aerobacter aerogenes* after freezing.

tryptose broth, lauryl tryptose broth with charcoal added, and, after the second freezing, the drop plate technic on lauryl tryptose bile agar. The expected decrease in total number of cells after freezing and thawing is evident, but the standard medium was much poorer in recovering the *Aerobacter aerogenes* than the other two media. This would indicate that at least some of the cells in a frozen product which we assume to have been killed, are capable of growing if we can place them in the proper environment. Attempts to create a more restricted environment by the two methods mentioned have not yet been found satisfactory due to the nature of the product. The drop plate method is only applicable when the bacterial populations are high and the presence of food particles in the shake tube masks the colonies present. Both of these difficulties stem from the fact that the bacterial count of frozen foods is of a relatively low order.

The coliform group has become well established as an indicator group in the fields of water and dairy microbiology. The food industry had found it a useful tool in maintaining good sanitary conditions. This paper has presented some of the problems that confront us in transferring a microbiological test from one product to another, in terms of technics as well as interpretation of results. The problems are not insurmountable. There are many challenges for good research—both basic and applied—in obtaining the full understanding that is necessary to establish the coliform group on a firm basis as one of the indicator organisms that may be used to measure the quality of our food products.

REFERENCES

1. American Public Health Assoc. "Standard Methods of Water Analysis."
   1st Ed. New York (1905).
2. American Public Health Assoc. "Standard Methods for the Examination of
   Milk." 6th Ed. New York (1934).
3. C. W. Darby and W. L. Mallmann. Studies on media for coliform organ-
   isms. *J. of Am. Water Works Assoc.* 31, 689–706 (1939).
4. R. F. F. Aea and O. A. Bushnell. Survival times of selected enteropatho-
   genic bacteria in frozen passionfruit nectar base. *Appl. Microbiol.* 10,
   277–279 (1962).
5. W. L. Mallmann and F R. Peabody. Coliforms determined by surface
   plating technique. *Water and Sewage Works* 109, 146–149 (1962).
6. W. H. Wright, A. A. Hendrickson, and A. J. Riker. Studies on the progeny
   of single cell isolations from the hairy-root and crown-gall organisms.
   J. Agr. Res. 41, 541–547 (1930).
7. American Public Health Assoc. "Recommended Methods for the Micro-
   biological Examination of Foods." 1st Ed. New York (1958).

Microbial Indexes of Food Quality:

Fecal Streptococci

C. F. NIVEN, JR.

American Meat Institute Foundation, Chicago, Illinois

To be of value, a microbiological index of food quality or pollution must perform a specific function, and the results from its use should be meaningful and interpretable. It must reflect the public health hazards that may be present due to an inherent condition or prior treatment of the food, and in some instances, it should give some indication as to keeping quality or spoilage potential of the food. Although the idea is not new (*1*), growing interest currently is being devoted to the feasibility of using the fecal streptococci as practical indexes of fecal pollution in water, as well as microbiological quality and sanitary history among a number of foods.

Definition

Nowadays, where the term "fecal streptococcus" is used, it becomes necessary to define the groups or species of microorganisms being discussed. Historically, there has been some degree of synonymy among the terms fecal streptococci, enterococci, and group D streptococci. Present-day authors, however, are inclined to use these terms rather loosely and, indeed, one sees diverse species limitations being employed especially with reference to the fecal streptococci.

In 1938, Sherman (*2*) stated in a publication on the enterococci and related streptococci: "The enterococcus, as the term is commonly used among bacteriologists, has about as much biological meaning as 'the bear' . . . a screen behind which the investigator could hide his ignorance of the organisms with which he worked." Today, we see an even more confused state in the literature, to the point that a convenient, meaningful, and useable term may have to be abandoned because of its abuse.

In his classical review, Sherman (*3*) divided the genus *Streptococcus*

into four major groups, each of which could be separated from the other on the basis of temperature limits of growth, in combination with easily recognizable physiological characteristics. One of the groups, the enterococcus group, was comprised of four species recognized at that time; namely, *Streptococcus faecalis*, *Streptococcus zymogenes*, *Streptococcus liquefaciens*, and *Streptococcus durans*. The first three species were considered to be very closely related, but could be separated on the basis of their hemolytic and proteolytic capacities. Sherman actually suggested that they be considered varieties of one species. All belonged to serological group D.

Subsequent studies by numerous authors have resulted in general agreement that *Streptococcus faecium* (*4*) is a definite entity within the enterococcus group. Lake *et al.* (*5*) have suggested two species with respective varieties as indicated in Table I. Shattock (*6*) has tabulated some of the

TABLE I

STREPTOCOCCAL SPECIES BELONGING TO LANCEFIELD'S SEROLOGICAL GROUP D

Enterococci	Other streptococci
S. faecalis var. *liquefaciens* var. *zymogenes* *S. faecium* var. *durans*	*S. bovis* *S. equinus*

salient characteristics which differentiate the enterococcal species according to the modern system of classification. In addition, it has been reported that *S. faecalis* and varieties obtain energy for growth from pyruvate, serine, and citrate, with an attendant requirement for the growth factor lipoic acid (*7*, *8*). Malate and arginine are also utilized as energy sources, but no lipoic acid requirement is demonstrable in these fermentations (*9*). *Streptococcus faecium* and *S. durans* var. do not utilize these substrates as energy sources, but further differ from *S. faecalis* var. in that they generally require folic acid in a defined medium (*5*).

The term enterococcus, where used in this restricted sense, and using the newer concepts of speciation, remains a practical and useable vernacular name which is meaningful from the taxonomic point of view. A plea is hereby made to retain this term to denote only those species or varieties as indicated in Table I.

Group D *Streptococcus* is easier to define, since literally any *Streptococcus* which possesses the group D antigen necessarily belongs to this group. Sherman (*2*) and Niven (*10*) noted that *S. bovis* may cross-react with group D sera. Shattock (*11*) conclusively proved that *S. bovis* possesses an antigen identical to group D. More recently, Smith and Shattock (*12*) have demonstrated that *S. equinus* also possesses the group D antigen. Therefore, the group D streptococci encompass all species listed in Table I, and only await a more precise speciation of those microorganisms commonly found in the alimentary tract of the cow, horse, and pig, and to determine which of these streptococci having prescribed physiological entities possess the group D antigen. The species belonging to group D, according to present day concepts, are presented in Table I.

The term fecal streptococcus, is difficult, if not impossible, to delineate at this time. In its strictest sense, only the enterococci would be included. Shattock (*13*) proposed that fecal streptococci be limited to those species belonging to Lancefield's group D, such definition being accepted by Mossel *et al.* (*14*).

In its broadest sense, the fecal streptococci might encompass all those species which are commonly found in, or whose habitat is in, the intestine of man and warm-blooded animals (*15, 16*). If carried to extremes, practically all streptococcal species with the exception of Sherman's "lactic group" might be included. Certainly, under such conditions, *S. salivarius* should have to be included, since it may at times comprise by far the largest majority of the streptococci in the human intestine (*17*). Such a concept would appear to create an unnecessarily complex, cumbersome, and chaotic situation when it comes to the detection and use of streptococci as indices of food quality or pollution. Furthermore, when the literature is perused, one senses that there is a tendency to define fecal streptococci as those which can grow on a particular selective or differential medium, rather than delimiting the group to distinct taxonomic entities.

No attempt will be made here to define fecal streptococci, but it would appear that restricting its use either to the enterococci or to the group D streptococci would be of practical value to both the food microbiologist and the public health investigator.

Habitat

As stated at the outset, the index microorganism employed for food quality should be of fecal origin in order to be useful. There is no doubt in anyone's mind that the intestine of man and animals is the habitat of all group D streptococci. Nevertheless, it is known that these streptococci, especially the enterococci, can become established in a food processing

plant as a common contaminant, and their introduction into foods does not imply direct fecal contamination.

Sherman (*18*) noted that group D streptococci, especially the proteolytic varieties, are commonly found on plants. In a series of reports, Mundt and associates (*19, 20, 21, 22*) reported the widespread occurrence of enterococci in soils, on wild and cultivated plants, and from insects. These authors, however, concluded that their studies did not preclude the possibility of these organisms having originated from mammalian fecal material. In spite of the original source of these streptococci, their presence leaves some doubt as to their significance in chilled or frozen vegetables and fruit juices which have not been heated sufficiently to kill them.

Streptococcus bovis is associated with bovine animals and perhaps the pig (*23*), whereas *S. equinus* is found in the gut of the horse and pig. Both, however, may occasionally be found in the human intestine. Disagreement persists as to whether these two species should be included in tests for food quality. Various attempts have been made to either devise selective media which will exclude these microorganisms, or else devise simple means for their differentiation so that more exact information as to source of fecal pollution can be ascertained (*15*). Others would prefer to include all group D streptococci (*14*). While admittedly fecal pollution of any type is esthetically undesirable in foods, strict interpretation as to pollution and health hazards may exert undue limitations on some classes of foods. For example, the presence of *S. bovis* in either raw or pasteurized milk should not be received with a critical eye, and indeed, should be expected at times.

Of greater current interest is the attempt by several investigators to differentiate between *S. faecalis* and *S. faecium* in water and food, and to draw conclusions as to their significance with respect to source of pollution. *Streptococcus faecalis* and varieties are known to be common inhabitants of the human intestine and less commonly associated with other animals. However, clear-cut host specificity appears not to exist. Bartley and Slanetz (*24*) noted considerable variation in the predominant types of fecal streptococci from different individuals, and even in the same individuals when examined at different times. In our laboratory, we have found similar variations from one human to another and, in fact, *S. faecium* did not appear to be uncommon at all in the human intestine (unpublished data). Conversely, *S. faecalis* is common in the chicken gut, especially from those fed diets containing low levels of tetracycline antibiotics (*25, 26*).

Barnes (*27*) employed the reduction of tetrazolium as a means for distinguishing between *S. faecalis* and *S. faecium*. This substance was

incorporated into a thallous acetate agar medium for the selective and differential detection of the group D *Streptococcus* species (*28, 29, 30*). Barnes and Ingram (*31, 32*) noted the predominance of *S. faecium* over *S. faecalis* in the gut and on the skin of pigs. They concluded that pasteurized canned hams spoil much more commonly from growth of *S. faecium* because this species is associated with the pig, whereas *S. faecalis* would denote contamination of human origin. Spoilage of pastureized canned hams by *S. faecium* has been confirmed by many laboratories, including our own. However, it would seem that common spoilage by *S. faecium* is due to its greater heat tolerance, thus affording a better opportunity to survive the marginal heat processing to which such hams are subjected. Therefore, it is questionable that the type of enterococcal spoilage denotes accurately the source of contamination.

Because of the ubiquity of both *S. faecalis* and *S. faecium*, it would appear premature at this time to assess greater significance of one over the other as an index of food quality. It should be recalled that Orla-Jensen (*4*) originally described *S. faecium* on the basis of cultures isolated from the human intestine. *Streptococcus durans* also commonly occurs in the human intestine (*18*). If, however, further evidence indicates that a species distinction would be desirable in food examinations, such might be accomplished easily by drawing upon the unique energy sources for growth by *S. faecalis* in developing new selective plating media. This may have already been done, though perhaps unwittingly, by Reinbold *et al.* (*33*) who devised an azide-ditetrazolium agar in which citrate serves as the energy source. This medium deserves further study to determine its specificity.

Selective Media

It is not intended here to review critically the various media that have been proposed for the detection of fecal streptococci in water, sewage, and foods. Suffice it to say that advantage has been taken of the versatility of the fecal streptococci, particularly the enterococci, to grow under diverse environmental conditions, and to tolerate a number of substances in concentrations that are inhibitory to most other groups of bacteria. The majority of the media depend upon added sodium azide to inhibit the catalase-positive bacteria. It is surprising that so many of these media perform as well as they do, considering the similarity of characteristics shared by other lactic acid bacteria which might allow them to grow also. It is possible that restrictions in the flora of the samples examined play an important part in their success.

Some examples of proposed selective media are presented in Table II.

TABLE II

SELECTIVE OR DIFFERENTIAL MEDIA FOR DETECTING FECAL STREPTOCOCCI

Authors and media designations	Selective or differential agents	Specified conditions
Broth	Azide, 0.5[a]	
Hajna (34) (BAGG)	Brom cresol purple, .015	45°C
Litsky et al. (35, 36) presumptive confirmatory (EVA)	Azide, 0.2 Azide, 0.4 Ethyl violet, 0.005	
Kenner et al. (16) (KF)	Azide, 0.4 Brom cresol purple, .015	Tetrazolium, 0.1, for agar medium
Agar	Azide, 0.5	39.5°C
Packer (37); Mossel et al. (14)	Crystal violet, 0.002	in blood agar base
White and Sherman (38) Dack et al. (39)	Azide, 0.1 Penicillin G, 100 units	
Slanetz and Bartley (40) (M-Enterococcus)	Azide, 0.4 Tetrazolium, 0.1	Membrane filter technique
Barnes (28, 30) (TITG)	Thallous acetate, 1.0 Tetrazolium 0.1	pH 6.0
Horie and Saheki (41) (ELA)	Azide, 0.225 Esculin, 1 Ferric ammonium citrate, 2	pH 9.0

[a] Grams per liter where indicated.

Index of Water Pollution

The use of fecal streptococci as an index of water pollution appears to be more popular in Europe than in America. Their poor acceptance here seems to stem from their relatively low recovery rates in comparison with the coliform bacteria, the lack of a standardized testing procedure, poor quantitative agreement among the proposed methods, and some hesitation in interpreting the significance of the various species detected.

There is general agreement among the various investigators that enterococci are usually present in lower numbers than the coliforms in surface and polluted waters, and that they tend to die more rapidly. In light of the relatively greater fastidious nature of the enterococci, such findings should not be too surprising. The enterococci are considerably more exacting than the coliforms in their nutritive requirements and available energy sources for growth. Horie (42) noted that enterococci diminished more rapidly than *Escherichia coli* in well water, while in sea water the reverse

was true. No appreciable growth of enterococci occurred in well water upon the addition of 250 ppm of peptone and yeast extract. On the other hand, 25 ppm of the nutrients resulted in remarkable growth of *E. coli*. Both microorganisms tended to die rather rapidly in sea water.

Leinenger and McCleskey (*43*) considered that enterococci offer certain advantages over coliforms as indicator organisms for water in that they apparently never multiply in waters, as some coliforms do, but disappear rather rapidly when added to streams or lakes.

Litsky *et al.* (*44*) found that a correlation of +0.9 exists between the numbers of coliform bacteria and enterococci in sewage from settling tanks. The population density of coliform bacteria was approximately thirteen times that of enterococci. Croft (*45*) found 21% as many enterococci as coliforms in flowing water, 23% as many in impounded water, 42% in wells, and 10% in sewage.

Thus, it would appear that the enterococcus or fecal streptococcus index might serve as an effective supplement to the coliform index in examining waters of various types, but it is unlikely that it could serve effectively as a substitute.

Index of Sanitary History of Frozen Foods

There appears to be remarkable agreement among various laboratories as to the high degree of persistence of enterococci as compared with coliforms in foods that are maintained in a frozen condition. Thus, it seems that enterococcus indexes in combination with total counts would have distinct advantages over the coliform index in assessing the sanitary history of the frozen foods prior to freezing. This would be particularly true among foods that are precooked sufficiently to reduce the bacterial population and to kill the fecal streptococci and coliforms prior to freezing. In such instances, one can determine with a reasonable degree of accuracy the effectiveness of in-plant sanitation regardless of the length of time the food has been held in a frozen state. For those foods that are frozen without being precooked, different interpretations may be in order.

Advantages of the fecal streptococcus index for frozen orange concentrate have been pointed out by Hahn and Appleman (*46*), Ferraro and Appleman (*47*), and Larkin *et al.* (*48*); for frozen fruits and vegetables by Burton (*49*) and Larkin *et al.* (*50, 51*); frozen meat pies by Kereluk (*52*) and Kereluk and Gunderson (*53*); frozen chicken pies by Raj *et al.* (*54*); and Hartman (*55*); and precooked frozen dinners by Zabarowski *et al.* (*56*).

Index of Spoilage Potential

Previous mention has been made of the not infrequent spoilage of pas-

teurized canned hams due to extensive growth of *S. faecium*. Spoilage makes itself apparent by the presence of a sour odor and flavor, and by poor color retention after removal from the cans. No swelling of the cans is apparent. *Streptococcus faecium* is particularly suited to survival and growth in this food because of its relative heat resistance, salt tolerance, and ability to grow at normal refrigerator temperatures. This microorganism is capable of surviving the minimum processing temperatures required by regulatory officials, and the processor has learned to cook to an internal temperature of at least 70°C in order to reduce the spoilage hazard.

Rieman (*57*) has demonstrated the persistence of group D streptococci in ham curing brines, and pointed out that particular care must be exercised to maintain their population levels at a minimum in the brines in order to reduce the spoilage incidence. This is especially true among European establishments where the curing brines may be used more than once. Some establishments have set standards for group D streptococcus levels for both curing pickles and pasteurized hams, especially if they are intended for export trade.

Utility in Food Fermentations

Despite the stigma attached to the enterococci because of their association with fecal pollution, insanitary handling of foods, occasional low grade infections, and food poisoning, they are also associated with several desirable food fermentations. Their wide temperature limits of growth, their homofermentative nature, ability to grow in rather high salt concentrations, as well as their relative tolerance toward a number of chemicals, and ability to grow under anaerobic conditions, predispose their ubiquity in nature, and especially in foods. Hardly any food not receiving terminal heating can be prepared feasibly without being contaminated with these microorganisms. If held above the freezing point for a reasonable length of time, they will grow.

Many desirable food fermentations are accompanied by more or less extensive enterococcal growth. It would be virtually impossible to prepare the large class of fermented sausages consumed the world over without some contamination and growth of the enterococci. Indeed, ten Cate (*58, 59*) claimed that they play an important part in aroma development of these sausages by participating in the early fermentation. They would be ideally suited to serve as pure culture starters for the fermented sausages.

Recognizing the potential value of group D streptococci for the rapid ripening of cheddar cheese prepared from pasteurized milk, Dahlberg and Kosikowsky (*60*) proposed the use of a particular enterococcus culture

as a starter. This culture has been fed to human volunteers in large quantities without producing any ill effects (*39*). Cheese manufactured with this culture has achieved some commercial success.

It is entirely possible that other intentional food fermentations by group D streptococci could achieve similar success. The occasional food poisoning outbreak in which enterococci are allegedly implicated impedes further investigations along these lines.

It is not within the scope of this discussion to cite evidence in favor of, or against, the food poisoning potentialities of the enterococci. Suffice it to say that the arguments in favor of their toxigenic capacities are decidedly equivocal (*61*). The question begs to be resolved.

Concluding Comment

The effectiveness of any test for quality depends upon intelligent interpretation. From the evidence presented, it is obvious that fecal streptococci, along with total counts, can serve effectively as indices of food quality, particularly among the precooked frozen foods. Also, they can serve as useful supplemental indices in evaluating other foods and drink. On the other hand, a strict requirement for low population levels of fecal streptococci in such foods as milk, cheeses, and other dairy products, fermented sausages and vegetable foods, would virtually eliminate these foods from the human diet. It should be kept in mind that enterococci, though of fecal habitat, can establish themselves and grow in or on food substances far removed from the original contaminating source. Therefore, an interpretation of the fecal streptococcus population in terms of amount of fecal contamination in the food can approach the ridiculous. Nevertheless, this does not detract from the usefulness of the test for some classes of foods.

The utility of enterococci in some food fermentations, and the establishment of maximum permissible numbers in other foods, though seemingly illogical, may be entirely feasible. If the strains employed prove to be safe, and if they serve a unique and useful function, there is no logical reason for prohibiting their use.

There is a dearth of information concerning the persistence of the viruses in foods, especially frozen foods. It is possible that the fecal streptococci will prove to be superior to the coliform organisms in assessing the safety of foods with respect to hazards from the infectious hepatitis virus, as well as others. Our present state of knowledge, however, does not permit us to proceed any further than mere speculation.

Acceptance of the coliforms as index microorganisms by the early bacteriologist is a tribute to him and has contributed greatly to the elevation

of the health standards of the human population. Though the choice had merit, we must not take for granted that it is the best test for all classes of foods, especially among those prepared foods that were unheard of a half century ago. As so aptly stated by Buttiaux and Mossel (*62*), "No reproach can be levelled at these original investigators other than that they were not prophets." As we accept changes in food preparation and handling practises, the bacteriologist must be willing to accept newer testing procedures that will ensure maximum safety.

REFERENCES

1. C. A. E. Winslow and G. T. Palmer. A comparative study of the intestinal streptococci from the horse, cow and man. *J. Infect. Diseases* 7, 1–16 (1910).
2. J. M. Sherman. The enterococci and related streptococci. *J. Bacteriol.* 35, 81–93 (1938).
3. J. M. Sherman. The streptococci. *Bacteriol. Rev.* 1, 3–97 (1937).
4. S. Orla-Jensen. The lactic acid bacteria: A monograph. A. F. Høst, Copenhagen, 1919.
5. D. E. Lake, R. H. Deibel, and C. F. Niven, Jr. The identity of *Streptococcus faecium. Bacteriol. Proc.* p. 13 (1957).
6. P. M. F. Shattock. Enterococci. *In* "Chemical and Biological Hazards in Foods," pp. 303–319. Iowa State Univ. Press, Ames, Iowa, 1962.
7. R. H. Deibel, J. B. Evans, and C. F. Niven, Jr. Lipoic acid requirement for anaerobic utilization of pyruvate as an energy source by *Streptococcus faecalis. Bacteriol. Proc.* p. 114 (1958).
8. R. H. Deibel and C. F. Niven, Jr. Lipoic acid and the fermentation of serine and malate by *Streptococcus faecalis. Bacteriol. Proc.* p. 164 (1960).
9. R. H. Deibel. Arginine as an energy source for the growth of *Streptococcus faecalis. Bacteriol. Proc.* p. 163 (1960).
10. C. F. Niven, Jr. Serological grouping of the enterococci. Ph.D. Thesis, Cornell University, 1939.
11. P. M. F. Shattock. The streptococci of group D: The serological grouping of *Streptococcus bovis* and observations on serologically refractory group D strains. *J. Gen. Microbiol.* 3, 80–92 (1949).
12. D. G. Smith and P. M. F. Shattock. The serological grouping of *Streptococcus equinus. J. Gen. Microbiol.* 29, 731–736 (1962).
13. P. M. F. Shattock. The identification and classification of *Streptococcus faecalis* and some associated streptococci. *Ann. Inst. Pasteur Lille* 7, 95–100 (1955).
14. D. A. A. Mossel, H. M. J. van Diepen, and A. S. de Bruin. The enumeration of faecal streptococci in foods, using Packer's crystal violet sodium azide blood agar. *J. Appl. Bateriol.* 20, 265–272 (1957).
15. K. E. Cooper and F. M. Ramadan. Studies in the differentiation between human and animal pollution by means of faecal streptococci. *J. Gen. Microbiol.* 12, 180–190 (1955).

16. B. A. Kenner, H. F. Clark, and P. W. Kabler. Fecal streptococci. I. Cultivation and enumeration of streptococci in surface waters. *Appl. Bacteriol.* 9, 15–20 (1961).

17. C. F. Niven, Jr., K. L. Smiley, and J. M. Sherman. *Streptococcus salivarius* in the human intestine. *J. Bacteriol.* 43, 113–114 (1942).

18. J. M. Sherman and H. U. Wing. *Streptococcus durans. J. Dairy Sci.* 20, 165–167 (1937).

19. J. O. Mundt, A. H. Johnson, and R. Khatchikian. Incidence and nature of enterococci on plant materials. *Food Res.* 23, 186–193 (1958).

20. J. O. Mundt and A. H. Johnson. Physiological properties of streptococci isolated from plants. *Food Res.* 24, 218–223 (1959).

21. G. N. Eaves and J. O. Mundt. Distribution and characterization of streptococci from insects. *J. Insect Path.* 2, 289–298 (1960).

22. J. O. Mundt. Occurrence of enterococci: Bud, blossom, and soil studies. *Appl. Microbiol.* 9, 541–544 (1961).

23. P. Raibaud, M. Caulet, J. V. Galpin, and G. Mocquot. Studies on the bacterial flora of the alimentary tract of pigs. II. Streptococci: Selective enumeration and differentiation of the dominant group. *J. Appl. Bacteriol.* 24, 285–306 (1961).

24. C. H. Bartley and L. W. Slanetz. Types and sanitary significance of fecal streptococci isolated from feces, sewage, and water. *Am. J. Public Health* 50, 1545–1552 (1960).

25. S. D. Elliot and E. M. Barnes. Changes in serological type and antibiotic resistance of Lancefield group D streptococci in chickens receiving dietary chlorotetracycline. *J. Gen. Microbiol.* 20, 426–433 (1959).

26. B. A. Kenner, H. F. Clark, and P. W. Kabler. Fecal streptococci. II. Quantification of streptococci in feces. *Am. J. Public Health* 50, 1553–1559 (1960).

27. E. M. Barnes. Tetrazolium reduction as a means of differentiating *Streptococcus faecalis* from *Streptococcus faecium* (Orla-Jensen). *J. Gen. Microbiol.* 14, 57–68 (1956).

28. E. M. Barnes. Methods for the isolation of faecal streptococci (Lancefield group D) from bacon factories. *J. Appl. Bacteriol.* 19, 193–203 (1956).

29. T. F. Medrek and E. M. Barnes. A note on the growth of *Streptococcus bovis* in thallous acetate and tetrazolium media. *J. Appl. Bacteriol.* 21, 79 (1958).

30. E. M. Barnes. Differential and selective media for the faecal streptococci. *J. Sci. Food Agr.* 10, 656–662 (1959).

31. E. M. Barnes and M. Ingram. The identity and origin of faecal streptococci in canned hams. *Ann. Inst. Pasteur Lille* 7, 115–119 (1955).

32. E. M. Barnes, M. Ingram, and G. C. Ingram. The distribution and significance of different species of faecal streptococci in bacon factories. *J. Appl. Bacteriol.* 19, 204–211 (1956).

33. G. W. Reinbold, M. Swern, and R. V. Hussong. A plating medium for the isolation and enumeration of enterococci. *J. Dairy Sci.* 36, 1–6 (1953).

34. A. A. Hajna. A buffered azide glucose-glycerol broth for the presumptive and confirmatory tests for fecal streptococci. *Public Health Lab.* 9, 80–81 (1951).

35. W. Litsky, W. L. Mallmann, and C. W. Fifield. A new medium for the detection of enterococci in water. *Am. J. Public Health* **43**, 873–879 (1953).

36. W. Litsky, W. L. Mallmann, and C. W. Fifield. Comparison of the most probable numbers of *Escherichia coli* and enterococci in river waters. *Am. J. Public Health* **45**, 1049–1053 (1955).

37. R. A. Packer. The use of sodium azide (NaN₃) and crystal violet in a selective medium for streptococci and *Erysipelothrix rhusiopathiae*. *J. Bacteriol.* **46**, 343–349 (1943).

38. J. C. White and J. M. Sherman. Occurrence of enterococci in milk. *J. Bacteriol.* **48**, 262 (1944).

39. G. M. Dack, C. F. Niven, Jr., J. B. Kirsner, and H. Marshall. Feeding tests on human volunteers with enterococci and tyramine. *J. Infect. Diseases* **85**, 131–138 (1949).

40. L. W. Slanetz and C. H. Bartley. Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium. *J. Bacteriol.* **74**, 591–595 (1957).

41. S. Horie and K. Saheki. Studies on enterococci as pollution indices of food and drink. X. A new agar medium for the selection of enterococci. *Bull. Japan. Soc. Sci. Fisheries* **26**, 623–626 (1960).

42. S. Horie. Studies on enterococci as pollution indices of food and drink. IX. Viability of enterococci in waters and foodstuffs. *Bull. Japan. Soc. Sci. Fisheries* **26**, 614–622 (1960).

43. H. V. Leinenger and McCleskey. Bacterial indicators of pollution in surface waters. *Appl. Microbiol.* **1**, 119–124 (1953).

44. W. Litsky, M. J. Rosenbaum, and R. L. France. A comparison of the most probable numbers of coliform bacteria and enterococci in raw sewage. *Appl. Microbiol.* **1**, 247–250 (1953).

45. C. C. Croft. A comparative study of media for detection of enterococci in water. *Am. J. Public Health* **49**, 1379–1387 (1959).

46. S. S. Hahn and M. D. Appleman. Microbiology of frozen orange concentrate. I. Survival of enteric organisms in frozen orange concentrate. *Food Technol.* **6**, 156–157 (1952).

47. F. M. Ferraro and M. D. Appleman. Microbiology of frozen orange concentrate. IV. Further studies of enterococci in frozen orange concentrate. *Appl. Microbiol.* **5**, 300–303 (1957).

48. E. P. Larkin, W. Litsky, and J. E. Fuller. Fecal streptococci in frozen foods. III. Effect of freezing storage on *Escherichia coli*, *Streptococcus faecalis*, and *Streptococcus liquefaciens* inoculated into orange concentrate. *Appl. Microbiol.* **3**, 104–106 (1955).

49. M. O. Burton. Comparison of coliform and enterococcus organisms as indices of pollution in frozen foods. *Food Res.* **14**, 434–438 (1949).

50. E. P. Larkin, W. Litsky, and J. E. Fuller. Fecal streptococci in frozen foods. I. A bacteriological survey of some commercially frozen foods. *Appl. Microbiol.* **3**, 98–101 (1955).

51. E. P. Larkin, W. Litsky, and J. E. Fuller. Fecal streptococci in frozen foods. II. Effect of freezing storage on *Escherichia coli* and some fecal streptococci inoculated onto green beans. *Appl. Microbiol.* **3**, 102–104 (1955).

52. K. Kereluk. Studies on the bacteriological quality of frozen meat pies. III. Identification of enterococci isolated from frozen meat pies. *Appl. Microbiol.* 7, 324–326 (1959).

53. K. Kereluk and M. F. Gunderson. Studies on the bacteriological quality of frozen meat pies. IV. Longevity studies on the coliform bacteria and enterococci at low temperatures. *Appl. Microbiol.* 7, 327–328 (1959).

54. H. Raj, W. J. Wiebe, and J. Liston. Detection and enumeration of fecal indicator organisms in frozen sea foods. *Appl. Microbiol.* 9, 295–303 (1961).

55. P. A. Hartman. Enterococcus: coliform ratios in frozen chicken pies. *Appl. Microbiol.* 8, 114–116 (1960).

56. H. Zabarowski, D. A. Huber, and M. M. Rayman. Evaluation of microbiological methods for the examination of precooked frozen foods. *Appl. Microbiol.* 6, 97–104 (1958).

57. H. Rieman. The survival of some group D Streptococci in ham curing brines. The microbiology of fish and meat curing brines. *Proc. Intern. Symp. Food Microbiol. 2nd, Cambridge.* p. 263–269 (1957).

58. L. ten Cate. De microbiologie der "geschwitzte" snijworst. *Tijdschr. Diergeneesk.* 85, 743 (1960).

59. L. ten Cate. Het aroma der snijworst. *Tijdschr. Diergeneesk.* 85, 859 (1960).

60. A. C. Dahlberg and F. V. Kosikowsky. The bacterial count, tyramine content and quality score of commercial American cheddar and stirred curd cheese made with *Streptococcus faecalis* starter. *J. Dairy Sci.* 32, 630–636 (1949).

61. J. H. Silliker and R. H. Deibel. On the association of enterococci with food poisoning. *Bacteriol. Proc.* p. 48 (1960).

62. R. Buttiaux and D. A. A. Mossel. The significance of various organisms of faecal origin in foods and drinking water. *J. Appl. Bacteriol.* 24, 353–364 (1961).

Low Temperature Organisms
as Indexes of Quality of Fresh Meat[1]

J. C. AYRES

Iowa State University, Ames, Iowa

If from no other than a practical point of view, the following ecological evaluation of organisms associated with meats must be limited to those that are capable of proliferating on the chilled product.

Sufficient evidence has accumulated to indicate that many of the microorganisms capable of reproduction at refrigeration temperature multiply even more readily and rapidly at 20°–25°C. For these organisms, the term *psychrophilic* or *cold loving* is not accurately descriptive (*1, 2*). Within the past 5 years, considerable attention has been directed to the selection of a less confusing term for microorganisms that proliferate at low temperatures. Mossel and Zwart (*3*) and Eddy and Kitchell (*4*) have suggested that the word *psychrotrophic* or *cold thriving* or increasing is more in keeping with the activities of organisms that are able to grow at 5°C or less within a certain time and that the word psychrophile should be used to imply that the microorganisms require a low optimum temperature. Thus, the low temperature organisms with which this paper is concerned probably include more psychrotrophs than psychrophiles.

Whatever the terms used to describe bacteria, molds, and yeasts that increase at low temperatures, these microorganisms produce innumerable defects that alter the value of the foods in which they grow. So ubiquitous are these forms of life and so profound and widespread the changes that they produce that it is difficult to keep meats at refrigeration temperatures even for short periods of time without observing one or more of the following signs of deterioration: unclean, fruity, aromatic, fermented, musty, stale, or putrefractive odor; sweet, sour, moldy or bitter, rancid flavor; red, green, pink, yellow, blue, grey, black, or fluorescent discoloration;

creamy, sticky, ropy or slimy consistency, body, or texture. In addition, evidence of the presence of low temperature organisms in or on foods may be noted by proteolytic, lipolytic, and lipoxidative changes and, under some circumstances, by the production of acid and gas.

These microorganisms originate from many sources. In the case of meats, they are transferred to the carcass from the hides and feet of the animals, from the air, water, soil, and sawdust with which the carcass comes in contact, from the hands of workers and from knives, saws, brushes, and swab cloths.

The prominence of low temperature organisms on flesh foods has long been known. For example, Glage in 1901 (5) reported that the moist surfaces of meat stored at low temperature and high humidity became covered with bacterial colonies. These organisms, which he called "aroma-bakterien," were oval to rod shaped with rounded ends and occurred occasionally in chains. They were motile aerobes which liquefied gelatin slowly and turned litmus milk alkaline. They grew well at 2°C but poorly at 37°C; the optimum temperature was thought to be 10°–12°C. Glage noted that a characteristic aromatic odor, which he considered rather pleasant in the early stages, accompanied their growth. As these organisms grew, the surface of the meat became covered with tiny droplike colonies which increased in size and finally coalesced to form a viscous or slimy coating.

Since Glage's early studies, a number of investigators have reported on the kinds of bacteria, molds, and yeasts that are associated with chilled meats. Haines (6, 7) and Empey and Vickery (8), and Lochhead and Landerkin (9) were among the first to identify the bacterial flora responsible for "slimy" beef and "off-odor" poultry respectively. These results indicated that a surprisingly homogeneous microflora proliferated on cold beef and poultry carcasses. For example, spoilage of beef held at 0°C by an organism of the genus *Pseudomonas* was described by Haines (6) but, in 1933 (7) he stated that ". . . with the exception of a certain number of organisms of the *Pseudomonas* group and a few *Proteus*, the bacteria growing on lean meats stored in the range 4°–0°C almost all belong to the *Achromobacter* group." Independently, Empey and Vickery (8) concluded that 95% of the flora of beef capable of growth at −1°C consisted of members of the genus *Achromobacter*, while the remainder were species of *Pseudomonas* and *Micrococcus*. They further indicated that during storage, the relative numbers of *Achromobacter* and *Pseudomonas* increased while those of *Micrococcus* decreased.

Lochhead and Landerkin (9) did not describe the odor that they observed developing on New York dressed chicken other than to state that

it was an initial sign of spoilage. Six predominant types of bacteria associated with this condition were identified as representatives of the genera *Achromobacter (3), Flavobacterium,* and *Micrococcus (2).*

At about this same time Ingram *(10)* observed that, when varying concentrations of salt were added to small quantities of fresh minced pork and the mixtures stored in the refrigerator, the surface of the meat was ". . . entirely covered by a fluid film containing a *Pseudomonas* of practically pure culture" where salt levels of less than 4% were incorporated with the meat. At salt concentrations between 4 and 10%, molds predominated. After comparing the morphology and cultural properties of the bacteria he had isolated from slimy beef with Glage's aromabakterien, Haines *(11)* concluded that the two organisms were identical or closely related. Empey and Scott *(12)* considered that the four principal genera of low temperature bacteria isolated from fresh meat and constituting the flora of the carcass were: *Achromobacter,* 90%; *Micrococcus,* 7%; *Flavobacterium,* 3%; and *Pseudomonas,* less than 1%. Similarly, Jensen *(13)* reported that bacteria found on the surfaces of refrigerated meat were usually species of *Achromobacter* and *Pseudomonas,* and Jepsen *(14)* found *Achromobacter* to be predominant in the slime layer of refrigerated meats. Further, he indicated that the ability of organisms to grow at 2°C was restricted to the genera *Achromobacter* and *Pseudomonas.*

More recent work *(15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32)* indicates that species of *Pseudomonas* have a relatively greater importance in causing off-odor and slime of flesh foods than was assigned to them by the earlier investigators. It should be emphasized that in many of the studies conducted prior to the publication of the fifth edition of Bergey's *Manual (33)* only those organisms which formed greenish fluorescent pigment soluble in water were considered to be pseudomonads. Colorless colonies having similar properties were classified in the genus *Achromobacter.*

Attempts by Ayres *et al. (17)* to relate the biochemical patterns of bacterial isolates from slimy chicken to those of species identified in the 6th edition of Bergey's *Manual (34),* revealed differences from previously named species. Also in 1950, Sulzbacher *(18)* isolated twenty-eight *Pseudomonas* and four *Achromobacter* strains from frozen pork and six *Pseudomonas* strains from frozen lamb; all grew rapidly at 2°C. While most of these organisms were considered to be pseudomonads, they did not fit any of the recognized species.

Kirsch *et al. (24)* described the main characteristics of a number of organisms that they had isolated from two samples of refrigerated hamburger. Nine of the isolates produced green pigment and were charac-

terized as being similar to *Pseudomonas aeruginosa*, but the great ma-
jority (532) were motile, nonpigmented pseudomonads. Of these, 147 were
considered related to *Pseudomonas geniculata* and 88 to *Pseudomonas
fragi*, and a further 62 differed from *P. geniculata* only by their failure to
liquefy gelatin and were tentatively classified as *P. rugosa*; but the rest
(235) were not identified as to species. Other organisms such as *Micrococ-
cus* and *Lactobacillus* also were found (*24*). The presence of the last two
genera has been substantiated by Halleck *et al.* (*30, 31*).

In 1954, Ingram and Hobbs (*25*) reported that *Pseudomonas fluorescens*
occurred in hams both before and after curing, and Kitchell and Ingram
(*26*) identified an organism resembling *P. multistriata* as the dominant
species in the sliming of pork at 15°C. Wolin, *et al.* (*28*) also studied the
bacterial flora of refrigerated fresh beef and reported that 90% of the
isolates were either *P. geniculata* or differed from it primarily by an
inability to liquefy gelatin. Among the miscellaneous forms, one was
identified as *P. fragi*.

In 1958, Brown and Weidemann (*29*) reassessed the taxonomy of the
129 psychrophilic meat spoilage bacteria isolated by Empey and Scott in
1939 (*12*) and concluded that almost all of these were pseudomonads.
Formerly, 66 of the stock cultures were listed as *Pseudomonas*, 56 as
Achromobacter while 7 were unclassified. Empey and Scott (*12*) previ-
ously had allocated organisms to the genus *Pseudomonas* largely on the
basis of the production of a water soluble green pigment. Barnes and
Shrimpton (*32*) observed that, in chickens at the time of spoilage, non-
pigmented *Pseudomonas* strains considerably outnumbered *Achromo-
bacter*. In their opinion, the growth of nonpigmented pseudomonads
during storage explained the poor correlation between the development of
off-odors and the appearance of fluorescence. Further, they indicated that,
when these pseudomonads were replaced by *Achromobacter* strains and
yeasts, as in chlortetracycline-treated chickens, the odor completely
changed.

Ayres (*22, 23*) compared the reactions of pseudomonads isolated from
refrigerated sliced beef with those of identified species within the genus
and suggested that these isolates were similar to six described species of
Pseudomonas; i.e., *P. fluorescens*, *P. fragi*, *P. convexa*, *P. taetrolens* or
incognita, *P. putida*, and *P. ambigua* and one species each of *Aeromonas*
and *Xanthomonas*. Immediately after processing of raw flesh foods,
chromogenic cocci, yeasts, molds, and spore-forming microorganisms
make up at least 75%, and sometimes more than 80%, of the total
population. However, upon storage at low temperatures (10°C or less),
pseudomonads and closely related forms increase so rapidly that the

chromogenic and miscellaneous flora usually represent only 1–2% of the ultimate population.

When cold meat surfaces are exposed to warm air, condensation takes place, and the meats are said to "sweat." Such conditions favor the development of microbial contamination. Most psychrotrophic micro-organisms require high levels of moisture for proliferation. Particularly, this is true in the case of the pseudomonads which move in a thin film of moisture to form a slime on cut meat surfaces, skin, or fat layers. Often they appear initially in damp pockets, such as those between the fore leg and breast of the carcass or between the hind quarter and flank. When these surfaces become dry, microbial activity is reduced and eventually halted.

It is generally recognized that water requirements of some molds and yeasts are much less exacting than similar bacterial needs (*35*). Tomkins (*36*), Scott (*37*), Heintzeler (*38*), and Snow (*39*) demonstrated that spores of *Alternaria*, *Aspergillus*, *Penicillium*, and *Cladosporium* germi-nated in the range 0.80 and $0.90a_w$. Recently Scott (*40*) reported that, since the metabolism of microorganisms proceeds only in an aqueous environment, the approximate combinations of temperature and a_w at which growth of *Pseudomonas fluorescens* and *Candida* sp. occur in a supercooled environment depends upon the vapor pressure equilibrium of the remaining unfrozen water and ice. From idealized curves representing the temperature $-a_w$ limits for these two organisms at $-3°C$, the a_w limit for *P. fluorescens* is approximately 0.97, while that for the psychrophilic mold is less than 0.92. While it is likely that activities of enzymes of various microorganisms vary in the concentrations of salts required to halt individual metabolic processes, there is no known explanation for the different water activity requirements of different forms of life.

A sticky condition not to be confused with bacterial sliming was cited by Tomkins and Smith (*41*). This stickiness was found to be caused by extensive growth of molds prior to the development of aerial hyphae. Talayrach (*42*) was one of the first workers to identify molds responsible for the contamination of chilled meats. Later, Massee (*43*), Brooks and Kidd (*44*), and Wright (*45*, *46*) indicated that the discolored black spots appearing on chilled beef, veal, mutton, and lamb shipped to England from Argentina were due to the fungus threads of *Cladosporium herbarum*. Except for its disagreeable appearance, this defect was considered harm-less and entirely superficial. However, the mold was shown to be able to grow and produce the black spots on meat maintained at a temperature several degrees below the freezing point. In the main, the activity of these organisms was restricted to surfaces.

Mycelia of mold of the families Mucoraceae and Thamnidiaceae growing on cured meats were observed to produce "whiskers" in chill rooms maintained at high humidities. *Rhizopus*, *Mucor*, *Thamnidium*, and *Penicillium* were the genera commonly incriminated (*44, 45, 46, 47, 48, 49, 50*).

Semeniuk and Ball (*51*) made a rather extensive study of molds occurring on meat held in cold storage and concluded that only the following reached any importance from the standpoints of frequency of occurrence and extent of development: *Thamnidium* (including *Chaetostylum*), *Mucor*, *Cladosporium*, *Sporotrichum*, and *Penicillium*.

Wolf and Wolf (*52*) and Jensen (*53*) reviewed the literature on fungi found on meats. In addition to the molds already described, their listings include: *Alternaria*, *Aspergillus*, *Botrytis*, *Dematium*, *Hormodendrum*, *Monilia*, *Monascus*, and *Oospora*. However, as Semeniuk and Ball (*51*) state, the occurrence of many of these organisms has been recorded only occasionally and usually occurs when the meat is exposed to temperatures above 0°C.

In studies reported by Jensen (*53*) and Ayres (*20, 23*) of molds growing on refrigerated beef, the same genera recorded by the early workers were encountered. However, Ayres indicated that species of *Penicillium* were the most commonly isolated, with *Cladosporium*, *Thamnidium*, *Mucor*, and *Rhizopus* next in order. Single instances were reported in which *Monilia*, *Alternaria*, and *Sporotrichum* were identified. Also, *Aspergillus* was encountered occasionally on meat held at a temperature of 10°C or higher.

Recently Gunderson (*54*) isolated 113 different species in molds from frozen convenience food products and, from these, 52 were able to grow at, or below, 5°C. Twenty-two species of *Penicillium* were identified, but the single, most frequently encountered mold growing at 5°C was *Auerobasidium pullulans* (*Pullularia pullulans*). This species was exceptionally abundant in blueberry fruit fillings. Where meat products were concerned, *Aleurisma carnis* was the most commonly found.

Only a few low temperature yeasts have been reported. Strains of organisms belonging to four asporogenous and two sporogenous genera of yeasts; i.e., *Rhodotorula* (*22, 42, 44*); *Torulopsis* (*20, 22*); *Trichosporon* (*55*); *Candida* (*22, 35, 55*); *Saccharomyces* (*50*); and *Wardomyces* or *Hansenula* (*50*) have occasionally been recovered from refrigerated meat. With the exception of *Hansenula*, these same genera frequently have been isolated from poultry meat (*27, 56, 57*) and, in addition, three species of *Trichosporon* have been reported (*58*).

Recently, Ingram and Barnett (*59*) reported that, of about 350 strains of yeasts isolated from stored poultry, a large proportion appeared to be

Candida lipolytica and *Trichosporon infestans*. None of the strains produced ascospores and only twenty-three were found to ferment in glucose; these latter were considered characteristic of *Candida tropicalis*. A few strains were identified as *Trichosporon pullulans, Candida zeylanoides* and *Candida mycoderma* and unidentified pink yeasts. These results contrast with those reported by Walker and Ayres (*58*) wherein *Trichosporon pullulans, Torulopsis glabrata* and *Torulopsis famata* were those most commonly found.

Prior to the introduction of antibiotic dips or mists, deteriorative effects traceable to these organisms were rarely observed. However, when meats treated with one of the tetracyclines were stored for too long a time in the cold, microbial growth was rampant and the product became slick or sticky and developed a fermented, yeasty odor. There has been some concern that pathogenic yeasts may be among those that persist but, at the present time, these have not been recovered under the usual storage conditions.

It is of interest to note and germane to an understanding of the activities of low temperature yeasts that Lawrence *et al.* (*60*) observed that the predominant types of yeasts developing in Concord grape juice stored at temperatures of approximately 0°C belong to the four genera, *Candida, Torulopsis, Hanseniaspora,* and *Saccharomyces.*

Another type of spoilage, putrefactive in nature, occasionally occurs in the deep tissue of large pieces of meat such as the hind quarters of pork and beef, even though the meat is held at low storage temperatures. In the industry, terms such as ham souring, bone stink, or bone taint are used to identify this condition. Haines (*11*) indicated that there were at least two types of bone taint: (1) true "souring," an anaerobic production of a volatile evil smelling fatty acid or related compound, and (2) true putrefaction or "green bone." Certain species of the genus *Clostridium* are associated with ham souring (*11, 61, 62, 63, 64, 65*) and with the development of bone stink or taint (*64, 65, 66, 67, 68*).

While the presence of *Clostridium botulinum* in chilled meats is seemingly quite exotic, Riemann (*69*) recently reported that type E botulism may be more common than heretofore suspected. Beginning with the observation of Dolman *et al.* (*70*) that a strain of *C. botulinum* type *E* grew and produced toxin at 6°C in pickled herring, this organism has been associated as an occasional pathogen of flesh foods. Ohye and Scott (*71*) confirmed this finding and, independently, Pederson (*72*) reported an outbreak of type *E* botulinum in Denmark wherein marinated herring was the food involved.

Later, Ohye and Scott (*73*) observed that minimum temperature re-

quirements were 8°–10°C less than, and maximum temperatures 5°C below, those tolerated by types A and B. Some strains of type E have been found to form toxin slowly at 3°C (74).

According to Meyer (75), C. botulinum has been isolated from the liver of healthy as well as diseased cattle, dogs, and fish. Blood pudding, liver sausage, smoked pork and ham, meat balls, liver paste, salmon, sturgeon, and mackerel have all been found (69) to permit growth of and toxin production by this organism. To date, however, fresh chilled meats have not been implicated in outbreaks of botulism.

Jensen and Hess (65) catalogued various types of ham souring and asserted that salt-tolerant bacteria which grow at 0° to 3.3°C in bone marrow cause any kind of souring. They considered that any of the following bacterial genera might be responsible: Achromobacter, Bacillus, Clostridium, Micrococcus, Pseudomonas, Proteus, Serratia, and a miscellany of streptobacilli and other organisms. Their observations are deserving of further study since most of these organisms are rather sensitive to relatively low salt concentrations, and the few that are not grow poorly in the range 0° to 3.3°C. Inasmuch as most of these contaminants are mesophiles rather than low temperature organisms, the presence of this defect almost certainly indicates poor refrigeration, poor pumping of curing agents, or both.

The importance of lipolytic activities of microorganisms in meat has been recognized for over 30 years. Several unpleasant tastes and odors in the fat of stored meat were considered by Lea (76) and Haines (7) to be caused by microorganisms growing in the layer of fat covering the muscular tissue and in the adjacent muscle. Lea found that, although the fat of beef carcasses stored in still air at 0°C had a tainted odor at 15 days, the meat remained good for 25 days. At 6 weeks, however, he judged the meat to be tainted. Later Lea (77) stated that tainted fat may contain several million microorganisms per gram. In addition, various molds, bacteria, and particularly yeasts produce fat soluble red pigments. At least one yeast was found to produce a colorless chromogen which becomes deep red in the presence of iron salts.

Collins (78) reported that, for 159 bacterial cultures that he studied and identified, frequently microorganisms capable of decomposing protein also were able to hydrolyze fat. Haines (11) and Vickery (79, 80) showed that some of their isolated strains of Achromobacter and Pseudomonas, and all of the yeasts, were lipolytic. To this group Jensen and Grettie (18) and Lea (77) added many molds including species of Aspergillus, Penicillium, Mucor, Rhizopus, Monilia, Oidium, and Cladosporium.

Nonmotile bacteria, similar to Alcaligenes viscosus except that they did

not produce ropiness and were almost spherical, commonly were isolated
(17, 82) on off-odor or slimy chicken. While these organisms were com-
paratively inert on the usual diagnostic media, they showed pronounced
ability to hydrolyze fats.

A number of studies have been made on the biochemical activity of the
genera *Pseudomonas* and *Achromobacter* at different temperatures. Nashif
and Nelson (83, 84) found that *Pseudomonas fragi* had an optimum tem-
perature at around 15°C for lipase production and little activity at 30°C
even though high counts were reported at the latter temperature. They
also reported that several other gram-negative bacteria produced maximal
quantities of lipase around 20°C. The following year Greene and Jezeski
(85) indicated that a proteolytic strain of *Pseudomonas* which liquefied
gelatin at 0, 10 and 20°C lost this ability at higher temperatures. Peterson
and Gunderson (86) observed that lipase action of psychrophilic bacteria
in frozen foods stored at defrost apparently is greater than that of the
proteinases and proceeds at low temperatures and in the frozen state.
Alford and Elliott (87) stated that the amount of enzyme produced per
cell of *P. fluorescens* at 5°C was equal to that produced at 20°C. At 30°C
production was very slight even though good cell yields were obtained.
Also, Alford (88) and Alford and Pierce (89) demonstrated that a few
degrees increase in temperature was able to inhibit gelatin liquefaction,
lipolysis, citrate utilization, fermentation of xylose and arabinose, and
action on litmus milk apparently without affecting the growth. These
findings cast considerable doubt on the negative biochemical reactions
that have been reported for psychrotrophs and psychrophiles at optimal
incubation temperatures. They likewise point up the need for uniformity
in the selection of temperatures for which results are reported.

Several attempts have been made to impose standards for the numbers
of microorganisms that should be present in or on meats. For example,
Marxer (90) set a limit of a million bacteria per gram of ground meat
and contended that meat having counts in excess of this standard was on
the verge of decomposition. Weinzirl and Newton (91, 92), however,
believed that Marxer's standard was too low and that, if such a level were
required, almost all ground meats would be condemned. They proposed a
limit of ten million organisms per gram as being more realistic of ground
meats in the market. LeFevre (93) criticized this higher count as repre-
senting meat "perilously near to . . . putrefaction" and returned to
Marxer's limit of tolerance for chopped meat of a million organisms per
gram. LeFevre (93) further suggested that the bacterial load associated
with the product could be of value for detecting the use of improper
materials and defects in the handling of the product. Despite this, and

other contemporary work (94) indicating that bacterial analyses give warning of spoilage before it is evident to the senses, the importance of these organisms was largely discounted by many of the early workers. For example, Zweifel (95) attempted to isolate organisms of public health significance and, failing in this objective, surmised that the bacterial count was without value and that little was to be gained merely by estimating numbers of organisms. Cary (96) indicated that little importance could be attached to the bacterial count, and Hoffstadt (97) and Weinzirl (94) concluded that there was no correlation between freshness of meat and its bacterial content. Unfortunately, the 37°C incubation temperature used by these workers favored recovery of mesophilic bacteria; many of these organisms grow poorly—if at all—at refrigeration temperatures (i.e., below 10°C) according to Haines (11). Cary (96) and Brewer (98) used a 20° as well as a 37°C incubation temperature but still reported that the appearance of a moderately fresh meat sample could not be taken as an index of its bacterial count.

Beginning with the work of Schmid (99), Haines and Smith (100) and Empey and Vickery (8), limits in the saleability of meats were associated with a bacterial count of about 5–$6 \times 10^7/cm^2$. At about this same time [1933–4, viz. Elford (101)], the city Portland, Oregon, passed an ordinance making it unlawful to sell hamburger or other unseasoned ground meats having an average bacterial count exceeding $1 \times 10^7/gram$. Elford reported that in the 2½-year period after this ordinance went into effect, there was not a single violation of this standard. In view of the fact that reported counts were required to represent the logarithmic average of at least four samples taken on separate days and that, generally, improvements followed notices of high counts, this result is not too surprising. Again, unfortunately, these suggested standards did not receive general acceptance. In fact, prominent bacteriologists (13, 102) considered that the value of standards of this nature were of debatable value. Apparently, insofar as ground fresh meats are concerned, improvements in mechanical refrigeration have not reflected similar improvements in microbiological quality. Modern advances in sanitation, food inspection and mechanical refrigeration have extended shelf life of food products, but no assurance has been given that the quality of ground meat offered to the consumer has become better. Ayres (19) observed that packages of ground beef purchased from a number of retail outlets contained loads ranging from 1.5 to 40 million organisms/gram. Kirsch et al. (24) reported aerobic plate counts in the range of 1.4 to 25 million per gram from 20 samples of market hamburger; Rogers and McClesky (103) determined the total count on ground beef purchased in 24 retail markets and indicated that 96

samples of ground beef contained an average of 192.5 million bacteria per gram when incubation was about 7°C.

Jensen (13), while critical of procedures that merely detected numbers of microorganisms, did concede that regulatory agencies must have some criteria and limits of numbers to serve to guide the processing of foods so that incipient spoilage does not result. To this might be added the fact that, while most of these microorganisms are considered harmless to man when viewed in accordance with their public health significance (104) their impact on the wholesomeness of food products, when considered aesthetically and psychologically, is quite detrimental and renders foods unfit for human consumption.

In conclusion, this paper has sought to point out that at 5°C or less, the microflora that proliferate on stored meats is quite restrictive. Among bacteria, the pseudomonads are pre-eminent although occasionally *Achromobacter* spp. have been associated with off odor, slime, lipolysis, and proteolysis. These two genera, being relatively sensitive to salt, are less commonly incriminated than are molds as spoilage agents of cured meats. Mold mycelial growth associated with stickiness, discolorations and other surface defects usually turns out to belong to the genera *Penicillium, Cladosporium, Mucor, Thamnidium,* and *Rhizopus.* Yeasts, such as *Candida, Torulopsis, Rhodotorula Trichosporon, Saacharomyces,* and *Hansenula* are only infrequently incriminated but do, upon occasion, give rise to characteristic "fermented" odors, sliminess, and lipolysis. Many other bacteria, molds, and yeasts have been able to persist at low temperature, but proof of their relation to specific defects appears lacking. For example, the flora causing defects of the deep tissue is generally mesophilic and so does not multiply under proper refrigeration conditions.

Present state of knowledge does not permit similar generalization for all foods. However, from the limited comparisons that this investigator has made, a marked similarity of flora in other food products has been noted. It is well known that, upon the cutting and grinding of fresh meats, the microbial population quickly changes from a few organisms per gram to millions. Therefore, much has been said regarding the matter of bacterial standards especially for comminuted meats. The decision is especially perplexing when attempts are made to control numbers of organisms having no known significance to public health. Yet, in those instances where policing of the product has been enforced, the level of sanitation has improved and the product is, aesthetically at least, more wholesome. Also, since it has been shown that pseudomonads, molds and yeasts continue to reproduce even when the product is kept cold, their presence in large numbers is evidence of incipient spoilage. For these reasons alone, a "cut-

off" point of one million organisms per gram for comminuted meats, such as was suggested by Marxer (90) seems quite reasonable. For wholesale cuts, it should be relatively easy to maintain levels of less than 100,000. While there is no magic in this number and it has little more significance than 75,000 or 150,000, its enforcement would, in the long run, lead to more satisfactory product reaching the retailer. Also, the one million maximum for comminuted meats would serve as a guide to protect the consumer from buying meat that had already reached an incipiently spoiled condition. A working limit wherein 4 of 5 or 9 of 10 of the lots analyzed did not exceed 100,000 for comminuted meats eventually should be possible and such a criterion would be preferable to a 1,000,000 "standard."

REFERENCES

1. J. L. Ingraham. Growth of psychrophilic bacteria. *J. Bacteriol.* **76**, 75 (1958).
2. J. L. Ingraham and J. L. Stokes. Psychrophilic bacteria. *Bacteriol. Rev.* **23**, 97 (1959).
3. D. A. A. Mossel and H. Zwart. The rapid tentative recognition of psychrophilic types among Enterobacteriaceae isolated from foods. *J. Appl. Bacteriol.* **23**, 185 (1960).
4. B. Eddy and A. G. Kitchell. The use and meaning of the term 'psychrophilic.' *J. Appl. Bacteriol.* **23**, 189 (1960).
5. F. Glage. Ueber die Bedeutung der Aromabakterien für die Fleischhygiene. A. Fleisch-u. Milchhyg. **11**, 131 (1901).
6. R. B. Haines. The growth of microorganisms on chilled and frozen meat. *J. Soc. Chem. Ind.* **50**, 223 T. (1931).
7. R. B. Haines. The bacterial flora developing on stored lean meat, especially with regard to "slimy" meat. *J. Hyg.* **33**, 175 (1933).
8. W. A. Empey and J. R. Vickery. The use of carbon dioxide in the storage of chilled beef. *Australia J. Council Sci. and Ind. Res.* **6**, 233.
9. A. G. Lochhead and G. B. Landerkin. Bacterial studies of dressed poultry. I. Preliminary investigation of bacterial action at chill temperatures. Sci. Agr., **15**, 765 (1935).
10. M. Ingram. The microbiology of pork and b acon. *Rept. Food Invest. Board Lond. No. 77.*
11. R. B. Haines. Microbiology in the preservation of animal tissues. *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. Board Special Rept. No. 45* (1937).
12. W. A. Empey and W. J. Scott. Investigation on chilled beef. Part I. Microbial contamination acquired in the meat-works. *Australia Commonwealth Sci. Ind. Res. Org. Bull.* **126** (1939).
13. L. B. Jensen. Microbiological problems in the preservation of meats. *Bacteriol. Rev.* **8**, 161 (1944).
14. A. Jepsen. Bacteriology of pork. Microbial contamination acquired in the meat-works. *Roy. Vet. Agr. Coll. Copenhagen, Den.* Yearbook p. 40 (1947).

15. M. Ingram. Benjamin Ward Richardson Lecture. Part II. Hygiene and Storage. *J. Roy. Sanit. Inst.* 69, 39 (1949).

16. M. Ingram. Factors controlling the occurrence of various bacteria in semi-preserved meats. *Ann. Inst. Pasteur Lille*, p. 136 (1955).

17. J. C. Ayres, W. S. Ogilvy, and G. F. Stewart. Post-mortem changes in stored meats. I. Microorganisms associated with the development of slime on eviscerated cut-up poultry. *Food Technol.* 4, 199 (1950).

18. W. L. Sulzbacher. Survival of microorganisms in frozen meat. *Food Technol.* 4, 386 (1950).

19. J. C. Ayres. Some bacteriological aspects of spoilage of self-service meats. *Iowa State Coll. J. Sci.* 26, 31 (1951).

20. J. C. Ayres. Microbiological implications in the handling, slaughtering, and dressing of meat animals. *Adv. in Food Res.* 6, 109 (1955).

21. J. C. Ayres. Effect of sanitation, packaging and antibiotics on the microbial spoilage of commercially processed poultry. *Iowa State Coll. J. Sci.* 34, 27 (1959).

22. J. C. Ayres. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. *Food Res.* 25, 1 (1960).

23. J. C. Ayres. The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. *J. of Appl. Bacteriol.* 23, 471 (1960).

24. R. H. Kirsch, F. E. Berry, G. L. Baldwin, and E. M. Foster. The bacteriology of refrigerated ground beef. *Food Res.* 17, 495 (1952).

25. M. Ingram and B. C. Hobbs. The bacteriology of "pasteurized" canned hams. *J. Roy. Sanit. Inst.* 74, 1151 (1954).

26. A. G. Kitchell and M. Ingram. A comparison of bacterial growth on fresh meat and on frozen meat after thawing. *Ann. Inst. Pasteur Lille* 8, 121 (1956).

27. H. W. Walker and J. C. Ayres. Incidence and kinds of microorganisms associated with commercially dressed poultry. *Appl. Microbiol.* 4, 345 (1956).

28. E. F. Wolin, J. B. Evans, and C. F. Niven, Jr. The microbiology of fresh and irradiated beef. *Food Res.* 22, 682 (1957).

29. A. D. Brown and J. F. Weidemann. The taxonomy of the psychrophilic meat-spoilage bacteria: a reassessment. *J. Appl. Bacteriol.* 21, 11 (1958).

30. F. E. Halleck, O. C. Ball, and E. F. Stier. Factors affecting quality of prepackaged meat. IV. Microbiological studies. B. Effect of package characteristics and of atmospheric pressure in package upon bacterial flora of meat. *Food Technol.* 12, 301 (1958).

31. F. E. Halleck, O. C. Ball, and E. F. Stier. Factors affecting quality of prepackaged meat. IV. Microbiological studies. C. Effects of initial bacteria count, kind of meat, storage time, storage temperature, antioxidants, and antibiotics on the rate of bacterial growth in packaged meat. *Food Technol.* 12, 654 (1958).

32. E. M. Barnes and D. H. Shrimpton. The effect of the tetracycline compounds on the storage life and microbiology of chilled eviscerated poultry. *J. Appl. Bacteriol.* 21, 313 (1959).

33. D. H. Bergey, R. S. Breed, and E. G. D. Murray. "Bergey's Manual of Determinative Bacteriology." 5th Ed. Williams & Wilkins, Baltimore, Maryland (1939).

34. R. S. Breed, E. G. D. Murray, and N. R. Smith. "Bergey's Manual of Determinative Bacteriology." 6th Ed. Williams & Wilkins, Baltimore, Maryland (1948).

35. W. J. Scott. Water relation of food spoilage microorganisms. *Adv. in Food Res.* 7, 83 (1957).

36. R. G. Tomkins. Studies of the growth of molds. I. *Proc. Roy. Soc. B* 105, 375 (1929).

37. W. J. Scott. The growth of microorganisms on ox muscle. I. the influence of water content of substrate on rate of growth at −1°C. *J. Council Sci. Ind. Res.* 9, 177 (1936).

38. I. Heintzeler. Das Wachstum der Schimmelpilze in Abhangigkeit von der Hydraturverhältnissen unter verschiedenen Aussenbedingungen. *Arch. Mikrobiol.* 10, 92 (1939).

39. D. Snow. The germination of mold spores at controlled humidities. *Ann. Appl. Biol.* 36, 1 (1949).

40. W. J. Scott. Available water and microbial growth. *Proc. Low Temperature Microbiol. Symp.* p. 89. Campbell Soup Co., Camden, New Jersey (1961).

41. R. G. Tomkins and E. C. Smith. "Stickiness" and growth of moulds. *Cold Storage* 35, 56 (1932).

42. M. J. Talayrach. Conserves de viandes par les procédés frigorifiques. *Ann. Hyg. Publ. Med. legale* 45, 166 (1901).

43. G. Massee. On the discoloured spots sometimes present on chilled beef, with special reference to "black spot." *J. Hyg.* 12, 489 (1912).

44. F. T. Brooks and M. N. Kidd. The "black spot" of chilled and frozen meat. *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. Spec. Rept.* 6 (1921).

45. A. M. Wright. Moulds on frozen meats. *J. Soc. Chem. Ind. London* 42, 488 T (1923).

46. A. M. Wright. Black spots on frozen meats. *New Zealand J. Sci. Technol.* 7, 352 (1925).

47. C. N. Jensen. Fungus flora of the soil. *N.Y. State Agr. Expt. Sta. (Geneva, N.Y.) Bull.* p. 315 (1912).

48. A. Monvoisin Les moisissures des viandes congelees. *Rec. Med. Vet.* 94, 306 (1918).

49. C. Bidault. Sur les moisissures des viandes congelees. *Compt. Rend. Soc. Biol.* 85, 1017 (1921).

50. F. T. Brooks and B. A. Hansford. Mould growths upon cold-store meat. *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. Spec. Rept.* 17 (1923).

51. G. Semeniuk and W. C. Ball. Some molds associated with meat in cold storage lockers in Iowa. *Proc. Iowa Acad. Sci.* 44, 37 (1937).

52. F. T. Wolf and F. A. Wolf. Chemical agents for the control of molds on meats. *Mycologia* 42, 344 (1950).

53. L. B. Jensen. "Microbiology of Meats." 3rd Ed. Garrard Press, Champaign, Illinois (1954).

54. M. F. Gunderson. Mold problem in frozen foods. *Proc. Low Temperature Microbiol. Symp.*, Campbell Soup Co., Camden, New Jersey, 1961.

55. J. Lodder, W. C. Sloof, and N. J. W. Kreger-Van Rij. The classification of yeasts. *In* "The Chemistry and Biology of Yeasts. Academic Press, New York, 1958.

56. A. N. Njoku-Obi, J. V. Spencer, E. A. Sauter, and M. W. Eklund. A study of the fungal flora of spoiled chlortetracycline treated chicken meat. *Appl. Microbiol.* 5, 319 (1957).

57. F. G. Wells and W. J. Stadelman. Development of yeast on antibiotic treated poultry. *Appl. Microbiol.* 6, 420 (1958).

58. H. W. Walker and J. C. Ayres. Characteristics of yeasts isolated from processed poultry and the influence of tetracyclines on their growth. *Appl. Microbiol.* 7, 251 (1959).

59. M. Ingram and J. A. Barnett. IV. Low Temperature Research Station, Cambridge, United Kingdom. *Yeast News Letter* 11, No. 1, (Mimeographed Sheets) May (1962).

60. N. L. Lawrence, D. C. Wilson and C. S. Pederson. The growth of yeasts in grape juice stored at low temperatures. II. The types of yeast and their growth in pure culture. *Appl. Microbiol.* 7, 7 (1959).

61. C. N. McBryde. A bacteriological study of ham souring. *U.S. Dept. Agr. Bur. Animal Ind. Bull. No. 132* (1911).

62. E. A. Boyer. A contribution to the bacteriological study of ham souring. *J. Agr. Res.* 33, 761 (1926).

63. W. H. Tucker. "Studies on *Cl. putrificum* and *Cl. putrefaciens.*" Institute of American Meat Packers, Chicago, Illinois (1929).

64. T. Moran and E. C. Smith. Post-mortem changes in animal tissues. The conditioning or ripening of beef. *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. Board Special Rept. No. 36* (1929).

65. L. B. Jensen and W. R. Hess. A study of ham souring. *Food Res.* 6, 273 (1941).

66. W. J. Howarth. Some matters of interest in the inspection of imported meat. *Proc. Cold Storage Ice Assoc.* 14, 46 (1917).

67. W. G. Savage. Further investigations on the distribution of Gaertner group bacilli in domestic and other animals. *J. Hyg.* 17, 34 (1918).

68. R. B. Haines and W. J. Scott. Anaerobic organisms associated with "bone taint" in beef. *J. Hyg.* 40, 154 (1940).

69. H. Riemann. Anaerobe Toxins. *In* (J. C. Ayres, A. A. Kraft, H. E. Snyder, and H. W. Walker, eds.), "Chemical and Biological Hazards in Foods," Iowa State Univ. Press, Ames, Iowa (1962).

70. C. E. Dolman, H. Chang, D. E. Kerr, and A. R. Shearer. Fish-borne and type E botulism: two cases due to home-pickled herring. *Can. J. Public Health* 41, 215 (1950).

71. D. F. Ohye and W. J. Scott. The temperature relations of *Clostridium botulism*, types *A* and *B*. *Australian J. Biol. Sci.* 6, 178 (1953).

72. H. O. Pederson. Botulisme. *Nord. Veterinar Med.* 5, 700 (1953).

73. D. F. Ohye and W. J. Scott. Studies in the physiology of *Clostridium botulinum* type E. *Australian J. Biol. Sci.* 10, 85 (1957).

74. C. F. Schmidt. Personal communication (1962).

75. K. F. Meyer. The status of botulism as a world health problem. *Bull. World Health Org.* 15, 281 (1956).

76. C. H. Lea. Chemical changes in the fat of frozen and chilled beef. II. Chilled beef. *J. Soc. Chem. Ind.* 50, 215 T (1931).

77. C. H. Lea. Rancidity in edible fats. *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. Board Special Rept. No. 46* (1938).

78. M. A. Collins. The action of lipolytic bacteria on some simple triglycerides and some natural fats. *Iowa State Coll. J. Sci.* 8, 187 (1933).

79. J. R. Vickery. The action of microorganisms on fat. I. The hydrolysis of beef fat by some bacteria and yeasts tolerating low temperatures. *J. Council Sci. Ind. Res.* 9, 107 (1936).

80. J. R. Vickery. The action of microorganisms on fat. II. A note on the lipolytic activities of further strains of microorganisms tolerating low temperatures. *J. Council Sci. Ind. Res.* 9, 196 (1936).

81. L. B. Jensen and D. P. Grettie. Action of microorganisms on fats. *Food Res.* 2, 97 (1937).

82. W. S. Ogilvy and J. C. Ayres. Post-mortem changes in stored meats. II. The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. *Food Technol.* 5, 97 (1951).

83. S. A. Nashif and F. E. Nelson. The lipase of *Pseudomonas fragi.* I. Characterization of the enzyme. *J. Dairy Sci.* 36, 459 (1953).

84. S. A. Nashif and F. E. Nelson. The lipase of *Pseudomonas fragi.* II. Factors affecting lipase production. *J. Dairy Sci.* 36, 471 (1953).

85. V. W. Greene and J. J. Jezeski. Influence of temperature on the development of several psychrophilic bacteria of dairy origin. *Appl. Microbiol.* 2, 110 (1954).

86. A. C. Peterson and M. F. Gunderson. Role of psychrophilic bacteria in frozen food spoilage. *Food Technol.* 14, 413 (1960).

87. J. A. Alford and L. E. Elliott. Lipolytic activity of microorganisms at low and intermediate temperatures. I. Action of *Pseudomonas fluorescens* on lard. *Food Res.* 25, 296 (1960).

88. J. A. Alford. Effect of incubation temperature on biochemical tests in the genera *Pseudomonas* and *Achromobacter. J. Bacteriol.* 79, 591 (1960).

89. J. A. Alford and D. A. Pierce. Lipolytic activity of microorganisms at low and intermediate temperatures. III. Activity of microbial lipases at temperatures below 0°C. *J. Food Sci.* 26, 518 (1961).

90. A. Marxer. Beitrag zur Frage des Bakteriengehaltes und der Haltbarkeit des Fleisches bei gewöhnlicher Aufbewahrung. *Fortschr. Vet.-Hyg.* 1, 328 (1903).

91. J. Weinzirl and E. B. Newton. Bacteriological analyses of hamburger steak with reference to sanitary standards. *Am. J. Public Health* 4, 413 (1914).

92. J. Weinzirl and E. B. Newton. The fate of bacteria in frozen meat held in cold storage and its bearing on a bacteriological standard for condemnation. *Am. J. Public Health* 5, 833 (1915).

93. E. LeFevre. A bacteriological study of hamburger steak. *Am. Food J.* 12, 140 (1917).

94. J. Weinzirl. Concerning the relation of the bacterial count to the putrefaction of meat. *Am. J. Public Health* 14, 946 (1924).

95. E. Zweifel. Bakteriologische Untersuchungen von rohem Hackfleisch, mit besonderer Berucksichtigung der Bacillen der Paratyphusgruppe. *Zentr. Bakteriol. Parasitenk. Abt. I. Orig.* **58**, 115 (1911).

96. W. E. Cary. The bacterial examination of sausages and its sanitary significance. *Am. J. Public Health* **6**, 124 (1916).

97. R. E. Hoffstadt. The bacteriological examination of ground beef. *Am. J. Hyg.* **4**, 33 (1924).

98. C. M. Brewer. The bacteriological content of market meats. *J. Bacteriol.* **10**, 543 (1925).

99. W. Schmid. Einfluss von Temperatur und Feuchtigkeit auf das Baktereinwachstun auf gekuhltem Fleisch. *Z. Ges. Kalte Ind.* **38**, 1 (1931).

100. R. B. Haines and E. C. Smith. The storage of meat in small refrigerators. *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. Board Special Rept. No. 43* (1933).

101. W. C. Elford. Bacterial limitations in ground fresh meat. *Am. J. Public Health* **26**, 1204 (1936).

102. F. W. Tanner. "The Microbiology of Foods," 2nd Ed. Garrard Press, Champaign, Illinois (1944).

103. R. E. Roberts and C. S. McCleskey. Bacteriological quality of ground beef in retail markets. *Food Technol.* **11**, 318 (1957).

104. M. Ingram. Fatigue musculaire, pH et proliferation bacterienne dans la viande. *Ann. Inst. Pasteur* **75**, 139 (1948).

Detection of Microbial Pathogens

in Foods

R. ANGELOTTI

Robert A. Taft Sanitary Engineering Center,
U.S. Public Health Service, Cincinnati, Ohio

The food microbiologist is handicapped by inadequate methods. The analytical problem of enumerating small numbers of specific disease-producing organisms in foods is one not encountered in diagnostic bacteriology, yet many of the methods commonly employed by food microbiologists are those originally proposed and found adequate for clinical bacteriology. Microorganisms in foods are often subjected to such debilitating environmental factors as freezing, drying, pH extremes, heat, and chemical additives. Singly, or in combination, these factors often affect the response of the organisms when attempts are made to cultivate them in media originally designed for diagnostic purposes. Additionally, the ratio of pathogenic organisms to related but nonpathogenic organisms, such as that existing among salmonellae and coliform bacteria, is such that the pathogen represents only a small portion of the total population, is extremely difficult to enumerate, and may be culturally inhibited by the numerically superior and related groups present. Efforts have been made to develop quantitative methods for use in analytical food micro-biology. However, they have not been wholly successful to date. For example, a selectively quantitative method for enumerating coagulase-postive staphylococci remains to be developed. Though improved media, such as staphylococcus 110 medium (*1*) and tellurite glycine agar (*2*), have been reported, neither is wholly adequate. The recent publication of Raj and Liston (*3*) describing an MPN technique involving incubation of food samples in an anaerobic broth, followed by confirmation on staphylococcus 110 egg-yolk agar, appears promising. However, the technique remains to be evaluated by other laboratories.

Probably the most difficult group to quantitate is *Salmonella*, and the

methods commonly suggested require incubation of food samples in selective enrichment media of the type available in the several modifications of tetrathionate or selenite broths. However, data indicate these media lose their selectivity in the presence of various food constituents, resulting in the inability to recover salmonellae (4, 5). Nonselective enrichment in lactose broth has been reported by North (6) as yielding greater MPN values of salmonellae in dry-egg products than obtained with two formulas of tetrathionate broth tested, or with cystine-selenite broth, and that "skips," as observed in the selective enrichment broths, did not occur. However, this method also remains to be evaluated for use with other foods.

Many foods contain some disease-producing bacteria. These organisms are ingested daily in a diversity of products with apparently no ill affects. However, sickness may develop when these organisms are present in food in unusually large numbers. Because of the individual variation in resistance to infection displayed by humans and of the variation in virulence possessed by the microorganisms, it is difficult to select a safe and permissible concentration of pathogens in foods. The resistance of children to infection may be quite different from that displayed by adults; and aged, debilitated, or convalescent individuals in the population represent an additional group with singular resistance to infection. Due to this problem, the examination of foods for the presence of pathogenic bacteria presents a uniquely different problem from that of examining foods for indicator organisms, because detection of extremely low numbers of pathogens is necessary. Though procedures for this purpose have been proposed by various control and regulatory laboratories as a result of their individual needs, the matter of choice becomes quite difficult because of the many conflicting reports relative to the merits of the different methods.

In spite of this controversy, and fully recognizing that any choice of methods, regardless of the extent of laboratory effort expended to justify the choice, runs an excellent chance of becoming obsolescent and may appear to some as arbitrary, the Food Microbiology Unit of the Public Health Service Milk and Food Program has undertaken an evaluation of presently employed microbiological methods. This evaluation was prompted by the several requests received annually from state and local health departments for methods applicable to foods and by our own research needs for reliable methods.

Our attention was directed primarily toward the development of methods for the detection and enumeration of pathogenic bacterial species in foods. Two procedural lines of investigation were undertaken: (a) methods applicable to foods implicated in foodborne disease outbreaks

in which the causal organism may be expected in relatively large numbers and (b) methods suitable for indicating the sanitary quality of foods and in which pathogenic species may be present in small numbers.

Routine laboratory examinations of foods involved in outbreaks of gastroenteritis are often inadequate. Generally, these examinations are perfunctory because the laboratory workers may be unfamiliar with the necessary procedures for performing critical microbiological analyses; or they may be unaware of the significance of the numbers as well as the types of organisms present. Our experience has been that to rely primarily upon the incubation period as a means of indicating the type of organism for which the food should be examined often results in not establishing the etiology of the outbreak. Many times attack rate data and other helpful epidemiological information are not available until after bacteriological examination is initiated. For this reason, we require that as many as possible of the menu items involved in an outbreak be submitted to us for examination when our assistance is sought. As a matter of course, we routinely determine the number of organisms per gram of each of the following groups for each menu item: aerobic "total count," coliform organisms, salmonellae-shigellae, fecal streptococci, coagulase-positive staphylococci, and clostridia. Thus, multiple analyses for several groups of organisms are conducted simultaneously on multiple food samples. Experience has shown that under these circumstances the use of multiple-tube dilution techniques is not practical, in spite of their better sensitivity in some instances than direct plating procedures, because of the large number of tubes involved. Therefore, the methods presently recommended by our laboratory for the investigation of foods involved in foodborne disease outbreaks are direct plating techniques. When applied to foods in which one or more etiologic agents occur in relatively large numbers, these methods have proved to be very satisfactory.

In order to be in a knowledgeable position regarding enrichment procedures used to enumerate small numbers of pathogenic bacteria in foods, these methods have been evaluated also. However, for most laboratories we recommend that they be restricted to use for examining single food items of suspected quality rather than be applied to outbreak foods. Thus, for each of the bacterial species for which determinations are commonly made, because of either the pathogenic nature of the organism or its relationship to sanitary quality, we have selected both a direct plating procedure and an enrichment method.

Though *Proteus, Pseudomonas,* and *Bacillus* species, in addition to other minor groups, have been reported to be the causative agents of food poisoning, we have directed our attention primarily to those groups re-

sponsible for the major share of the foodborne disease outbreaks in this country. Presently, methodology studies are still in progress in our laboratories. Particular emphasis is being directed toward the enumeration of coagulase-positive staphylococci and to the detection of enterotoxin directly in foods. Consequently, evaluations of methods for detecting and enumerating the minor groups of pathogenic organisms in foods have yet to be accomplished.

The remaining portions of this report are devoted to descriptions of pertinent bacteriological methods which we are presently employing in our laboratories for detecting and enumerating coagulase-positive staphylococci, clostridia, salmonellae-shigellae, and fecal streptococci.

Microbiological Methods for the Detection and Enumeration of Pathogenic Bacteria in Foods

Pathogenic types of bacteria are present in perishable foods. Coagulase-positive staphylococci are found in prepared foods which have experienced intimate human contact. Salmonellae are present in small numbers in some raw foods and may be expected to occur in products containing these raw ingredients which do not receive a terminal heat treatment sufficient to destroy them. *Clostridium* species, particularly *Clostridium perfringens*, are ubiquitous and enter foods as soil-, fecal-, and in some instances tissue-borne contamination. Fecal streptococci also exist in foods, and though their role as human pathogens or indicators of fecal contamination is controversial, it may be assumed that contamination with certain of these organisms is, in part, from human sources. Therefore, microbiological methods applied to foods, which merely indicate the presence of pathogenic species with no reference to the numbers per gram at which they occur, yield insufficient information to assess the hazard associated with the product. Consequently, with the exception of those few products for which a zero tolerance for pathogenic organisms has been stipulated and applied, as in certain heat-processed egg products labeled as free of salmonellae, methods which enumerate rather than merely detect pathogens are of greater utility.

Within this frame of reference, the methods listed below are recommended for enumerating as well as detecting commonly encountered foodborne pathogenic bacteria.

I. General methods and materials as employed in the Food Microbiology Unit, Milk and Food Branch, United States Public Health Service, Robert A. Taft Sanitary Engineering Center.

A. Diluent—Phosphate buffered distilled water prepared according to Standard Methods for the Examination of Dairy Products (7).

Initial dilution of food and subsequent 10-fold serial dilutions are prepared with this diluent.

B. Homogenization—Mechanical blending at approximately 8000 rpm for 2 minutes at room temperature. The homogenized suspension is allowed to stand for 2 minutes in order that the foam may subside and heavy particles settle. Determinations of the liquid supernatant material are performed.

C. Initial dilution of food for direct plating methods—As required by circumstance, initial food blends are made at either the 1:5 or 1:10 dilutions as follows: 50 grams of food added to 200 milliliters of phosphate buffered dilution water equals a 1:5 dilution and 50 grams of food added to 450 milliliters of phosphate-buffered dilution water equals a 1:10 dilution.

D. Glassware, equipment, and supplies—Pipettes, dilution bottles, Petri plates, counters, incubators, etc., conform to the specifications outlined in Standard Methods for the Examination of Dairy Products (7).

II. Staphylococci

A. Initial cultivation

1. Solid medium

a. Medium: Staphylococcus medium 110 (1).

b. Incubation: 35°C for 48 hours.

c. Procedure: Transfer 0.1-milliliter portions of serial dilutions of food homogenate to duplicate Petri plates of poured, dried *Staphylococcus* medium 110. Smear inoculum over the surface of the plates with a bent, sterile, glass rod to obtain even distribution of growth. Count the number of pigmented and nonpigmented staphylococcal-type colonies developed on the incubated plates and determine the ratio between the two. Pick representative colonies of both types in the same ratio and test for coagulase production. By this means, it is possible to estimate the total number of coagulase-positive staphylococci (pigmented and nonpigmented) present per gram.

2. Liquid medium

a. Cooked meat 10% salt (NaCl) broth (Bacto-cooked meat medium with 10% NaCl added).

b. Incubation: 35°C for 24 hours.

c. Procedure: Transfer 1-milliliter portions of each decimal dilution into five tubes containing 10 milliliters of cooked meat 10% salt broth.

B. Confirmation of staphylococci—Following incubation of inoculated cooked meat salt broth tubes, transfer material by loop from each tube to the dried surface of *Staphylococcus* medium 110 plates to obtain isolated colonies. Incubate plates at 35°C for 48 hours and observe for pigmented staphylococcal type colonies. Pick representative colonies and test for coagulase production. A most probable number (MPN) of coagulase-positive staphylococci is obtained by this confirmation of cooked meat salt broth tubes (*8*).

III. Salmonellae-shigellae

   A. Initial cultivation

   1. Solid medium (not suitable for shigellae or *Salmonella typhosa*)

      a. Medium: Brilliant green sulfadiazine agar (0.06 milligram sulfadiazine per milliliter) (*4*).

      b. Incubation: 35°C for 24 hours.

      c. Procedure: Transfer 0.1-milliliter portions of serial dilutions of food homogenate to duplicate Petri plates of poured, dried, brilliant green sulfadiazine agar. Smear inoculum over the surface of the plate with a bent, sterile glass rod to obtain even distribution of growth. Count the number of pink-white or pale red colonies surrounded by red medium developed on the incubated plates and record as the number of suspected salmonellae per gram. Confirm typical *Salmonella* colonies according to the biochemical and serological schema of Edwards and Ewing (*9*).

   2. Liquid medium

      a. Media: Lactose broth (*10*) and selenite-cystine broth (*6*).

      b. Incubation: Lactose broth for 24 hours at 35°C, and selenite-cystine broth for 6 to 7 hours at 35°C.

      c. Procedure: [Adapated from North (*6*).] Prepare an initial 1:10 dilution of food by adding 60 grams of sample to 540 milliliters of lactose broth in a mechnical blender cup and homogenizing as described in Section I. From the initial 1:10 dilution of food homogenate remove five 100-milliliter aliquots and place in sterile bottles. Also remove five 10-milliliter aliquots, five 1-milliliter aliquots, and five 0.1-milliliter aliquots. Add each of these last three sets of aliquots to separate tubes of lactose broth containing 10 milliliters per tube. This results in a total of twenty samples as follows: five bottles containing 10 grams of food

each; five tubes containing 1 gram of food each; five tubes containing 0.1 gram of food each; and five tubes containing 0.01 gram of food each. Incubate inoculated bottles and tubes for 24 hours at 35°C. By means of a loop, transfer material from bottles or tubes of lactose broth displaying growth to small tubes containing 1.0 milliliter of selenite-cystine broth. Incubate selenite-cystine tubes for 6 or 7 hours at 35°C. Streak each tube of selenite-cystine broth on brilliant green sulfadiazine agar to detect salmonellae; streak *Salmonella-Shigella* (SS) agar to detect shigellae and *Salmonella typhosa*. Reincubate tubes of selenite-cystine broth for full 24 hours, reexamine for growth which may not have been evident after the initial incubation, and streak positive tubes to plates of the agar media described above. Incubate plates at 35°C for 24 hours and select suspected salmonellae- and shigellae-type colonies for biochemical and serological identification. Calculate the MPN (most probable number) of salmonellae and shigellae per gram according to the procedure outlined in Recommended Methods for the Microbiological Examination of Foods (*8*).

IV. Fecal streptococci
  A. Initial cultivation
    1. Solid medium
        a. Medium: KF streptococcus agar (*11*).
        b. Incubation: 35°C for 48 hours.
        c. Procedure: Transfer 1-milliliter portions of appropriate decimal dilutions of food-homogenate to duplicate Petri plates and pour with KF streptococcus agar. After incubation, fecal streptococci appear as brick-red or pink subsurface colonies. Surface colonies appear as pink, or pink colonies with a small white periphery.
    2. Liquid media
        a. Media: Azide dextrose (AD) broth (*12*) and ethyl violet azide (EVA) broth (*13*).
        b. Incubation: Azide dextrose broth, 35°C for 24 hours; ethyl violet azide broth, 35°C for 48 hours.
        c. Procedure: Transfer 1.0-milliliter portions of each decimal dilution of food-homogenate to each of five tubes of azide dextrose broth. Following incubation, transfer a loopful of growth from each AD broth tube displaying turbidity

(growth) and transfer to a tube of EVA broth. Following incubation, EVA tubes are observed for turbidity and the formation of a "button" of blue sediment in the bottom of each tube. An MPN determination of the number of fecal streptococci present may be obtained from the number of positive EVA tubes, according to the instructions given in Recommended Methods for the Microbiological Examination of Foods (8). False-positive reactions may occur in EVA broth, due to growth of *Lactobacillus plantarum*, *Pediococcus cerevisiae*, and *Streptococcus mitis*. However, growth of *Streptococcus salivarius*, *Streptococcus lactis*, *Streptococcus pyogenes*, and *Streptococcus uberis* also occurs, but with the formation of a white "button" of sediment. Confirmation of positive EVA broth tubes is advisable and is accomplished by streaking a loopful of material from each positive EVA tube to the surface of poured, dried plates of KF streptococcus agar. The confirmation plates are incubated at 35°C for 24 to 48 hours and observed for the development of the pink colony of fecal streptococci described above.

V. Clostridia

Until recently, little need has existed for detecting pathogenic clostridial species in food other than *Clostridium botulinum*. Recent reports indicate that clostridial food poisoning may be more prevalent than once suspected, and that *Clostridium perfringens* is responsible for most of the clostridial outbreaks reported. To date, examination of foods for pathogenic clostridial species has been restricted, for the most part, to foods incriminated in outbreaks of food poisoning and in which large numbers of these organisms occur. As a consequence of their high concentrations in foods involved in such outbreaks, and because their significance as indicators of sanitary quality is not established, techniques for detecting these organisms have been restricted to isolation procedures rather than quantitative methods. Described below are methods which may be employed successfully to detect *C. perfringens* and *C. botulinum* in foods involved in food-poisoning outbreaks. Enumeration of *C. botulinum* is cumbersome, and the methods are directed primarily to detecting the toxin. *Clostridium perfringens* may be enumerated quantitatively from outbreak foods by the method described, though caution is indicated when attempts are made to enumerate less than ten organisms per gram.

A. *Clostridium perfringens*
   1. Initial cultivation
      a. Medium: Sulfite-polymyxin-sulfadiazine agar (SPS) *(14)*.
      b. Incubation: 35°C for 24 hours anaerobically.
      c. Procedure: Transfer 1-milliliter portions of appropriate serial dilutions of food homogenate into duplicate Petri plates and pour with SPS agar. Sulfite-reducing clostridia, of which *C. perfringens* is a member, produce black colonies on this medium.
   2. Confirmation of *C. perfringens*
      a. Medium: Motility-nitrate medium (Bacto nitrate broth with 0.3% agar added).
      b. Incubation: 37°C water bath until growth is evident (usually between 12 and 24 hours).
      c. Procedure: Pick representative black colonies from SPS agar plates and stab inoculate to nitrate-motility medium. After incubation, observe for type of growth (motile or non-motile), and test culture for nitrate reduction. Prepare a Gram stain of growth. Black colonies which give rise to gram-positive, nonmotile rods, capable of reducing nitrate, and which grow anaerobically, are confirmed as *C. perfringens*.

B. *Clostridium botulinum*
   All of the six toxin types produced by *C. botulinum* (A, B, C_a, C_b, D, and E) are pharmacologically similar but antigenically distinct. However, almost all human cases of botulism are due to ingestion of A, B, or E type toxins. Consequently, examinations for types A, B, and E toxins are conducted most commonly.
   1. Demonstration of botulinus toxin in suspected food
      a. Procedure: Passively immunize a minimum of two mice (approximately 25 grams), each with 0.5-milliliter intraperitoneal injections of types A, B, and E antitoxins. Inject an additional set of four mice with 0.5-milliliter intraperitoneal injections of sterile physiological saline. Grind a small portion of the suspected food in a sterile mortar containing sterile sand. Add a small aliquot of sterile physiological saline to facilitate grinding. Centrifuge the homogenized material in the cold at high speed to obtain a clear supernatant. Do not filter. Dilute the clear supernatant 1:10 with sterile physiological saline to avoid nonspecific reactions in injected animals sometimes caused by putrefactive bacterial

products such as ammonia, amines, and other convulsant or lethal factors. Four to six hours after passively immunizing the mice, inject all the mice, including those which received saline, with 0.5 to 1.0 milliliter of the diluted food supernatant. Observe for labored breathing, constricted abdomen, and muscular paralysis, usually followed by death of the animal. Symptoms often occur within a few hours after injection, but may take as long as 3 to 4 days. Unprotected mice (injected with saline) will die if high potency botulinus toxin is present in the food. Mice not passively protected against the specific type toxin present in the food will also die.

2. Cultivation of *C. botulinum* from suspected food

   a. Media: Bacto-fluid thioglycollate medium or Bacto-cooked meat medium.

   b. Incubation: 35°C for 4 days anaerobically.

   c. Procedure: Heat three tubes of either liquid medium for 10 minutes in a boiling water bath to drive off dissolved oxygen and cool rapidly under tap water. Tubes should be of large volume and filled to at least $\frac{2}{3}$ capacity. To each tube add 1.0 gram or 1.0 milliliter of suspected food. Similarly inoculate a second set of three tubes and heat for 10 minutes at 80°C to kill vegetative cells. Incubate both sets of inoculated tubes in an anaerobic jar or under sterile petrolatum in the air. Growth in the unheated tubes indicates the presence of vegetative anaerobic organisms in the food. Growth in the heated tubes indicates the survival of spores and the presence of spore-forming anaerobic organisms in the food. Remove aliquots aseptically, prepare Gram stains, and test for botulinus toxins as previously described. Production of toxin which is neutralized by specific botulinus antitoxin and cellular morphology typical of *C. botulinum* is accepted as confirmatory evidence for the identification of this organism. Should cultural and biochemical identification be necessary or desirable, the tests outlined in Bergey's Manual (15), may be performed. The details for performing these tests are available in the Manual of Microbiological Methods (16).

REFERENCES

1. G. H. Chapman. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis,

plasma coagulation, mannitol fermentation, and the Stone reaction. *J. Bacteriol.* 51, 409–410 (1946).

2. E. Zebovitz, J. B. Evans, and C. F. Niven, Jr. Tellurite-glycine agar: A selective plating medium for the quantitative detection of coagulase-positive staphylococci. *J. Bacteriol.* 70, 686–690 (1955).

3. H. Raj and J. Liston. Detection and enumeration of coagulase-positive staphylococci. *Bacteriol. Proc.* A72, 68 (1961).

4. M. M. Galton, W. D. Lowery, and A. V. Hardy. *Salmonella* in fresh and smoked pork sausage. *J. Infect. Diseases* 94, 232–235 (1954).

5. J. H. Silliker and W. J. Taylor. Isolation of salmonellae from food samples. II. The effect of added food samples upon performance of enrichment broths. *Appl. Microbiol.* 6, 229–232 (1958).

6. W. R. North. Lactose pre-enrichment method for isolation of *Salmonella* from dried egg albumin. *Appl. Microbiol.* 9, 188–195 (1961).

7. American Public Health Association. "Standard Methods for the Examination of Dairy Products," 11th Ed., pp. 51–58, 60. Am. Public Health Assoc., Inc., New York (1960).

8. American Public Health Association. "Recommended Methods for the Microbiological Examination of Foods," pp. 134–135. Am. Public Health Assoc., Inc., New York (1958).

9. P. R. Edwards and W. H. Ewing. "Identification of Enterobacteriaceae," 2nd Ed., Burgess Publishing Co., Minneapolis, Minnesota (1962).

10. American Public Health Association. "Standard Methods for the Examination of Water and Wastewater," 11th Ed., p. 486. Am. Public Health Association, Inc., New York (1960).

11. B. A. Kenner, H. F. Clark, and P. W. Kabler. Fecal streptococci I. Cultivation and enumeration of streptococci in surface waters. *Appl. Microbiol.* 9, 15–20 (1961).

12. W. L. Mallman and E. B. Seligman. A comparative study of media for the detection of streptococci in water and sewage. *Am. J. Public Health* 40, 286–289 (1950).

13. W. Litsky, W. L. Mallman, and C. W. Fifield. A new medium for the detection of enterococci in water and sewage. *Am. J. Public Health* 43, 873–879 (1953).

14. R. Angelotti, H. E. Hall, M. J. Foster, and K. H. Lewis. Quantitation of *Clostridium perfringens* in foods. *Appl. Microbiol.* 10, 193–199 (1962).

15. Bergey's Manual of Determinative Bacteriology, Society of American Bacteriologists, 7th Ed. Williams & Wilkins, Baltimore, Maryland (1957).

16. Manual of Microbiological Methods, Society of American Bacteriologists, McGraw-Hill, New York (1957).

Discussion by J. B. Evans

North Carolina State College, Raleigh, North Carolina

The present session is devoted to an evaluation of "microbial indices of food quality." Therefore, it is incumbent on us first to define what we

mean by food quality and then to evaluate methods that may be a measure of it. Dr. Silliker has divided quality into four components: (1) sanitary quality, (2) organoleptic quality, (3) safety, and (4) utility. Others undoubtedly use other criteria or focus attention on only one or two of these components.

At first glance it would seem that the highest quality would be directly correlated with the lowest bacterial load, and for many items this undoubtedly is correct. However, if this low population is the result of destruction of a heavy bacterial population by processing or is the result of gradual death of the bacterial population during prolonged storage, it is not even a good index of bacteriological damage. Dr. Silliker has clearly shown the limited applicability of total counts to the measurement of each of the components of quality.

For the majority of perishable foods, however, the best quality is achieved by keeping the bacterial population at the minimum attainable level at every step of production, processing, and marketing. Thus, if line samples are taken on a given product in a given food plant, total bacterial counts can be an excellent index of quality.

The viable bacteria that may be found in a food item at any particular time are the net result of many dynamic factors, including (1) the indigenous flora, (2) contaminants added during production and processing, (3) multiplication of some of this flora during processing and marketing, (4) death of some of the flora during processing and marketing.

The bacteria recovered by any quantitative procedure are further limited by the sampling, the media used, and the method of incubation. Differing results between liquid and solid media with similar ingredients may be oxygen or CO_2 effects.

In many instances the sampling procedure is the greatest source of variability. Composite samples often tell us more about the product than would a single sample, but also often tell us much less than multiple samples that are judiciously taken. For example, separate surface and deep samples are highly desirable in many situations. Dr. Peabody has shown the wide differences that may occur between presumably similar samples such as the two halves of a frozen meat pie.

All media and methods are selective, and we might be more accurate to find a term to replace "total bacterial count." What we really mean is a count of the bacteria that survive our sampling and dilution procedures and grow on the complex medium used at the temperature chosen for incubation. We are all aware that we are not counting the thermophiles, halophiles, or obligate anaerobes with our usual plating procedures. In some instances there may be equally important groups of bacteria that

are not detected and of which we are unaware. For this reason it often is a fruitful procedure to prepare a Gram stain of a loopful of material from the first dilution of each sample or directly from the sample.

I will illustrate several of these points by a rather old example of some work that was done with Dr. Niven early in the history of the American Meat Institute Foundation. It was found that greenish discolored hot dogs, bologna, and other products often gave a low total bacterial count even on meat infusion media despite the fact that direct smears revealed large numbers of small gram-positive rods. When a suitable medium was developed it was found that the viable count was extremely high. These bacteria have unusually high requirements for manganese and thiamine that were not met by the usual media. Furthermore, it was found that these bacteria were confined to the surface of some discolored products and to the interior of other products. The obvious conclusion was that the high surface counts resulted from multiplication of contaminants after processing and the high interior counts resulted from multiplication of survivors of under-processing. The weakness of a plug sample in such instances is clear.

Counts of coliform bacteria or fecal streptococci as indexes of quality are predicated on the assumption that these organisms represent fecal contamination and that the number of them present is indicative of the extent of this fecal pollution. Thus, they may be measures of sanitary quality and indicators of safety. The preceding speakers have clearly illuminated some of the pitfalls of these assumptions. It would seem that the greatest need may not be for new and improved methods of detecting these groups of bacteria, but rather a restriction of such tests to specific situations where they should be meaningful and a greater sophistication in their interpretation. Thus, we must know more about (1) the physiology and ecology of these organisms, (2) the natural flora of foods and food ingredients, and (3) the effect of production, processing, and marketing on this flora. Much progress along these lines is evident in Dr. Niven's discussion of the fecal streptococci.

Examples of the importance of such studies are numerous and a recitation of them before this group would be an instance of belaboring the obvious. The work of Dr. Mundt on the widespread natural occurrence of enterococci has been a major contribution in this area. The work of several groups concerning the presence of "coliform" bacteria in frozen orange juice concentrate is another contribution with wide implications. Each of you could supply other examples, I am sure.

In conclusion, it might be well to subdivide our definition of quality as Dr. Silliker has done. Intuitively most of us think of "bacteriological

quality" as the absence of a health hazard, the absence of filth or fecal pollution, and a minimum of bacterial deterioration as measured organoleptically. Despite the nebulous character of these criteria, they form the basis for our use of microbial indexes of quality and show that this topic is inseparable from other major topics discussed at this conference (e.g., food borne diseases, microbial standards, spoilage problems, and research needs).

Discussion by M. D. Appleman

The University of Southern California,
University Park, Los Angeles, California

The review of the activity of psychrophilic and/or psychrotrophic microorganisms presented so ably by Dr. Ayres recalls this group although not necessarily omnipresent is ubiquitous. They have been studied in relation to milk and ice cream by many, decades ago, to flesh foods by Haines (*1, 2*) and Ingram (*3*), to chicken by Ayres *et al.* (*4*) and Mallmann *et al.* (*5*) and to fish by Shewan (*6*). At the present time there are two major philosophical approaches to the study of these organisms and as a result the frontiers of knowledge are being advanced at an ever-increasing rate. The investigators working in both fields are aware of the work of the other and not infrequently we see both approaches integrated in one study. The first has included the more theoretical or academic studies upon factors concerned with the metabolic activity of organisms at low temperatures and I need to recall to your attention that research of only a few including Halvorson *et al.* (*7*), Ingraham (*8*), Ingraham and Stokes (*9*), Jezeski and Olsen (*10*), Peterson and Gunderson (*11*) and Scott (*12*). The second approach has arisen through the work related to public health in which the activities of microorganisms including reproduction or toxigenicity at low temperatures as well as total flora have been studied by Angelotti *et al.* (*13*), Dack *et al.* (*14*), Hartzell (*15*), Larkin *et al.* (*16*), and many others. Although the organisms of public health significance, whether they be pathogens or indicators of fecal pollution, do not classify as psychrophiles in any sense, their activity at low temperature is a matter of importance. It is also important to be aware of the competitive effect of the psychrotrophic group with the organisms of public health significance [Peterson *et al.* (*17*)].

The importance of nonmicrobial causes of spoilage should not be disregarded with foods stored at low temperatures. Effects of oxidative

rancidity have been extensively studied but recently in the excellent work of Shank et al. (18) on sour round the importance is shown of a lipoic block, or an extreme demand on lipoic acid that could not be satisfied. This condition previously had been thought due to mesophiles functioning at low temperatures or to psychrophilic microorganisms.

The question relative to microbial standards mentioned by Dr. Ayres emphasizes that we must have a standard microbiology. Gorseline (19), Peterson and Gunderson (11) and others have recently commented upon and gathered data necessary for a standard microbiology. Dr. Ayres has given reasons why a certain count (1,000,000 per gram) under standard microbiology would be a logical "cut-off point" or legal limit for comminuted meats in the retail trade. Whatever the exact number might be there is no excuse for the 10–100,000,000 per gram all of us have seen in sausage emulsion or comminuted meats in the retail trade when state trim is used.

Dr. Angelotti has reemphasized that the methods of examination for organisms of pollution or public health hazard cognate to food materials are not necessarily the methods utilized for other purposes in the laboratory. In foods the organism(s) sought might be very low in number in comparison to a tremendous contaminating microflora, the foodstuff might affect the selectivity of the medium and processing conditions might affect the microorganisms with subsequent inability to grow upon a selective medium. The effect of the foodstuff pointed out by many investigators including Martinez and Appleman (20) is such that many media and methods which would otherwise be selective are rendered useless. Two concepts must be considered relative to the examination of foodstuffs: was the food the causative agent of an outbreak of disease, and is the food safe for the ultimate consumer. As Dr. Angelotti points out this necessitates the detection of specific pathogens or their products such as the toxin of Clostridium botulinum and the detection of organisms serving as indicators of sanitary quality.

The techniques of examination have been carefully worked out with alternate procedures using solid and liquid enrichment media where possible. It is an excellent idea to use more than one method of procedure as certain strains of microorganisms are overly sensitive upon different media [Yamanoto et al. (22)]. In our laboratory we have found that a few strains of salmonellae are unusually sensitive to sulfadiazine. This interferes with their recovery when isolation is attempted using brilliant green sulfadiazine agar. We are also in agreement with Taylor and Silliker (23) in that we have found that with certain Salmonella spp our recoveries increase greatly as the time of incubation is extended. However,

in our experience in attempts to recover *Salmonella* from meat pies, selenite broth is preferred to cystine selenite broth; the cystine greatly enhances the growth of *Aerobacter* spp which then tend to overgrow the slower growing *Salmonella pullorum* and *S. gallinarum*. Incubation in selenite for 24 to 48 hours appears optimum. In the protocol of Dr. Angelotti, however, there is the use of alternate procedures of direct plating, enrichment and preenrichment that should result in the recovery of the microorganisms sought if they are in the detectable range. Preenrichment media for *Salmonella* spp may be lactose broth such as used in this protocol to mannitol broth used in others, both of which may give satisfactory results.

ACKNOWLEDGMENT

REFERENCES

1. R. B. Haines. The growth of microorganisms on chilled and frozen meat. *J. Soc. Chem. Ind.* **50,** 223T (1931).
2. R. B. Haines. The bacterial flora developing on stored lean meat, especially with regard to "sliming" meat. *J. Hyg.* **33,** 175 (1933).
3. M. Ingram. The microbiology of pork and bacon. *Gt. Brit. Dept. Sci. Ind. Res. Food Invest. No. 77* (1934).
4. J. C. Ayres, W. S. Ogilvy, and G. F. Stewart. Post-mortem changes in stored meats. 1. Microorganisms associated with the development of slime on eviscerated cut-up poultry. *Food Technol.* **4,** 199 (1950).
5. W. L. Mallmann, L. E. Dawson, B. M. Sultzer, and H. S. Wright. Studies on microbiological methods for predicting shelf-life of frozen poultry. *Food Technol.* **12,** 122–126 (1958).
6. J. M. Shewan. A review of the microbiology of frozen and cured fishing products. *Proc. Symp. Cured Frozen Fish Technol.* Goteborg, Sweden (1963).
7. H. O. Halvorson, J. Wolf, and V. R. Srinvasan. Initiation of growth at low temperatures. *Proc. Low Temperature Microbiol. Symp.* pp. 27–40. Camden, New Jersey (1961).
8. J. L. Ingraham. Newer concepts of psychrophilic bacteria. *Proc. Low Temperature Microbiol. Symp.* pp. 41–56. Camden, New Jersey (1961).
9. J. L. Ingraham and J. L. Stokes. Psychrophilic bacteria. *Bacteriol. Rev.* **23,** 97 (1959).
10. J. J. Jezeski and R. H. Olsen. The activity of enzymes at low temperatures. *Proc. Low Temperature Microbiol. Symp.* pp. 139–155. Camden, New Jersey (1961).
11. A. C. Peterson and M. F. Gunderson. Role of psychrophilic bacteria in frozen food spoilage. *Food Technol.* **14,** 413–417 (1960).
12. W. J. Scott. Available water and microbial growth. *Proc. Low Temperature Microbiol. Symp.* pp. 89–105. Camden, New Jersey (1961).

13. R. E. Angelotti, M. J. Foter, E. Wilson, and K. Lewis. Time–temperature effects on salmonellae and staphylococci in foods. I. Behavior in broth cultures and refrigerated foods. Technical Report F59-2. Robert A. Taft Engineering Center, Cincinnati, Ohio (1959).

14. G. M. Dack, E. Wheaton, M. N. Mickelson, and M. N. Schuler. Public Health significance of microorganisms in frozen pot pies. *Technical Service Bull. No. 13* (1960).

15. S. E. Hartzell. The microbiology of frozen foods. *Proc. Low Temperature Microbiol. Symp.* pp. 263–283. Camden, New Jersey (1961).

16. E. Larkin, W. Litsky, and J. E. Fuller. Incidence of fecal streptococci and coliform bacteria in frozen fish. *Am. J. Public Health* 46, 464–468 (1956).

17. A. C. Peterson, J. J. Black, and M. F. Gunderson. Staphylococci in competition. I and II. *Appl. Microbiol.* 10, 16–22, 23–30 (1962).

18. J. L. Shank, J. H. Silliker, and P. A. Goeser. The development of a nonmicrobial off-condition in fresh meat. *Appl. Microbiol.* 10, 240–246 (1962).

19. H. E. Gorseline. Historical development of the modern frozen food industry. *Proc. Low Temperature Microbiol. Symp.* pp. 5–25. Camden, New Jersey (1961).

20. J. Martinez and M. D. Appleman. Certain inaccuracies in the determination of coliforms in frozen orange juice. *Food Technol.* 3, 392–394 (1949).

21. R. Yamanoto, W. W. Sadler, H. E. Adler, and G. F. Stewart. Comparison of media and methods for recovering *Salmonella typhimurium* from turkeys. *Appl. Microbiol.* 9, 76–80 (1961).

22. W. I. Taylor and J. H. Silliker. Isolation of salmonellae from food samples. IV. Comparison of methods of enrichment. *Appl. Microbiol.* 9, 484–486 (1961).

Open Discussion

DR. GORESLINE: The series of papers on Microbial Indices of Food Quality affords an excellent opportunity to obtain a better understanding of the various facets of the problem and gives us a better idea of how we should attack our own particular problems. At this time the meeting is open for discussion on any phase of this subject.

DR. LITSKY: In 1957 I published a survey showing that the frozen pies had quite a bit of bacterial activity. Under a grant from the Public Health Service we repeated our work this year. We find that we now need better techniques. Two weeks ago we think we made a major breakthrough. We are concentrating the organisms in the food and putting them on nontoxic media. This technique, believe it or not, is giving us four times as many streptococci as with the conventional MPN and twice as many as with the KF medium. This slide shows a plate inoculated with a 1:2 dilution of a sample yielding a zero MPN. Each one of those colonies was confirmed as a fecal streptococcus.

DR. GORESLINE: I am glad, Dr. Litsky, that you came prepared with a slide. Is there anyone else who would like to add to the proceedings?

DR. JAY: Regarding the recovery of coagulase-positive staphylococci from meats, I would like to say that in my studies staphylococcus medium 110 fortified with egg yolk (egg yolk agar) and coupled with the anaerobic liquid medium of Raj and Liston proved to be far superior to four other such media including mannitol salt agar, tellurite-glycine agar, the Vogel and Johnson modification of the tellurite-glycine medium, and polymyxin agar. The egg yolk medium should be surface plated and incubated at 45°C for 48 hours while the Raj-Liston medium is held at 37°C for a similar period. In the event that no egg yolk precipitating colonies develop within this period on egg yolk, cultures in the Raj-Liston medium should then be streaked onto an egg yolk plate and incubated at 45°C.

DR. GORESLINE: Do you have anything to say about the method of preparation?

DR. JAY: The egg yolk medium is prepared by adding the yolks of two fresh eggs to a liter of the staphylococcus medium 110 previously cooled to about 50°C. It may be used fresh or stored in the refrigerator and used later.

DR. GORESLINE: Breaking the egg by ordinary means with the hands into the agar cooled to 50°C and then pouring?

DR. JAY: Yes.

DR. HURST: We have been working along similar lines in our laboratory and Dr. Parker published a paper on a complex medium containing egg yolk for enumeration of staphylococci. We found that the toxicity of the selective agents can be reduced by the addition of sodium glycocholate to the medium. Why it works we don't know but it certainly does.

I would like to comment on the MPN counts and the results which were presented very briefly by Doctor Angelotti. He showed, for instance, that there was a difference between some media of 3000 and 13,000. He didn't have time to explain the details of the MPN technique and I assume he did it the same as we do, namely five tubes per sample dilution. And if that is so, then the difference between 3000 and 13,000 would still be within the confidence limits.

I would also like to ask him how he added the organisms. I should imagine when the foods were deliberately contaminated some of the recoveries which you reported would depend to some extent on the method which is used in contaminating the food.

DR. ANGELOTTI: Relative to the method that we employ for contaminating our experimental foods, we prepare our cultures in appropriate media standardized to the desired combination turbidemetrically and make plate counts of our standardized suspensions. We then take this suspension, add the proper aliquot to the food and the food is homogenized in a mechanical blender and a plate count is made from the blended sample. This then is our base count against which the comparisons are made. As you could see from some of the data presented, when we estimate that we add 1.5 organisms per gram or 1.5 per milliliter, it is necessary that we plate out as much as 10 grams of food.

DR. ORDAL: I would like to comment on the importance of relating methods to the technology of food processing and manufacture. We are in an age in which we are experiencing rapid changes in food technology and food handling processes. So far there has been relatively little said in regard to the evaluation of microbiological procedures as they are affected by technological change. Dr. Peabody touched on it when he was discussing coliforms. I would like to report a finding with respect to the ability of staphylococci to grow on

the salt-containing selective media that are so commonly used for these organisms. In many of our foods, if we don't heat sterilize, we do use some heat treatment. The question then arises, how effective are these salt-containing media when they are used to recover viable but heat-injured staphylococci. I can only report on a model system where a suspension of staphylococci was heated to 90 to 95% destruction and the selective media compared to trypticase soy agar for the ability to recover the remaining viable cells. The salt-containing selective media recovered only a small per cent of viable organisms as compared to trypticase soy agar. However, if we suspended the heated cells in trypticase soy broth and incubated the mixture at 37°C for about 2 hours and then plated on the two agars the results were comparable. The heat-injured cells recovered and were able to grow out on the selective media. The heat-injured cells did not recover if the trypticase soy broth dilution was kept at refrigeration temperatures. We established that what we observed was recovery of heat-injured cells and not multiplication of some cells by following the changes in population by plating on both media at ½ hour intervals. The lag time was close to 3½ hours and after this time the growth curves measured on the two media was essentially the same.

The point I wanted to emphasize is that, the heat-injured cells are viable cells but certain conditions are necessary for their recovery before they can grow on the salt-containing selective media. The heat-injured cells apparently do not recover at refrigeration temperatures.

DR. GORESLINE: Are you saying that you should provide for limited periods of growth in food?

DR. ORDAL: I have only provided an example where recovery on a selective medium was poor following a common processing procedure, namely, the application of heat. What I am implying is, that we must be cautious in the interpretation of such microbiological data and we must do so in the light of the processing and handling conditions that the food has gone through prior to examination.

DR. GORESLINE: This is what worries me with regard to studies using inoculated foods. When we use broth cultures and add these organisms to food in which all of the conditions are given with the idea that we are imitating or simulating what you have in nature, it seems to me this is open to question. I would like to have someone tell me how long they think the period of incubation ought to be, because I feel if it is too long you have one situation, and if it is too short, you have another.

DR. KAMPELMACHER: I would like to direct some questions to Dr. Angelotti. I would like to have you discuss further what amount of a given food sample should be tested. I have seen and experienced that this is a very important point. I am speaking about salmonellae now.

Also, I would like to ask what is your method of isolation? What are your incubation periods for your enrichment cultures and, if you have colonies on your plates, how do you screen them further, especially in connection with salmonellae and shigellae. Do you find shigellae in different foods very often?

DR. ANGELOTTI: I don't think I can remember all the questions but I will start backwards and you may remind me of the others. Relative to *Shigella* organisms, in the 7 years that I have been at the Center we have never encountered a *Shigella* outbreak.

Next I think you were talking about salmonellae; how do we know we have

salmonellae on the plate. I am not quite sure I understood the question relative to screening.

DR. KAMPELMACHER: If you have your colonies on the plates what further tests do you make?

DR. ANGELOTTI: The use of sulfadiazine in the medium reduces the growth of some of the interfering organisms, particularly *Proteus*. We have an idea that the organisms are salmonellae if the agglutination tests are positive. If we want to go further, we would follow the outline of Edwards. Now what was another question?

DR. KAMPELMACHER: What time do you make the streaks of the enrichment broth?

DR. ANGELOTTI: The technique was described by North using an incubation in lactose broth for a 24-hour period. This material is then inoculated into small tubes of Selinite broth, incubated for 7 hours, and if growth occurs in the broth, it is streaked on brilliant green agar. If growth does not occur after 7 hours, the Selinite is incubated for a full 24-hour period and streaked again.

Now on the other question of sampling, there is an admirable series of articles by Hartman and Huntsberger bearing on sampling techniques. They indicate that there are a number of important variables, such as how much food to use and the number of samples to test. One must make some kind of decision based on the number of people he has working in his laboratory and the number of samples they must analyze in a given period of time. Oftentimes such a decision results in a situation which is far from best but it is that which is workable in your laboratory. I am not prepared to say at the moment that one 50-gram sample from a thousand pounds is as good as five hundred 10-gram samples from the same thousand pounds. As a matter of routine, we use 60 grams in 540 ml and we make our determinations from this sample.

DR. GORESLINE: I think you will have to agree, it makes it difficult for people who are trying to get standardized methods.

DR. LEWIS: I should like to comment on the ultimate necessity for standardization of methodology, if we are going to deal with food in international trade, between states, and among communities. We cannot all go our own ways and come up with uniform results. At this time we could not get agreement, perhaps, even within the Public Health Service, let alone anywhere else, on what these standard methods should be. Nevertheless, we must come to some kind of agreement wherever we set up a microbiological standard for control purposes or for whatever reason. The only mechanism I have been able to visualize, by which we could make progress toward this goal is to adopt a reference against which we can make future comparisons. Some groups are now using one set of methods and others use a completely different set, so there is no basis of comparison. I should like to urge that consideration be given to accepting reference methods for use in future studies as a baseline for comparison.

DR. BUCHBINDER: I would like to make a brief comment on Dr. Peabody's observation of the lack of information of some coliforms isolated on plates. My reference is to a similar problem we have with harbor water in New York City which is brackish water and has very high MPN even though we spent over two hundred million dollars for sewage disposal plants at the end of the war. We found that plate count for coliforms gave very many more colonies or high results than by the MPN method. Now we found many of these

colonies did not ferment lactose on a 24–48 hour plate. But if you let them go for 3, 4, or 7 days they would ferment. We never did anything about it because we didn't want to get involved; it would be a tremendous job.

DR. MOSSEL: It has been noted rather frequently that foods "containing" enteropathogenic Enterobacteriaceae nevertheless appeared to be "free" from coliform or other currently used indicator organisms. Causes of this discrepancy might be:

(a) The difference between the amount of food examined in the two types of tests: 10–25 gram in the examination of foods for *Salmonella* and *Arizona* as against 0.1–1 gram in current tests for indicator organisms.

(b) With most solid media used for the enumeration of coliforms lactose negative or slow lactose fermenting Enterobacteriaceae are often overlooked

(c) When liquid enrichment media are used, the anaerogenic strains are missed amongst the lactose positive Enterobacteriaceae. For reasons (b) and (c), it is much better to replace the use of lactose media by those containing glucose. Particularly recommended for this purpose are:

(i) Buffered brilliant green bile glucose peptone water as an enrichment medium for larger weights of food;

(ii) Crystal violet neutral red bile glucose agar as a poured plating medium for 1:10 dilutions of foods and for confirmation of the enrichment cultures referred to under (i).

Virtually all Enterobacteriaceae grow copiously in the first medium and form characteristic colonies in the latter. The growth of other bacteria commonly occurring in foods is completely suppressed, with the exception of aeromonads, pseudomonads, and certain achromobacters. Although the latter bacteria are as undesirable in heat-treated foods as, for example, aerobacters of nonfaecal origin, the bacteriologist may wish to recognize these non-Enterobacteriaceae. This can be done by a combination of two simple tests. Aeromonads can be recognized by their positive oxidase reaction, pseudomonads and achromobacters by their oxidative rather than fermentative attack on glucose, if any.

DR. MUNDT: There were two positive statements made about using the streptococci as a sanitary indicator. I want to sound a note of caution in here because our work indicates that these organisms don't always necessarily arise as a result of pollution. In fact you can find them in practically every package of frozen food. We have gone into the processing plants and picked these organisms out of the air. It make me wonder if these are not just part of the total microbial population of the products rather than organisms which might have some specific sanitary significance.

DR. NIVEN: I don't think we have any argument at all. In fact, I believe I did mention in my paper that these organisms have not definitely been established as normal plant contaminants. We should remember too that the enterococci will grow at very low temperature and it is especially true of *Streptococcus faecium*, a common spoilage organism in canned ham.

DR. LISTON: Apropos of the remark made, I would like to make a plea for common sense in this application of data. I was thinking particularly in reference to the chairman's remarks on temperature of incubation and total count. Certainly under certain circumstances total counts are meaningless. However, in the case of enterococci, the fact is that these organisms are not normally present on fresh fish food. Nevertheless they do appear after freez-

ing. This is an indication of contamination. I would also remind you that Dr. Mossel made an excellent suggestion that we might use an index to determine whether contamination is precooking or postcooking. One other thing if I may. With reference to freezing temperature and also the physiological state of the organisms that we all consume, the fecal streptococci are the least sensitive. Recent experiments we have done indicate this might not be true for all organisms.

DR. SLANETZ: I don't think that we should leave the impression that fecal streptococci are not good indicators of fecal contamination for certain purposes. While their use for indicating the sanitary quality of foods certainly needs further study, I am sure you will agree that fecal streptococci are very useful indicator organisms in the field of water pollution.

Limitation of Microbial Levels

in Chilled and Frozen Foods

R. PAUL ELLIOTT

Western Regional Research Laboratory, Albany, California

In recent years there has been increasing interest in standards for bacterial numbers in various foods, and yet very few standards have actually been put into law. The city of New York and the U. S. Quartermaster Corps have announced tolerances and the State of Massachusetts has promulgated official bacteriological standards for precooked frozen foods. Other government agencies in the United States have not. Canada has used several of them with good success.

The purpose of this paper is not to take a stand for or against microbiological standards, but rather to summarize, from the scientific literature, important facts which have a bearing on such standards, particularly as applied to chilled and frozen foods.

Are bacteriological standards desirable? Administrators would find bacteriological standards convenient to help them decide on the acceptability of a food, because standards have the advantage of putting questions of safety and quality of foods on a numerical basis. Organizations that have no recourse to factory inspection may find them useful; that is, standards may be desirable when bacterial counts are the only data available on which to make a decision of suitability. An agency might apply standards to goods coming from outside its jurisdiction, especially to foods coming from countries that have high disease rates and poor sanitation. Standards are also used successfully in purchase specifications, as, for example, those of the U. S. Quartermaster Corps. Some agencies, particularly the Public Health Service, have developed codes of recommended practice for various food industries. Recommended bacterial limits are often incorporated in such codes (1). They have proved their value as guides or aims.

The U. S. Food and Drug Administration sometimes sets unannounced administrative bacteriological tolerances for its own use, particularly for a few imported foods. But these are flexible and always linked to public

health hazard, attainability, accessory chemical and organoleptic data, and to factory inspection information (if available). On the other hand, the inspectional programs of the U. S. Food and Drug Administration and of state and local agencies offer a "control at the source" which may be a better safeguard than sampling, particularly if used in conjunction with food handling codes and an educational program in proper food handling methods.

Procedures for freeing foods of bacteria may be more effective in public health protection than specifications of permissible numbers. Microbial control of milk showed a coincident reduction in the incidence of milk-borne epidemics. But the question arises: Was this because of standards or because of the general application of pasteurization?

How is microbial level related to danger to health? Dr. Hobbs has reported (*2*) that foods suspected of causing food poisoning usually contain 1 to 10 million bacteria per gram, whereas most "normal" foods contain 10,000 or fewer per gram. But in another publication she reported *Salmonella* on meats where there were low total viable counts and no fecal organisms (*3*). In the United States, salmonellae in small numbers have caused infections from dried egg yolk and dried yeast, both of which had very low total counts. Dried milk containing no viable staphylococci has caused food poisoning due to enterotoxin formed before the drying process killed the cells.

The complete absence of pathogens from foods is desirable, but often unnecessary and sometimes impossible; furthermore, standards may not accomplish this end. Spores of *Clostridium botulinum* and small numbers of *Staphylococcus aureus* are harmlessly ingested. For healthy adults, this is also true of *Salmonella*, although low levels of *Salmonella* have caused illness in babies. Dr. Hobbs has stated (*4*) that, whereas the elimination of the *Salmonella* group should be regarded as essential and practicable, to aim for freedom from staphylococci would be difficult and from *Clostridium perfringens* impossible. Pathogenic strains of staphylococci are constantly carried on the skin or in the nose by one-third to one-half of all normal individuals. Thus, any food handled by human beings, regardless of excellent sanitation and handling procedures, is likely to contain them in small numbers. Spores of both *C. perfringens* and *C. botulinum* are widely distributed.

Food poisonings generally occur because a protein food is neglected after it is cooked. Laboratory studies have shown that food poisoning organisms will grow in such a food held too long in the danger zone, 40° to 140°F. Obviously, a high level of food poisoning organisms in a food *is* a health hazard.

It would be difficult to specify the precise number of cells of a food poisoning organism that would warrant condemnation. A zero level cannot be demonstrated by sampling, because methodology is inadequate to detect small numbers.

Spoilage at refrigeration temperatures is accompanied by bacterial growth to tremendous numbers, but no food poisoning bacteria can grow at such temperatures. We have made an extensive literature survey on this subject, and a review is in preparation. The lowest recorded growth temperature for *Staphylococcus aureus* is 6.7°C; *Salmonella*, 6.7°C; *Clostridium botulinum* types A, B, and C, 10°C, and type E, 3.3°C.[1] Thus, a food held for long periods in an efficient refrigerator might have a high total count, but this count would be unrelated to the question of public health.

Foods generally decompose before they become poisonous. This serves to warn the consumer and, in addition, sometimes the food poisoning bacteria cannot compete with the normal spoilage flora. Thus, high numbers of harmless spoilage bacteria would *protect* the consumer. More research is needed in this field. Dr. Gunderson has suggested (5) that frozen precooked foods be permitted to retain their proper proportion of natural flora. One might even venture to suggest that known nontoxic spoilage organisms be used to inoculate a precooked food. A bacteriological standard limiting numbers of spoilage bacteria might be contraindicated, if the concern is solely public health.

How are standards and sanitation related? Merely announcing a standard often improves plant sanitation indirectly by calling attention to the question of bacteria in the minds of food producers and handlers. Dr. Thatcher has said, "Without exception, the introduction in Canada of the few microbiological standards has marked a positive trend towards improvement . . . in the practice of hygiene by the industries concerned. . . ."(6)

However, a standard can have a somewhat different effect, as the U. S. Food and Drug Administration found when the mold count tolerance on tomato products was announced many years ago. One firm was found shooting for the tolerance rather than for the sanitation and the adequate sorting that the tolerance was meant to encourage.

An agency that sets a tolerance for foods coming from outside its

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[1] *Clostridium botulinum* type E is very rare, probably primarily because its spores are easily killed by heat. Thus, a heating that would kill the vegetative cells of spoilage bacteria would most likely also kill type E. Furthermore, at this lowest temperature limit, type E has produced toxin only after 4 to 6 weeks of incubation in laboratory media.

jurisdiction would probably, at first at least, create a "sorting" procedure wherein the low-count food would be admitted and that which did not meet the tolerance would be shipped elsewhere. Perhaps only the food packed in the morning hours or immediately after clean-up would meet the tolerance, whereas that packed in the afternoon would not. A standard might have to be country-wide or even international to correct this problem.

Any bacteriologist knows that counts—viable or microscopic—determined on a product along a production line give a good picture of the sanitation of that line. But the absolute numbers are not as important as the relative levels at each station. A count on the final product cannot reflect the plant sanitation unless the levels of bacteria on the raw product and those at various steps in the processing are also known. This is particularly true if a period has elapsed after the product was packed, during which growth or death of microorganisms might have occurred.

Are low levels of bacteria attainable? In the survey of 1958 and 1959, the U. S. Food and Drug Administration found that, while the inspection team was in the plant, the levels of bacteria in the final product were often lower than levels in the same product packed prior to the inspection. The conclusion was that lower levels of microbial content could readily be attained by the precooked frozen food industry. In fact, a gradual improvement has been seen in recent years.

But a firm that fails to attain a low count by proper handling can cover up its failure by a terminal cook, by adding a preservative, or by storing the food in the freezer until the count drops below the standard.

How is microbial level related to degree of decomposition and to future shelf-life? Bacterial level is one of the best indicators of degree of decomposition. Usually the total numbers reach 10^6 or more per gram at the time decomposition is detectable to human senses, although some exceptions in oysters have been described by Boyd and Tarr (7) and in chicken pies by Peterson and Gunderson (8). In the latter work, 10,000 psychrophilic bacteria per gram gave detectable and 100,000 per gram gave unacceptable off flavors. Bacterial level can therefore affect consumer acceptance, since these levels are well within the range of counts found in commercial pies on today's market. It is obvious that bacterial growth in a food will invariably cause alteration of the food.

The shelf-life of a food held at a temperature permitting bacterial growth decreases markedly if the numbers of spoilage bacteria present at the beginning of the growth period are high. For example, if beef has 10^5 bacteria per square centimeter, it will keep about 7 days at $0°C$, whereas if it has 10 per square centimeter, it will keep about 18 days. Fish with

10^5 bacteria per gram will keep about 6 days at 0°C, whereas fish with 10^3 per gram will keep about 12 days. The same is true for all foods subject to spoilage by bacterial growth. This factor has been stressed as an argument for imposing bacteriological standards to control quality.   *

How valuable would a fecal indicator standard be? The coliform group (except *Escherichia coli*) and enterococci are widely distributed, so their presence alone is not conclusive evidence of fecal contamination. *Escherichia coli* is unavoidable in vegetables from heavily manured soils or in animal products such as eggs, poultry, or red meats. Coliforms could not be used for indicating fecal pollution of southern oysters, orange juice, fermented products, or even egg whites, because of their natural occurrence in the raw product or their use in fermentation. Isolation of a fecal indicator organism warrants investigation of its source or means of entry into the food, but does not necessarily warrant condemnation of the food unless it can be shown that the organism does indeed represent filth, insanitary practice, or a potential hazard.

In frozen storage, *E. coli* dies quickly, other coliforms less so, and the enterococci are highly resistant. The question then arises as to which of these is the most valuable. Is *E. coli* best because its presence indicates a more recent and therefore more potentially hazardous contamination, or are the enterococci best because they preserve a picture of insanitary practice?

How do formulation, processing, and storage influence bacterial numbers? Bacterial level is a dynamic thing, depending on many factors. Foods of different formulation, though basically alike, may differ in pH and in content of moisture, spices, nutrients, or solutes, all of which would affect bacterial survival or growth. Processing procedures, particularly heating and cooling, would affect their level drastically. Freezing and storage will cause a drop in numbers. Whereas milk and water are relatively homogeneous commodities varying little between areas of production, frozen and chilled foods show vastly greater variations, so that they must be considered individually.

Milk and water are easily handled in closed systems after pasteurization or chlorination, whereas solid foods, in the present stage of our technology, cannot easily be handled without contamination.

Methods of sampling and analysis are inadequate. Methodology is perhaps the greatest problem in the enforcement of bacteriological standards. Infection is never uniformly distributed throughout a lot, so that one could not be certain of the absence of a pathogen by a sampling procedure, no matter how carefully performed. Furthermore, methods of isolation of the more fastidious pathogens such as *Brucella* are not

reliable. Bacteria that have been injured by freezing or heating may require special treatments or nutrients to coax them into growth on laboratory media.

.Even the so-called "standard" plate count is affected significantly by subtle differences in procedure. Multiple-tube determinations, by their very title "Most Probable Numbers," are recognized as highly inaccurate, although this fact is often not remembered in recommendations for microbiological standards. A direct microscopic count requires extreme care in sample preparation because of the minute amount actually examined under the microscope. Two authors (9) have recommended that collaborative studies, such as those conducted for chemical analyses by the Association of Official Agricultural Chemists, be undertaken.

The nature of the inoculum may also affect isolation of bacteria or interpretation of results. The coliform test in orange juice is unreliable, because sugars in the juice are carried into the lactose broth medium. In egg analyses the inoculum affects selectivity of enrichment media and therefore recoveries of salmonellae.

The only fully reliable method for demonstrating the toxicity of foods infected with staphylococci is by animal or human feedings or animal inoculation tests. Most laboratories do not conduct animal tests, and the coagulase test does not always parallel toxin formation.

For products stored near the freezing point, a count of bacteria viable at 37°C would be valueless to measure quality or future shelf-life, but a count of bacteria that can grow near the freezing point would have some meaning.

Regulations must include a description of sample selection and preparation, diluent, medium, time and temperature of incubation, and must also recognize the existence of variations in analytical results.

Existing laboratory facilities and personnel are inadequate. An effective enforcement of a bacteriological standard will require an expansion in laboratory facilities both in enforcement laboratories and in the laboratories of the industries controlled. For this reason, standards should be applied only where the need is evident. Any standard that is not enforced probably will not be adhered to by those in the industry for whom the standard is primarily meant, i.e., the firms with poor sanitation and handling procedures.

Bacteriological standards will be hard to defend in court. If total viable bacterial count reflects spoilage, quality, or grade, but not public health hazard, there is some doubt that food enforcement agencies whose primary responsibility is the protection of public health have the legal right to enforce such a bacteriological standard. Unless the law clearly states that

standards relating to quality may be enforced, a legal contest may be decided in favor of the owner of the food. If some of the suggested standards were enacted into law, a large proportion of the foods being marketed would be subjected to legal proceedings, if the agency were to enforce the letter of the law. Such drastic action might result in curtailment of the enforcement agency's powers or funds by the legislative body. It seems wise to introduce a standard on a tentative basis, and use it primarily as an educational stimulus. Standards might be useful in a court action if accompanied by strong accessory evidence, such as factory inspection or chemical results. A great deal of background information and years of tentative use of a standard would be necessary before it could stand alone in court as evidence of unsuitability of a food.

But then, the question arises: Why has not the food industry contested in court, the few standards now on the law books? One obvious reason may be that the enforcement agencies involved have no intention of enforcing a standard to the letter. Then, too, it would be a very unwise businessman who would defend in court his right to produce a precooked food with a count of 500,000 per gram. Even if he were to win, the publicity which might attend the case might ruin his business. In view of this kind of economic pressure on the food industry, we bacteriologists have a moral obligation to be reasonable in our suggestions concerning standards.

Recommendations: A previous publication (*1*) presents microbial limits recommended by advocates of microbiological standards for the several types of frozen and chilled foods, and a list of food handling codes. For many products there is general agreement on 100,000 per gram as the recommended level. However, before one should accept this as agreement, one should consider the words of William T. Sedgwick as quoted by Max Levine: "Standards are often the guess of one worker, easily seized upon, quoted and requoted, until they assume the semblance of authority" (*10*).

Dr. Ingram has suggested (*11*) that instead of relying on a series of bacteriological standards, we rely as far as possible on other procedures such as pasteurization, chemical tests for adequacy of heating, and plant-inspection procedures.

If standards are put into use, they will find their greatest success where their legality cannot be questioned.

If microbiological standards are deemed desirable, the following eight points, gleaned from a survey of the scientific literature, should be considered:

(1) A single set of microbiological standards should not be applied to foods as a miscellaneous group, such as "frozen foods" or "precooked foods."

(2) Microbiological standards should be applied first to the more hazardous types of foods on an individual basis, after sufficient data are accumulated on expected bacterial levels, with consideration of variations in composition, processing procedures, and time of frozen storage.

(3) When standards are chosen, there should be a definite relation between the standard and the hazard against which it is meant to protect the public.

(4) Methods of sampling and analysis should be carefully studied for reliability and reproducibility among laboratories, and chosen methods should be specified in detail as part of the standard.

(5) Tolerances should be included in the standard to account for inaccuracies of sampling and analysis.

(6) At first, the standard should be applied on a tentative basis to allow for voluntary compliance before becoming a strictly enforced regulation.

(7) Microbiological standards will be expensive to enforce.

(8) If standards are unwisely chosen they will not stand in courts of law.

REFERENCES

1. R. P. Elliott and H. D. Michener. Microbiological standards and handling codes for chilled and frozen foods: A review. *Appl. Microbiol.* 9, 452–468 (1961).
2. B. C. Hobbs. *Intern. Congr. Microbiol., 6th Rome* 3, 288–289 (1953).
3. B. C. Hobbs and J. Greenwood Wilson. *Monthly Bull. Ministry Health Lab. Serv. Gt. Brit.* 18, 198–206 (1959).
4. B. C. Hobbs. *J. Roy. Soc. Health* 80 (4), 267 (1960).
5. M. F. Gunderson. *In* Conference on Frozen Food Quality p. 74–78. *U. S. Dept. Agr.*, Western Utilization Research and Development Division, Albany, California (ARS–74–21) (1960).
6. F. S. Thatcher. *J. Appl. Bacteriol.* 18, 449–461 (1955).
7. J. W. Boyd and H. L. A. Tarr. *Fisheries Res. Board Can. Progr. Rept. Pacific Coast Stat.* 105, 12–13 (1956).
8. A. C. Peterson and M. F. Gunderson. *Food Technol.* 14, 413–417 (1960).
9. R. Buttiaux and D. A. A. Mossel. *Ann. Inst. Pasteur (Lille)* 9, 138–175 (1957).
10. Max Levine. *Food Technol.* 15, 29–33, 35–36, 38 (1961).
11. M. Ingram. *Food Technol.* 15, 4–6, 8–9, 12, 16 (1961).

A Discussion of the Microbiology

of Various Dehydrated Foods

H. E. GORESLINE

Armed Forces Food and Container Institute,
U.S. Army Quartermaster Research and Engineering Command

The dehydration of foods is one of the oldest industries practiced by man. From his early beginnings he sun-dried meat, fish, berries, etc., and later practiced the art of salting and smoking to assist in the preservation. Although the dehydration methods employed have been modified it has been only in recent years that truly novel and markedly improved operations have come into being. Most of these have been mechanical in nature and modern equipment and technology were necessary for their development. These include spray drying, continuous hot air drying, vacuum drying, and very recently freeze drying. It is interesting to note that most of these methods were developed, or received great impetus, by reason of a military need and, in most cases, the Armed Forces pioneered in the developments.

The products of the present day dehydration industry or of processes now in development can be divided into the following categories:

    Raw, dehydrated—rehydrated in cold water
    Raw, dehydrated—rehydrated during cooking
    Blanched, dehydrated—rehydrated in hot water or during cooking
    Cooked, dehydrated—rehydrated in hot or cold water
    Spray, belt, or pan dried products

Each of the above has its own microbiological problems. These are dependent, in large part, on the type of treatment the item receives as it passes from the raw state to its ultimate use. The sanitary practices that are employed, the temperature controls over the entire handling period and the rapidity of movement through the process all play important parts in the type of microbial flora that will be found in dehydrated food products. The microbiological problems encountered may be those concerned

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with the quality of the product, with spoilage and deterioration, or due to organisms of public health significance. They may be concerned with the natural flora, an acquired flora, or a potentially dangerous flora due to contamination.

Let us look at some of the groupings of dehydrated products and discuss their microbiology. Only those that illustrate different problems can be discussed here.

Raw dehydrated vegetables. This type of product may be used to make crisp fresh-like salads by rehydrating in cold water. Vegetables used may be cabbage, carrots, green peppers, sweet red peppers, etc. In order to keep the fresh crisp qualities of these vegetables they must not be blanched or cooked but must be used as they come from the field. The washing and the preparation will remove soil contamination and a large portion of the natural flora, but a number of organisms will remain representing those types commonly found on or growing in the folds of leaves or other parts of the plant. The numbers and the predominating types will depend upon the type of season of growth of the vegetable, the amount of soil contaminating it at the time of harvest, the thoroughness used in preparing the product for dehydration, and the manner of handling throughout the operation.

The question may be naturally raised as to the significance of the organisms found in such dehydrated products and the numbers to accept as normal. The flora of such products should be that of the organisms found in nature on plant material. Members of the genera *Lactobacillus, Leuconostoc, Pseudomonas, Achromobacter,* etc. and certain spore formers are to be found on such plant material. None of these is of public health significance. However, their presence in large numbers may be indexes of quality deterioration and use of low quality products. High numbers of certain types may indicate long holding of raw product before processing or poor sanitation of equipment with the resulting build-up of an in-plant flora. The presence of coliforms will require confirmation as to type since certain members are normal to field crops. If *Escherichia coli* is confirmed it signifiies human contamination through handling during processing, or animal contamination through farming practices, or ineffective animal control in the plant, and should be used as one index to possible contamination with organisms of public health interest.

It is essential that good control be exercised over the processing of this type of item since it will be consumed in essentially the raw state. The only chance to rid the product of potentially dangerous organisms is by the use of effective washing methods and by germicidal rinses.

The amount of research on the microbiology of this type of product is

limited and much more work must be done before reasonable, attainable, and factual numbers may be selected.

Blanched dehydrated vegetables. The blanching process not only inactivates the enzymes in the tissues of the vegetables, but also markedly reduces the microbial flora which is primarily located on the surface. Thus, the flora of blanched vegetables differs markedly from that of the fresh product both as to numbers and types. Pederson (*1*) has so aptly stated, "Bacterial counts should not be thought of as an indication of the quality of the food, but rather as an indication of the handling of the food between blanching and freezing." The types of bacteria found in the product as it goes to the dehydrator will be largely those acquired during the plant handling after the blanching step. Care needs to be taken to prevent cross contamination from the raw material and in maintaining cleanliness and good sanitation of equipment and working surfaces. The presence of coliforms and other index organisms gain access during handling since in a blanching operation the presence of such bacteria are indicative of a poorly controlled process.

The bacterial flora of the dehydrated vegetables should be low. In some types of driers heavy tray loading or nonuniformity of thickness will cause a stagnation of air movement through the wet product and give rise to rapid bacterial growth (*2*) in those areas. When this material is mixed with the balance of the material in the drier the entire batch shows low quality. In other driers improperly adjusted air movement will cause slow drying at some points and an increase in bacterial numbers due to the lower temperature allowing growth. One way to determine the source of high counts is to determine the types and their numerical distribution and tie these in with the various steps in the plant procedure. This type of product is intended for use by rehydration and cooking to the point of doneness. Although this gives a last chance to kill undesirable organisms it should not be used to excuse unsanitary operations that allow the contamination to take place. However, the fact that the product is to be cooked should be taken into consideration in choosing the type of requirement or standard to be set up.

Cooked dehydrated vegetables. Cooking of the vegetables to the point of doneness should produce a very low bacterial count and eliminate all potential pathogens. Except for the spores the bacteria found on the raw products are heat sensitive and thorough cooking will greatly reduce their numbers. Therefore, high counts in cooked dehydrated products can be attributed to faulty handling or cross contamination. Faulty handling during the dehydration, such as that discussed above, can give rise to an increase in count.

Since cooked dehydrated foods are intended for use by rehydrating with hot water, or in some cases with cold water, there is little opportunity for the killing of undesirable organisms that may be present. The consumer eats essentially what is in the package of dehydrated product, viable bacteria and all. For this reason it is very essential that every precaution possible be taken to prevent contamination with potentially dangerous organisms. Also essential is that test methods be devised to detect such types of contamination. The fact that no cooking is intended for this type of product should influence the selection of the type of microbial quality requirement for such products.

Raw dehydrated meat. The tissues of meat animals are essentially devoid of microorganisms. Veterinary inspection eliminates diseased tissues and good sanitary practices in slaughtering gives products with a relatively low flora on the surface that is acquired in the normal operations used in the preparation of the raw meat. Thus, carcasses, pieces, or blocks of beef, pork, or chicken, as received for dehydration, should have a flora limited largely to the surface areas of the meat and reflecting the care and sanitation used in the preparation. May (*3*) recently pointed out that the bacterial counts on the surface of chicken carcasses reflected the sanitary procedures in the poultry dressing plant and that the range of counts for a given plant was fairly constant. Thus, high-count plants had a tendency to consistently turn out high-count birds. This would mean that the bacterial count of the raw material delivered to the dehydrator could vary greatly depending upon the type of plant from which it was received. Since no heat or other bacteria killing process would be employed in making a raw dehydrated product the bacterial count of the final product would likely be high if made from products coming from a plant with poor sanitary history.

The flora of the meat placed in the dehydrator will reflect the manipulations in the preparation. The greater the degree of manipulation the greater is likely to be the total number of bacteria and the more complete their distribution in the product. Thus, higher counts can be expected in ground meats than in sliced pieces of meat. The effects of the holding or slow progress of the product during processing will be reflected in the chance for multiplication as will also a rise in temperature. The type of flora will be that acquired from the slaughtering and dressing operations, plus that acquired in the preparation operations.

In freeze dehydration the flora present in the prepared products will survive to a greater degree than when heat is applied. Sanitary index organisms are more likely to be found since these types from both the raw

meat preparations and the manipulative preparations will show up in the final product.

Cooked dehydrated meat. The cooking operation markedly reduces the bacterial flora of meat products. Almost all heat-sensitive organisms are killed and only the resistant ones and those in the spore state should remain. Therefore, the microbial flora of the product placed in the dehydrator should reflect very largely only that flora acquired in the preparation for dehydration. The types of organisms should be quite different from those of raw meat and represent those types that would grow on equipment and handling surfaces, plus that acquired from the hands and clothing of the workers. The latter should reflect the degree of personal cleanliness of the workers.

Dehydrated cooked poultry meat presents an example of the manipulation necessary to produce the dehydrated product. Poultry carcasses are cooked in large tanks to the proper state of doneness and then placed on tables where the deboning is done by hand. Since the pieces of meat are small and the bone structure of the birds is so varied hand deboning is a necessity. Since the deboning is done while the meat is warm there is an opportunity for organisms to multiply while the meat progresses through the plant. Also, since there is such intimate contact of the meat with the hands or gloves of the workers, as they debone, sort, and examine the meat for extraneous matter, the personal habits and cleanliness of the workers is likely to be reflected in the type of bacteria found in the final product.

Fully cooked meat products undergo rehydration with hot water, or in cases like shrimp, in cold water, and the type of flora consumed by the user is largely that of the dehydrated product. If the rehydrated product is not consumed at once but is allowed to stand at ambient temperatures, there is a good chance of rapid growth of the organisms contained. Organisms of public health significance could become a danger especially if competitor organisms are in the minority.

Spray-, belt-, or pan-dried products. The spray drying of nonfat milk, eggs, and other liquid foods has been practiced for many years. The bacterial content of the final powder reflects the quality of raw material and the type of handling employed in the plant. In the case of milk the high temperatures that can be used may reduce the bacteria to a low level. Thus, high counts or particular types of organisms may reflect recontamination in the plant handling. With competitive organisms out of the way there is a chance for almost pure cultures of potentially dangerous organisms to develop. There have been cases of toxin poisoning from dried milk, from which no potentially dangerous organisms could be isolated. This presents an unusual case in which organisms such as toxigenic *Staphylo-*

coccus can grow to large numbers in a low count product and produce enough toxin to cause food poisoning from the consumption of the product. However, in the subsequent treatment the organisms are killed and viable organisms of this type cannot be demonstrated in the final product. The toxin being heat stable is not destroyed during the processing and can cause food poisoning symptoms. This presents a case where bacterial counts or bacterial types of viable organisms are not sufficient to evaluate the quality or the safety of the product.

Egg products are in a somewhat different position in that the coagulation point of the protein prevents the use of high temperatures for pasteurization. As a consequence, the total flora cannot be reduced to a point where fairly rapid development of competitive organisms will not take place. The *Salmonella* are the organisms of most interest to public health workers. These organisms are normal inhabitants of the intestines of chickens and consequently are frequently found as contaminants in liquid egg products. Pasteurization at proper temperature and time values can be used to eliminate *Salmonella* from liquid whole egg and yolk and thus these organisms should not be found in properly treated spray dried products. However, it is extremely difficult to eliminate *Salmonella* from liquid egg white by the use of heat. In the dried state this can be done by exposure to heat by placing in a room held somewhere in the range of 120–130°F for a period of one week or until the product is free of viable *Salmonella*.

The production of belt-, pan-, or puff-dried products differ in that the period of drying is much longer than in spray drying and the temperature of drying is lower. Care must be taken to see that the temperature of exposure is above the growth range of bacteria to prevent buildup during drying. The bacteriology of these products reflect the quality of the raw material, the sanitary conditions during processing, and the contamination that may have occurred.

Packaging. Packaging materials have very low bacterial counts as manufactured and delivered for use. However, there are aspects of packaging used in the food industry that should be taken into consideration. These are storage and use of the packaging materials and the storage of the final packaged dry product. The warehousing of packaging material is very important and precautions should be taken against rodents, insects, and dirt. Rats and mice have a preference for materials, such as used for packaging, in which to live and have their young. The writer has seen several cases of gnawed holes in cases of packaging materials with urine stains and droppings evident on paper and plastic material contained in the boxes. There have been cases of dehydrated products being contami-

nated with insects through the packaging material. In such cases as these the contamination held against the dehydrated products were there through no fault of the dehydration process or the raw material, but resulted from carelessness and the improper handling of the packaging materials in which the product was packed.

The choice of packaging materials and the storage of the final product to prevent both rodent and insect damage is important. Ample protection should be provided and warehouse surveillance should be employed to see that the material in the packages is not contaminated. Public health workers and inspectors should make it a point to check on packaging material in order to prevent the contamination of an otherwise wholesome and acceptable item.

Specification Requirements, Standards, or Other Microbiological Quality Measures

From the foregoing discussions it can be seen that there are several facets to the microbial picture of dehydrated food products. The results obtained from samples of dehydrated foods will reflect the history of the processes through which they were made. The microbial flora will reflect the effectiveness of each manufacturing step designed to kill bacteria or to hold them in check. The type of organisms will also indicate the source of the contamination of undesirable bacteria.

It is evident that the evaluation of the results must be viewed with an understanding of the type of food processing techniques used and the use for which the product is intended. All of these things must be taken into consideration in establishing any type of measure of the microbiological quality of a product.

There must be enough data available upon which to base a requirement. Otherwise, it becomes a matter of use of "best judgement" and not that of known facts. A standard or requirement should be attainable in order to avoid endless controversy over its use and should be reasonable so that there can be a fairly broad base of manufacturing availability. A standard should be meaningful as to what it is supposed to do or what is accomplished by its use. If it is to protect the health of the public it should be designed to do so or if to reflect product quality it should be couched in attributes that reflect that quality.

The amount of meaningful microbiological data on dehydrated food products is quite low. For products that have been produced for many years, such as spray-dried milk and eggs, air-dried vegetables and fruits, etc., there is considerable information. It consists of total counts for the most part and does not indicate the types of organisms to be expected

under known conditions of operation. While such information is useful and does represent factual data, in the opinion of the writer it should be amplified by research designed to produce data of greater value in drawing up bacterial requirements.

Other types of products such as that being produced by the freeze-dried method have been available such a short time that very little microbiological data are available on commercial products. In the opinion of the writer cooperative research projects should be organized to study the effects of the various factors involved in the manufacture of such dehydrated foods on the microbiology of the end item. By so doing it will be possible to arrive at factual and meaningful microbiological values for various types of products and values that can be accepted by common agreement rather than those established by a control agency. This would also give the dehydration industry a better understanding of what goes on in its own ranks and an appreciation of being a part of a forward movement to improve food quality and wholesomeness on a practicable basis.

The presence of potential pathogens in foods is undesirable and should not be tolerated. However, there is much to be done in the area of methodology, sampling, culture media, and interpretation of results. The methods used to determine the microbial content of foods were not developed for food but have been altered to meet a need. As a consequence, what works for one type of food may not for a different type and thus truly reproducible and factual results are difficult to obtain. For example, at the present time there are several groups sifting through the many suggested methods and culture media that have been used to detect and/or enumerate *Salmonella* in food products. Products found "free" from *Salmonella* by one method may be found to be positive when assayed by another method. Reproducibility of results must also be improved before there is a clear understanding on the part of all concerned as to just what constitutes freedom of a product from potential pathogens. Similar information is needed for other types of bacteria and their significance.

It is urged that cooperative efforts be mounted to develop the data that are needed to fully understand the microbiological needs for good quality dehydrated food products. This work should be supported in part by research grants, by industry associations and by public agencies. It is believed that a well-organized cooperative effort will produce more useful and usable data in a much shorter period of time and at less expense than if left to independent effort.

REFERENCES

1. C. S. Pederson. "Significance of Bacteria in Frozen Vegetables." *Food Res.* 12, 429–438 (1947).

2. R. Vaughn and R. W. Kuneman. "Microbiological Survey." *Fruit Prod. J.* 24, 7, 196 (1947).
3. K. N. May. "Bacterial Contamination during Cutting and Packaging Chicken in Processing Plants and Retail Stores." Paper read before 22nd Annual Meeting of the Institute of Food Technologists, June 1962.

Open Discussion

DR. MALLMANN: I would like to ask Doctor Schmidt if he would make some comments from the standpoint of standards as it refers to the processor.

DR. SCHMIDT: We in industry, of course, are interested in the subject of standards, and possibly some of you may be interested in our problems. I am speaking of the quality control man. The quality control man has a rather wide variety of activities. He is concerned with physical conditions, microbiological conditions, nutritional conditions, and functional conditions within our plants.

We in the food industry are getting into many diversified products and the question arises as to what bacteriological standards and tests we should use for the ingredients. Recently, I had about 150 ingredients from about 178 suppliers. Therefore, when we set standards they must be practical and the tests must be in an economical range. There is another point that I want to make. We may have a supplier who is shipping $20,000 to $50,000 worth of products. It is my decision to accept or reject. If they have to be rejected and sent back at his expense, you can see the difficulty that occurs for this supplier. Therefore, the standards that are used must be well defined and they must be usable standards. This also involves products from other countries. What is a representative sample? Should I sample one lot and take the square root of the bacteriological counts? Must I reject a product that is over that particular number? These are many of the problems we have and I just wanted to say those few words because the whole problem of quality control is most difficult. Your point on the process control, Dr. Ingram, is very good. It is a subject we have to get into and very strongly. That is an in-line type of control where we must get decisions very quickly because we are dealing in large amounts of products and must make the right decision.

DR. MALLMANN: Doctor Thatcher has asked for a few minutes to talk about what transpired at the International Congress in Montreal.

DR. THATCHER: A meeting was held at Montreal just prior to the 8th International Congress of Microbiology to discuss microbiological standards for foods and specifically for certain groups of frozen foods. The meeting was sponsored by the Section on Food Microbiology and Hygiene of the I.A.M.S. with Dr. Betty Hobbs as Convenor. It was the writer's privilege to serve as Chairman. Representatives participated from the United Kingdom, the United States, Netherlands, and Canada. It is regretted that representatives invited from France, Germany, and Portugal were unable to attend.

The purpose of the meeting was to coordinate the knowledge of the microbiological condition of frozen foods, specifically frozen egg-products, frozen precooked meals, frozen boned meat, and frozen comminuted meats (hamburger), and to make recommendations that in the opinion of the group would

minimize the health risk to the consumer. The outlook was international, and with the intent that any recommendations should be applicable not only to those countries with the more advanced systems of food control.

The purpose was not to formulate legislation but to arrive at common principles that would aid in reaching agreement about realistic limits for the bacteriological content of the specific classes of foods.

The approach was to review for each food: (1) the evidence for health hazard; (2) tests that would be applicable for estimation of potential hazard, and for determination of the effects of treatment and adequacy of sanitation during processing; (3) sampling procedures; and (4) interpretation of specified limits. Reviews were presented of current food control policies prevailing in the countries represented. Common to all such policies was the recognition of the need for what Canadians refer to as "Control at the Source," i.e., emphasis on improved sanitation and hygiene throughout processing operations.

A number of statements were agreed upon with a view to publication. What follows is the Chairman's personal appraisal of salient comment. His colleagues have had no opportunity to approve this presentation, and any statement made should not be attributed literally to any one of them.

It was generally agreed that any recommendations to be offered by this Committee should have as their objective, the protection of the health of the consumer. The production of safe foods required that they be processed under good conditions of sanitation. To secure good sanitation and to estimate the safety of the final product it was recognized that specific foods should be marketed with a bacterial content not exceeding recommended limits. Whether such limits would be attained by the voluntary practice of industry or by legislation would depend on local conditions. Where appropriate, competent agencies should extend to industry (1) education in the microbiology of sanitation; (2) technical assistance, particularly in bacteriological tests for discovering and eliminating sources of potentially dangerous contamination. The need was expressed for a much improved effort on the part of industry in general to establish their own microbiological testing laboratories or else to make consistent use of commercial testing laboratories. Bacteriological analysis should be recognized as a part of the food production process. Particularly did this apply to manufacturers of foods produced in large volume, or involving new or modified technology.

Positive hazard was recognized if large quantities of food should have been subject to error, mechanical fault, or undetected contamination, or if new technology were introduced without regard for, or determination of, its selective effect upon the microbial ecology of the food.

Recommendations of microbial limits for incorporation into law would only be warranted under the following conditions:

(1) Where demonstrated health hazard established a need for control;

(2) Where adequate sanitation cannot alone give safety to a food;

(3) To avoid substandard products from one area being shipped to another area where standards were not in force;

(4) To protect against anticipated hazard from new foods where experience as to their relative safety is inadequate;

(5) To control the movement of foods from areas of the world where enteric disease was endemic at a high rate.

The incorporation of microbial limits into legalistic standards should be avoided unless both the *will and capability to enforce* were positive. Failure to enforce undermines the respect that is vital for the effective functioning of any regulatory agency, and encourages inadequate processing control.

It was agreed that a recommendation of specific microbial limits requires (1) a statement of an adequate method for determining the particular group of organisms under test; (2) an appreciation of the statistical significance of results obtained by the method; (3) a specified sampling procedure including a statement of the size of a test aliquot and the proportion of containers to be sampled; (4) the recommended limits must be practicable in terms of attainment under good commercial conditions of processing.

Discussion revealed that most control agencies practice some form of administrative discretion in interpretation of microbial limits, whether or not these limits are expressed in law. The essential purpose of establishing a limit is to provide maximum protection to the health of the consumer. Many factors should properly be considered in estimating the safety or hazard implied by a numerical statement of the bacterial content of a food. The parameter alone has limited intrinsic value.

The practice of exercising discretion aids in securing fair treatment of the owner of the food, and promotes concord between industry and the regulatory body. This discretion may also be used to make allowance for the inherent inaccuracies of determinative methods, of sampling procedures, and for other limitations imposed by lack of optimal numbers of analysts or of facilities.

All such limitations should be recognized, and microbial limits should accordingly be interpreted with the following factors in view:

1. The statistical significance of:

(a) the method of analysis,

(b) the sampling procedure;

2. Subjective factors related to knowledge of disease hazard peculiar to the food in question and and to the prevailing microbiological quality of the food produced within a particular factory. Examples of such interpretation include:

(a) the tolerance factors listed in the table; (tables 2 and 3 of Minutes)

(b) decision as to the proportion of food within a lot to be regarded as substandard before condemnation of the lot;

(c) the practice of warning prior to restrictive action against subsequent substandard lots;

(d) option for voluntary withdrawal of substandard foods;

(e) establishment of categories of acceptability, e.g., "acceptable," "acceptable on condition," "reject" (of U.S.–Canada agreement on shellfish).

Decision as to which of these administrative practices would be specifically pertinent would depend upon the nature of the food and the form of expression of the standard.

Statements expressing the conclusions of the group in relation to the specific foods are as follows:

Frozen Egg Products

The presence of *Salmonella* in egg products is a hazard to health. It is

therefore recommended that appropriate processing techniques be used to kill these organisms. It is further recommended that laboratory methods be used to verify that this has been accomplished and that no recontamination has occurred.

Frozen cooked sea-foods. (Shrimp, prawns, crab and lobster meats)

. International trade in frozen cooked sea-foods has reached large proportions. These products sometimes contain microorganisms indicative of potential health hazard. Disease outbreaks have been traced to such foods. It is proposed, therefore, that such foods be subject to bacteriological examination. For this purpose, useful criteria of microbiological quality include standard plate count, and numerical estimates of coliforms and coagulase-positive staphylococci.

The following values are believed to be commercially attainable and are recommended as maximum levels for this class of food:

(1) Standard plate count at 35°C   —100,000/gram
(2) Coliform content   —20/gram
(3) Coagulase positive staphylococci—100/gram

It is recommended that at least two of these determinations be made, with other tests to be added according to local need.

Frozen precooked complete meals and closely related products.

The frozen foods industry distributes very large amounts of frozen complete meals. The nature of the production and of the distribution methods for these foods implies that if serious contamination should occur, then disease outbreaks might be very widespread indeed. Therefore, it is imperative that frozen foods be manufactured under the best commercial conditions of sanitation. As a measure of such attainment, practical microbial limits should be used by both manufacturing and regulatory agencies. Such limits confer greater safety if used in association with inspection and bacteriological analysis at the factory.

Readily attainable limits agreed upon included:

(1) Standard plate count at 35°C   —100,000/gram
(2) Coliform content   —20/gram
(3) Coagulase-positive staphylococci—100/gram

It was also recommended that when a raw ingredient used in the manufacture of a frozen food is known to have a high probability of containing *Salmonella*, then the finished product should be subject to analysis for the presence of salmonellae. A positive determination would indicate an unacceptable product.

Frozen boned meat

Frozen boned meat is usually severely contaminated with *Salmonella*. Disease outbreaks have been attributed to this commodity. This committee deplores the situation as found in importing countries where severely contaminated meat is distributed because of the overlying need for protein foods. It recommends, further, that the entire production, processing, and handling of meat to be shipped as frozen boneless meat needs major improvement. Such improvements comprehend practices in animal husbandry, and in pre-slaughter treatment, the removal of *Salmonella* from animal feeds, improved sanitation in the boning plant with special effort to eliminate cross-contamination within

the plant, and the elimination of *Salmonella*-carriers from operations affording ready opportunity for introducing comtamination.

A serious health hazard prevails in a number of countries where the admission of severely contaminated meats serves to perpetuate and aggravate the prevailing rates of human and animal salmonellosis, and largely nullifies contemporary efforts to break the chain of contamination from animal to human. Efforts should be made to persuade government trade authorities to appreciate the gravity of the situation.

Tolerances for salmonellae in such meats should not be established. Rejection of imports beginning with the most generally contaminated and coupled with local efforts for improvement at the source were recommended.

Frozen Comminuted Meats

Frozen comminuted or minced meat is frequently very highly contaminated. This results from two main causes: (1) the use of trimmings and scraps that have been subject to many opportunities for contamination, (2) the comminuting process tends to distribute hazardous contamination throughout the product. While much of this food is eaten with impunity, nevertheless such foods have been implicated in very severe outbreaks, in one instance (in Sweden) involving 10,000 cases with 105 fatalities.

The committee expresses concern over the poor bacteriological quality of this food, and efforts towards improvement are strongly recommended. The committee doubts the justification of use of poor quality trimmings as an economic advantage to the processor in view of the existing data indicating potential and actual hazard.

It was agreed to recommend further study of this topic to a newly formed standing committee of I.A.M.S.

Recommendations for Education and Research

A shortage of professionals trained in the microbiology of foods, and indeed a shortage of bacteriologists in general, was a handicap to the safe development of new food technology and to the public health control of foods. This shortage could be critical to public safety as new processes emerge and new areas of the world develop and market processed foods. It was urged, therefore, that provision of appropriate courses in food microbiology and cognate studies be commended to Universities, and that every encouragement be extended to provision of post-graduate fellowships, with tenure in laboratories and institutes experienced in food research and control. Further, that postgraduate students in the appropriate field be encouraged to take advantage of the funds allocated for post doctoral fellowships by WHO and other agencies.

The committee's deliberations revealed many areas where microbiological research and investigation were needed to provide the data necessary for sound decisions in relation to the public health and developmental aspects of foods.

Examples of research currently needed included:

(1) Determination of the botulism hazard in frozen pasteurized eggs.

(2) Investigation of the *Staphylococcus* hazard in shrimp.

(3) The effect of a mixed bacterial flora on the production and/or activation of: (a) staphylococcal enterotoxin, (b) *Clostridium botulinum* toxins.

(4) The significance of transduction and transformation on staphylococci in relation to enterotoxin formation.

(5) The effect of storage temperature of foods on the bacterial plate count at different incubation temperatures.

(6) The effect of processing factors on the recoverability of *Salmonella* by specific selective plating media; e.g., temperature, storage life, ingredients of the food.

(7) Development of a satisfactory "field-test" for salmonellae.

(8) The effect of size of test aliquot on recoverability of salmonellae from foods.

(9) Improved methods for determination of coagulase-positive staphylococci in various types of food.

(10) Exploration of *Salmonella* reservoirs.

(11) The distribution and control of *Salmonella* in animal feeds.

(12) The effect of the formulation of a processed food on its microbiology, with special reference to multiplication of pathogens.

(13) The role of foods as virus vectors, and the longevity of enteric viruses in foods.

Methodology

Time did not permit comparative appraisal of the many methods in use among the several collaborators. A proposal was made that comparative methodology might well be a topic of study to be undertaken by the newly formed committee proposed by the Group to be a standing committee under the Section on Food Microbiology and Hygiene now existing as a permanent committee of I.A.M.S.

Publication

The meeting concluded with recommendations:

(1) to publish a synopsis of the proceedings of the meeting and of all agreed Group Statements;

(2) to forward copies of the manuscript for consideration by W.H.O. and F.A.O.

Microbial Spoilage Problems

of Fresh and Refrigerated Foods

R. H. VAUGHN

University of California, Davis, California

The development of mechanically refrigerated railway cars, ships, and trucks during the past century (*1*) thus permitting the transportation of freshly harvested or prepared foods from producing areas to marketing centers has had a dramatic effect on the eating habits and the general well-being of man. Eventually, this progress will also alter the meaning of fresh as applied to foods for, although the present definition reads "newly produced, gathered or made; hence not stored or preserved as by pickling in salt or vinegar, refrigeration etc.; as fresh vegetables, fruit etc., fresh tea, raisins etc." I submit that most perishable foods (fruits, vegetables, meats, etc.) remain fresh because they are properly refrigerated. Thus at the outset it is clear that this discussion of the microbial spoilage of fresh and refrigerated foods must, of necessity, forego semantics in order to meet the imposed time schedule.

Over the years many detailed studies of the spoilage of refrigerated market fruits and vegetables have been made. Some of the more common types of market spoilage (disease) common to California crops are listed in Table I.

In many cases a softening is manifested at some point during the progressive spoilage of the crop. Deterioration of the texture of these crops has been correlated with the elaboration of pectolytic enzymes by many of the microorganisms causing the various types of market "disease."

Evidence accumulated over the years would tend to substantiate the belief that the fungal pectolytic enzymes have low pH optima for activity whereas the bacterial enzymes have high pH optima. As will be noted from Table I, with a single exception (pears), the recognized market diseases are caused by fungi. In contrast, however, both fungi and bacteria cause market diseases of the vegetable crops.

TABLE I

Market Diseases of Crops in California Listed in Order of Importance*

FRUIT CROPS

Apple	*Apricot*	*Avocado*
Penicillium rot	Rhizopus rot	Anthracnose
Neofabraea rot	Brown rot	
Stempyllium rot	Aspergillus rot	
Pleospora rot	Penicillium rot	
Cladosporium rot	Alternaria rot	

Caneberries	*Cherry*	*Citrus*
Rhizopus	Rhizopus rot	Penicillium rot
	Brown rot	Geotrichum rot
	Penicillium rot	
	Alternaria rot	
	Cladosporium rot	
	Botrytis rot	

Fig	*Grape*	*Peach*
Alternaria rot	Botrytis rot	Rhizopus rot
Cladosporium rot	Diplodia rot	Brown rot (Monilia)
		Gilbertella rot
		Aspergillus rot
		Alternaria rot

Pear	*Plum*	*Strawberry*
Penicillium rot	Brown rot	Botrytis rot
Erwinia rot		Rhizopus rot

VEGETABLE CROPS

Artichoke	*Aaparagus*	*Cantaloupes & Honeydew Melons*
Botrytis rot	Bacterial soft rot	Sun scald
	Phytophthora rot	Cottony leak (Pythium)
	Fusarium rot	

Carrot	*Celery*	*Crucifers*
Bacterial soft rot	Sclerotinia rot	Sclerotinia rot
Rhizopus rot	Bacterial soft rot	Altenaria rot
Thielaviopsis	Septoria spot	Downy mildew
		Botrytis rot

Lettuce	*Onion*	*Potato*
Botrytis rot	Aspergillus rot	Bacterial soft rot
Internal browning	Bacterial soft rot	Black heart
Downy mildew	Botrytis rot	Jelly end rot
Bacterial soft rot	Downy mildew	Ring rot
Tip burn		

TABLE I—*Continued*

VEGETABLE CROPS

Snap beans	Sweet potato	Tomato, ripe
Rhizopus rot	Rhizopus rot	Rhizopus rot
Sclerotinia rot	Internal cork (unknown)	Alternaria rot
Botrytis rot	Pythium rot	Geotrichum rot
	Black rot	Stemphyllium rot
		Botrytis rot
Tomato, green wrap	*Watermelon*	
Botrytis rot	Rind rot	
Stemphyllium rot	Pythium & Phytophthora rots	

* Partial list prepared by J. M. Ogawa and E. E. Butler with help from E. E. Wilson and W. K. Kimble. Department of Plant Pathology, University of California, Davis, California. Consult (*2*) and (*3*) for a list applicable to all areas of the United States.

The association of the fungi with spoilage of the low acid vegetables suggests the possibility that the *in vitro* vs. *in vivo* pectolytic activities of the various organisms are different or different pectolytic enzymes capable of activity at pH are elaborated when the fungi are in contact with "living" tissue.

Until 1960 the well-described pectolytic enzymes produced by bacteria, yeasts, or molds were all thought to be hydrolytic in nature (*4*). In 1960 Albersheim, Neukom and Deuel (*5*) discovered an enzyme in a commercial enzyme preparation which had a mechanism for attacking pectin which was not hydrolytic in nature. This enzyme caused the degradation of pectin with an accumulation of an unsaturated digalacturonic acid. It was named pectin *trans* eliminase and appeared to attack the polymer in a random manner. As a result of this key discovery by these Swiss investigators one must suspect that many of the "polygalacturonases" described prior to 1960 may have to be recharacterized, to a degree at least.

More recently it has become apparent that a number of pectolytic *trans* eliminases may be produced by microorganisms. The work of Nagel and Vaughn (*6, 7, 8*) clearly established that three enzymes produced by *Bacillus polymyxa* were responsible for the degradation of pectin.

(1) Pectin methylesterase—causing demethylation of the molecule.

(2) Pectic acid or Polygalacturonic acid *trans*-eliminase—causing degradation of the pectic acid to short-chain polymers and eventually to unsaturated digalacturonic acid, normal digalacturonic acid, and a-D-galacturonic acid.

(3) Digalacturonase—splitting of the normal and unsaturated dimers to a-D-galacturonic acid which is further degraded by glycolytic system to give end products CO_2, H_2, etc.

The polymyxa enzyme appears to attack the pectic acid molecule in a random manner. The work of Starr and Moran (9) substantiates these findings. More recently Macmillan and Vaughn (10) have found another bacterial polygalacturonic acid trans-eliminase produced by a strain of Clostridium multifermentans which attacks the terminal groups to produce a preponderance of unsaturated digalacturonic acid. This organism also produces the demethylating enzyme so that, grown in vitro or in nature, the culture can cause the degradation of pectin as far as the dimer stage. However, it appears to be unable to produce the digalacturonase so in contrast to the Bacillus polymyxa it does not completely destroy the pectic material in the same manner.

These two trans-eliminases have several common properties:

(1) Optimum pH 8–9
(2) Require Ca^{++} ions
(3) Range of activity pH 5–9.5
(4) Optimum temperature 45°–55°C

Edstrom and Phaff (11) also have been studying a pectin trans-eliminase produced by a species of Aspergillus. This enzyme attacks the pectin directly without any necessity for demethylation. It is most active at low pH and peaks at 3.5–4.5 in activity without any requirement for Ca^{++} ions. However, if calcium is added (0.13 M) the enzyme still remains active at pH values up to at least 8.0 although its activity diminishes.

These latter in vitro experiments indicate that, contrary to our previous concept, pectolytic fungal enzymes probably can cause softening of the low acid fresh-refrigerated fruits and vegetables.

Although these interesting trans-eliminases have been found to be produced by bacteria and a mold, to date in our laboratory, we have not found the pectolytic yeasts to produce the same or similar enzymes (Saccharomyces fragilis and Rhodotorula glutinis).

One may ask, why such interest in pectolytic enzymes? There are a number of reasons:

(1) Softening is a major deteriorative change in fresh refrigerated fruits and vegetables as well as those that undergo processing.

(2) It may be possible through a better understanding of these enzymes, to find better ways and means for arresting activity of the enzymes or, perhaps, even prevention of their elaboration.

(3) Their study adds to our knowledge of their properties.

One may also ask, why not prevent growth of the microorganisms in the

first place? This problem has been, and still is, the subject of much interest. Such things as antiseptic ices, antibiotics, antifungal and antibacterial chemicals, and irradiations of various kinds have been used. However, at the present time there appears to be no substitute for careful, rapid handling of the freshly harvested crop so that it can be put into refrigeration at the proper temperature with the proper humidity and atmosphere in as short a time as possible.

REFERENCES

1. O. E. Anderson, Jr. "Refrigeration in America." Princeton Univ. Press, Princeton, New Jersey (1953).
2. American Society of Refrigerating Engineers, A.S.R.E. Data Book, Vol. 1, "Refrigeration application," New York, 1959.
3. American Society of Heating, Refrigerating and Air-Conditioning Engineers, A.S.H.R.A.E. Guide and Data Book. "Applications for heating, refrigerating, ventilating and air conditioning," New York, 1962.
4. A. L. Demain and H. J. Phaff. "Recent Advances in the Enzymatic Hydrolysis of Pectic Substances." *Wallerstein Lab. Commun.* 20, 110–119 (1957).
5. P. Albersheim, H. Neukom, and H. Deuel. *Helv. Chim. Acta* 43, 1422–1426 (1960).
6. C. W. Nagel and R. H. Vaughn. "The Characteristics of a Polygalacturonase Produced by *Bacillus polymyxa.*" *Arch. Biochem. Biophys.* 93, 344–352 (1961a).
7. C. W. Nagel and R. H. Vaughn. "The Degradation of Oligogalacturonides by the Polygalacturonase of *Bacillus polymyxa.*" *Arch. Biochem. Biophys.* 94, 328–332 (1961b).
8. C. W. Nagel and R. H. Vaughn. "Comparison of Growth and Pectolytic Enzyme Production by *Bacillus polymyxa.*" *J. Bacteriol.* 83, 1–5 (1962).
9. M. P. Starr and F. Moran. "Eliminative Split of Pectic Substances by Phytopathogenic Soft-Rot Bacteria." *Science* 135, 920–921 (1962).
10. J. D. Macmillan and R. H. Vaughn. "Studies on a Polygalacturonic Acid-*trans*-eliminase (PATE) Produced by *Clostridium multifermentans.*" In preparation (1963).
11. R. D. Edstrom and H. J. Phaff. "Preparation and Certain Properties of Pectin-*trans*-eliminase from *Aspergillus fonsecaens.*" In press (1963).

Microbial Spoilage of Canned Foods

C. W. BOHRER

National Canners Association, Washington, D.C.

In canning, preservation is accomplished by hermetically sealing the food product in a suitable container and destroying, by means of a heat treatment, the bacteria capable of spoiling the product. By definition one would expect spoilage in canned food to be nonexistent. While we know that this is not the case, microbial spoilage in canned food is relatively infrequent. Also, the bacterial agents responsible for spoilage in canned foods, especially those in the low-acid range, are limited to certain spore-forming types which are resistant to heat. In any method of food preservation, the first concern is the protection of the health of the consumer by controlling or destroying any pathogenic bacterial types. The only food poisoning organism of sufficient heat resistance to require special consideration when canning low-acid products is *Clostridium botulinum*.

Organisms Causing Spoilage

Heat processing studies conducted by the National Canners Association (N.C.A.) since 1918 have established the processes for low-acid products which will fulfill the basic requirement of adequacy to destroy the spores of *C. botulinum*. Minimum temperature and time values used in any canning process are based on the maximum conditions needed to destroy this organism. It has been well established that the typical nonpathogenic spoilage organisms have considerably greater heat resistance than *C. botulinum*. This fact provides, in effect, a built-in safety factor since the destruction of the more heat-resistant nonpathogens to prevent economic loss insures at the same time the destruction of *C. botulinum*. As mentioned previously, since heat is the indispensible element in canning preservation, the agents responsible for any spoilage in canned foods are usually heat-resistant spore-forming organisms which survive the heat treatment. The principal nonpathogenic spoilage types are the facultatively and obligately thermophilic flat sours which are represented by the

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type species, *Bacillus stearothermophilus*, and the mesophilic and thermophilic aerogenic anaerobes represented by nontoxic putrefactive types and *Clostridium thermosaccharolyticum*. As far as we know these canned food spoilage types are not of concern otherwise in food spoilage. Their importance in canning rests largely on the heat resistance of their spores.

Spoilage Relationships

Each type of organism appears to have certain food preferences governed for the most part by the pH of the canned product (1). Thus, the thermophilic flat sours and the putrefactive anaerobes are of primary importance in the low-acid food group (pH 5.3 and higher; examples: peas and corn). The thermophilic anaerobes, however, are most commonly encountered in connection with the medium-acid food group (pH 5.3 to 4.5; examples: asparagus and specialty products, such as spaghetti with tomato sauce). Only two spore-forming organisms are important in the acid food category (pH 4.5 to 3.7 range) namely the gas producing butyric anaerobes represented by *Clostridium pasteurianum*, which affect tomatoes, and the aciduric flat sours represented by *Bacillus thermoacidurans* that may become a problem in tomato juice. No spore formers have been found to spoil highly aciduric canned foods such as fruits in the pH 3.7 and lower range. Here the important spoilage agents are the yeasts, molds, and certain lactic acid-producing bacteria.

As a practical matter, pH 4.5 has been selected as the point above which products should be processed under pressure to a degree sufficient to destroy all food poisoning bacteria. Below pH 4.5 the spores of *Clostridium botulinum* and the majority of the other food spoilage organisms fail to germinate even though they may be present.

Spoilage Control

The degree of process necessary to preserve a canned food product is determined with regard to the probable kinds of spoilage bacteria that may be present and also their numbers. Obviously, these requirements lead to process suggestions that carry a factor of safety to guard against something more than an average contamination. It has been the practice of laboratories connected with the canning industry to make process suggestions on this basis. The heat processes derived from experimental procedures are suggested to the canning industry in N.C.A. bulletins (2) and (3). The processes given are not necessarily adequate if the food is exposed to extreme contamination by spoilage bacteria that may or may not be associated with unsanitary conditions. Control of contamination by strictly applying the principles of sanitation to the entire canning

procedure and by other appropriate means is a necessary consideration in designing any process.

Sources of Contamination

Bacteriological investigations in the canning plant itself have pointed up the causes of abnormal levels of bacterial contamination which might render the usual heat treatment for preservation inadequate (4).

The basic source of the spoilage organisms is usually the soil that clings to the various raw products brought into the canning plant. Proper washing and preparation procedures are usually sufficient to reduce to a minimum the number of microorganisms introduced in this manner. Bacterial contamination directly transferred to the canning plant through the medium of the raw product may be a factor in spoilage but only in exceptional cases. Mushrooms are one of the exceptions. Because of the structure of the mushroom it is difficult to wash, and the very conditions that are favorable for the development of the mushroom itself are conducive to the development of large numbers of the thermophilic spore-forming organisms. Other examples of this situation are spinach, grown in muck soils, and asparagus.

Equipment Contamination

Contamination sources which may become significant can develop within the canning plant itself. Under certain conditions, inadequate equipment or faulty operating practices may promote a buildup of the initial minimum contamination to the point where a spoilage hazard is created. Faulty operating practices are not necessarily associated with unsanitary conditions, per se. One fault is permitting heated canning equipment to remain within the thermophilic temperature range of 105°F to about 168°F. The N.C.A. as a result of its bacteriological studies in canning plants since 1926 is able to offer suggestions to canners for controlling the contamination sources within the canning plant.

Ingredient Contamination

Another source of contamination, and an important one, may be the ingredients which are added to certain products. Some of these ingredients, such as sugar, starch, powdered milk, spices, rice, etc., may be heavily contaminated with such thermophilic spore-forming spoilage organisms as the flat-sours and the thermophilic anaerobes. In the case of ingredients, contamination by the spoilage types may occur in two ways. The contamination may be added directly to the product with the ingredient

or alternatively the spoilage types present in the ingredient may contaminate the canning equipment through which they pass and there increase in number. The N.C.A. has published bacterial standards for sugar and has applied these standards to other ingredients. A spoilage problem encountered in recent years is associated with the use of frozen foods in canning mixed vegetables when one or more of the ingredients are out of season, or in the preparation of formulated products, such as soups. The frozen product may have picked up thermophilic contamination during its preparation. The thermophilic contamination has no significance in the frozen food but when the latter is used as an ingredient in canning the thermophilic spore contamination may constitute a spoilage hazard.

Commercial Sterility

It has been demonstrated that the higher the optimum temperature for the growth of a spore-forming organism the greater will be the resistance of its spores. The spores of the obligately thermophilic organism possess the highest heat resistance of all the spore forming spoilage types. If present in any considerable number it may not be feasible to attempt to destroy them in a product whose quality is markedly deteriorated by heat. However, since the contaminating type is obligately thermophilic the possibility of its producing spoilage in usual commercial distribution channels is quite remote. When spoilage would result only if the canned and processed product were stored at elevated temperatures, the product would normally be considered commercially sterile. The recent advent of hot vending machines, however, imposes on canned products intended for such distribution bacterial restrictions to a degree not heretofore necessary.

Acid Product Preservation

As indicated previously, food products with a pH below 4.5 can be given boiling water processes, since with the two exceptions mentioned, spore-forming spoilage types are of no concern. When the acid food product is placed in the can and processed in boiling water to give a center temperature of about 165°F for highly acid products such as sauerkraut, or 185°–190°F for less acid products such as tomatoes or tomato juice, there is little, if any, spoilage potential. In recent years in an attempt to speed up production some acid products, particularly juices, have been preserved by the hot-fill-hold procedure (5). The acid juice is heated to around 200°F, filled into cans, and the cans sealed and held for a time sufficient for the hot juice to sterilize the container. In this proce-

dure if the hold period is shortened, or if cold water or drafts strike the cans during the hold period, the full lethality of the heat process may not be attained and spoilage due to nonspore-forming lactic acid bacteria or yeasts may occur depending upon the degree of under-process. Since the food poisoning types do not grow in acid media the problem here is strictly one of economic loss.

Recontamination Following Processing

So far, we have only considered the efficiency of the heat treatment in the preservation of food material. We now come to the hermetically sealed container which plays its role in preservation by preventing recontamination of the product following the heat process. At the completion of the heat process it is necessary to cool the canned food product. In the case of some canned products, the hot cans may be stacked in open rows to permit good circulation and allowed to air cool. There is little, if any, potential for recontamination in air cooling principally because the can seams are dry. The usual practice, however, is to cool the heat processed cans in water. This water cooling may be accomplished either by spinning the cans under water sprays or by drawing crates of the processed cans through a cooling canal filled with cold water. Subsequent to this cooling, cans are conveyed to the casing or labeling machines by means of can runways, belts, or cables. During water cooling and the post-cooling handling of the cans in the cannery, we have the possibility of recontamination by organisms entering the sealed container. The reason for this is: (1) during the cooling operations the cans go from a condition of internal pressure to vacuum, and (2) immediately following the heat treatment the can seams are slightly expanded and the compound lining is somewhat soft or plastic. During this period, the cans are very susceptible to spoilage caused by rough handling since any severe stress or strain placed on the can could result in a temporary seam deformation with the possibility of contaminated water being drawn into the can by the internal vacuum.

The organisms which cause spoilage in the event of leakage are heterogeneous, free-living forms not subject to orderly classification in relation to canned food groups. Since a wide variety of organisms gain entrance in leakage of the container, spoilage is the quick and inevitable result, and except for very rare instances is gaseous in character resulting in swelling of the container.

The three main factors in spoilage resulting from post-processing can handling operations are: (1) the condition of the can double seams, (2) can abuse due to poor operation or adjustment of the filled can han-

dling equipment, and (3) the presence of bacterial contamination in cooling water or on can runways.

Spoilage due to leakage is kept at a minimum by frequent checks of the can seams to make sure that they are being properly rolled and formed. In addition to the usual attention to good seam construction, precautions must be taken in handling the cans before they are thoroughly cooled to prevent even small dents on or near the double seams. When filled cans are handled in automatic equipment at high speeds, small deformations of the seams may be more significant as spoilage factors than they are under slow speed, low-impact conditions. The installation of the many new labor-saving devices for handling filled cans has introduced certain hazards, which if not minimized, may result in some spoilage even with the best possible double seam construction. To keep these hazards at a minimum, rough handling of the container during the cooling or post-cooling handling are eliminated as far as possible (6).

The final factor is the bacterial condition of the cooling water. The bacterial quality of the cooling water should be the highest attainable. It is not possible to set any fixed bacterial standard for all cooling water which, if maintained, would insure against spoilage due to leakage. Theoretically, a perfect seam should exclude bacteria after the process regardless of the extent of contamination in the cooling water provided that the original perfect seams were not altered during the physical handling of the container. However, one cannot uniformly attain this degree of perfection under practical conditions. It is realized that a numerical limit of contamination of cooling water is wholly arbitrary, but the recommendation usually made is that cooling water be of potable quality. In terms of bacterial count a potable water should contain less than 100 bacteria per milliliter. At this level, there would be about five bacteria per drop and the can seams would have only to protect against the entrance of $\frac{1}{5}$ of a drop of water to prevent the entrance of a single bacterium. However, if the count were 1,000,000 bacteria per milliliter, the can seams would have to protect against the entrance of as little as $\frac{1}{50,000}$ of a drop of water to prevent recontamination. This latter condition demands a seam perfection which is not possible to attain under commercial conditions.

Where potable water is used in filling the cooling system and for make up the original contamination should be negligible. However, after some use, bacterial buildup can occur, and this is controlled by continuous chlorination. By treating cooling water with chlorine to a point where one part per million was indicated in the tank water, the counts were always below 50 per milliliter and in most cases were less than 20 per milliliter.

Once the canned product is cool and the compound set, the possibilities of recontamination are extremely remote, with actual breaking or puncturing of the can being about the only method of recontamination.

REFERENCES

1. E. J. Cameron and J. R. Esty. Comments on the microbiology of spoilage in canned foods. *Food Res.* **5,** 549–557 (1940).
2. Natl. Canners Assoc. Res. Lab. "Processes for Low-Acid Canned Foods in Metal Containers." Bull. 26-L, Ninth Ed., Washington, D.C., August 1962.
3. Natl. Canners Assoc. Res. Lab. "Processes for Low-Acid Canned Foods in Glass Containers." Bull. 30-L, Third Ed., Washington, D.C., June 1963.
4. C. W. Bohrer. A bacteriologist inspects pea and corn canning plants. *Canner* **109,** No. 10, pp. 12, 13, 14, 24, 30 (1949).
5. C. P. Collier and C. T. Townsend. Container sterilization for acid products by hot-fill-hold-cool procedures. *N.C.A. Information Letter*, No. 1472 (1954).
6. C. W. Bohrer and J. Yesair. Bacteriological studies on post-cooling can handling equipment. *N.C.A. Information Letter*, No. 1666 (1958).

The Limits of Edibility

of Defrosted Chicken Pot Pies

A. C. Peterson, M. J. Fanelli, and M. F. Gunderson

*Microbiological Research Department, Campbell Soup Company,
Camden, New Jersey*

In a series of papers, we have developed and stressed the concept that
defrosted frozen convenience foods become organoleptically unacceptable
before they are possible public health hazards. Our experiences in defrost-
ing chicken pies and following the development of a naturally occurring
Staphylococcus population have substantiated this (*1*). Present concepts
and, in some places, codes for handling frozen foods call for the mainte-
nance of −18°C temperatures or below. Nearly every frozen food manu-
facturer prints the admonition on his package, "If thawed, do not
refreeze!" In spite of this, defrost does occur. Housewives write to ask us
if frozen foods that have been thawed for a few hours can be refrozen
safely.

This laboratory has determined defrost temperatures, times and micro-
bial populations for a variety of convenience frozen foods (*2*) and has
attempted to formulate criteria by which defrost and its effects can be
recognized. Peterson and Gunderson (*3*) reported a correlation of defrost
time, temperature, bacterial count, and organoleptic evaluation of chicken
pies. Their report was not concerned primarily with the organoleptic
quality of the product, and they investigated only the effects of defrost
at 5°C.

The most applicable known research is the classical time-temperature
tolerance study of the Western Regional Laboratory of the United States
Department of Agriculture. These studies, however, did not include bac-
teriological analyses of the products. With exception of incubation at −7°
or −4°C, and then depending on the product, their study did not include
the effect of defrost. Hucker and David (*4, 5*) investigated the effect of
thawing and refreezing frozen vegetables and frozen chicken pies. They

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reported no change in microbial populations at 2°C and 7°C in vegetables. Chicken pies had an increase in total count after 10 hours at 21°C and 32°C. The defrost periods were short and there was no organoleptic evaluation of the products.

In view of the paucity of information concerning the effects of defrost time and temperature on bacteriological quality and on organoleptic acceptability and in view of that persistent question about the refreezing of a defrosted product, this study was undertaken.

Methods and Materials

Intact case lots of commercially produced, frozen chicken pot pies were used because such cases frequently represent consecutive units in production and are from the same batches of ingredients. While there may be appreciable variations in microbiological quality within a given case, these are less than those between cases. It is impossible to use a single pie with repeated samplings during an entire defrost experiment because the pies are simply not large enough and the integument of the pie is broken by sampling. Chicken pies representing two different manufacturers were selected. One of these manufacturers consistently produced pies of excellent microbiological quality. These were designated "Low Count Pies." The other manufacturer consistently produced pies with microbial counts beyond the standards of the industry and beyond those once considered in the Association of Food and Drug Officials of the United States model code for frozen foods. These were designated "High Count Pies."

A minimum of three pies was used for every time and temperature examined. Five such replicates were run, so that the data represent a total of fifteen samples at each time and defrost temperature for each brand of pies. All of the pies were defrosted from the hard frozen state (after storage at −25°C for at least 48 hours) in thermostated incubators, while still in their original cartons. The time intervals for defrost were selected to give frequent sampling during the time a chicken pot pie passed from organoleptically acceptable status to an unacceptable status and were based on previous research.

Eleven g samples were removed aseptically from each pie, being careful not to include an undue portion of the crust. The sample was added to a 99 milliliter sterile water blank containing glass beads and shaken on a mechanical shaker for 3 minutes before further appropriate dilutions were made. Total counts were made on Trypticase Soy (TS) agar[1] using duplicate plates and incubated for 24 to 36 hours at 37°C. A duplicate

[1] Baltimore Biological Laboratories, Baltimore, Maryland.

series of plates was incubated at 5°C for 21 days for the "psychrophile count." Coliform organisms were counted by plating on Violet Red Bile (VRB) agar[2] and incubating the plates at 37°C for 48 hours. Data on coliform organisms have not been included in any of the tables because very few of these organisms were found and they failed to multiply during defrost. Staphylococci were enumerated on Tellurite Glycine (TG) agar[2] plates which were incubated at 37°C for 24 to 36 hours.

For organoleptic evaluation, a single sample from each of the incubation periods at the same defrost temperature was presented to the taste panel. They were given an opportunity to inspect eight samples simultaneously. Samples were inspected in the defrosted, raw state, and the defrosted, baked state before opening the pie so that the crusts could be examined. Of course, the contents of the pie as well as the crusts were examined, smelled, and tasted. Determinations of the pH of the fillings were also made. From each defrost time, a sample was immediately blast frozen in a −40°C freezer. These samples were then stored for 1 to 2 weeks at −25°C and then given to the taste panel. In presenting the samples to the taste panel, the samples were arranged in order of increasing defrost time (severity of defrost treatment). This was done so that unsuspecting tasters might not put a very strong, badly flavored sample into their mouths and ruin their taste perception and unnecessarily or unreasonably bias them. Samples were identified by number only, and members of the panel did not know the temperature at which defrost had taken place nor the defrost interval which each pie had suffered. Considerable difficulty was observed with taste panel fatigue, and considerable care was exercised in presenting the series of samples to the panels to prevent any preconceived opinions being exercised or habit patterns formed. A prepared form was supplied to the participants which asked the panel member to specify whether the sample was acceptable or not visually, by odor, and by taste. Space was provided and specific instructions were given to comment on differences from the controls. Two panels were used in the organoleptic evaluation. One panel, comprised of senior product development research personnel and senior members of this department, was considered to be an experienced panel (A). Its members, particularly those in product research, have had years of experience in organoleptic evaluation of food products and all have worked in the development of the products examined.

A second panel, comprised of assistants in this department, was considered to be an untrained panel (B). By contrast, the inexperienced panel had only native abilities and noncommercial domestic experience. Identi-

cal samples were presented to both panels. Panel members were not screened or tested for taste perception. The flavor profile technique was not employed.

Photographs were made on Kodacolor film with a Rolleflex, 2.8E camera using 3200°K floodlights. Pictures were made of raw pies, baked intact pies, and baked pies with the top crust removed. A similar series of pictures was made of pies after being refrozen. Pictures were made of every time interval of defrost at every temperature level and of a sample from every replicate.

Data and Results

Figure 1 presents the results of the organoleptic evaluation of "low
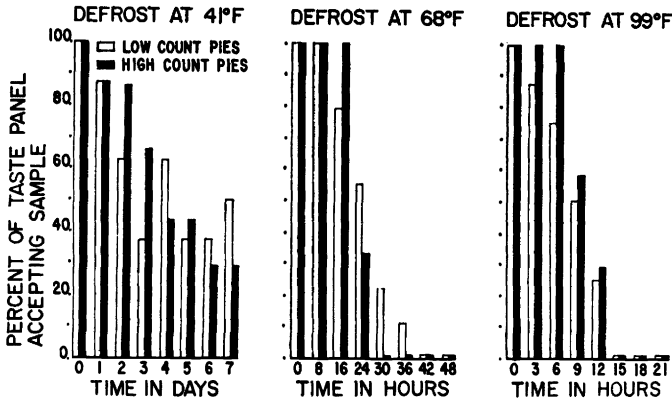
FIG. 1.   Effect of time and temperature of defrost on organoleptic acceptability of frozen chicken pot pies. Comparison of pies having low or high bacterial counts.

count" chicken pot pies. The percentage of the taste panel ruling the sample acceptable is plotted as a function of the defrost time at the three different defrost temperatures. Edibility in this study as a function of acceptability is not to be confused with desirability. Acceptability by the taste panel implied only a decision that the product could be eaten, not that it was good or that they liked it. These samples were all defrosted and refrozen and were examined by panel B. No effort was made to eliminate those individuals who can eat anything, and obviously did, from either panel. Surprisingly, while there was a drop in acceptability during defrost, a part of the panels found pies edible even after seven days at 5°C. At 20°C, complete inedibility was reached at 42 hours (100% rejection). If the less sensitive individuals (about 25% of the panel) were

discarded, unacceptability was reached at 30 hours. At 37°C unacceptability was reached at 15 hours after commencing defrost from the frozen state. Again, if the insensitive minority was eliminated, unacceptability was reached at about 12 hours. It is surprising how soon incipient organoleptic unacceptability begins. If one assumes an acceptability of 90% (to compensate for the ultrasensitive on the taste panel), incipient unacceptability begins at 24 hours at 5°C, at 16 hours at 20°C, and at 6 hours at 37°C.

As shown in Fig. 1, the results with high count chicken pies were comparable to those obtained with pies having low initial bacterial counts. The major difference was that organoleptic unacceptability was reached sooner, for example, at about 30 hours at 20°C. Probably, the organoleptic changes were more pronounced because the percentage of acceptability falls more sharply in fewer changes, or steps, in the diagram.

The influence of refreezing the samples after defrosting is shown in Fig. 2. Percentage of acceptability is plotted as a function of the time
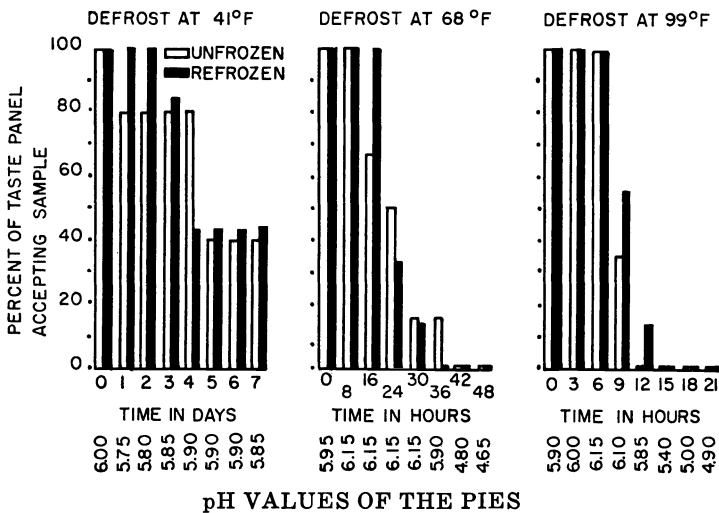
FIG. 2. Comparison of organoleptic acceptability of defrosted nonfrozen and refrozen chicken pot pies.

of defrost at the three defrost temperatures for low count chicken pies. These results were obtained with panel A. The net results compare very well with those obtained with panel B. It was found that the experienced panel tended to rule more sharply that the sample was acceptable or nonacceptable with fewer gradations between than the untrained panel. In this experiment, the untrained panel was found to be nearly as dis-

criminating as the experienced panel. Organoleptic unacceptability was, in general, reached one time period earlier at all temperatures with the experienced panel than with the untrained panel. Similarly, the decisions on acceptability were sharper, resulting in fewer stages in reaching agreement or 100% unacceptable status. Refreezing the sample, seemingly, had very little influence on the subsequent organoleptic evaluation of the product. Defrost results in impaired appearance, odor, and flavor and these losses in desirable characteristics increase as the time and temperature of defrost increase. Because refreezing does not reverse defrost effects and only slows down those actions due to defrost which are already in progress, *defrost effects are cumulative.* Thus, a series of mild defrosts at low temperatures would lead to a product whose wholesomeness was considerably impaired. Pies defrosted in any degree could always be recognized by the taste panels. Measurements were made of the pH of the contents of the baked pies and these are included in Fig. 2. The only conclusion reached was that an abrupt lowering in pH coincided with organoleptic unacceptability.

One important by-product of this study was the discovery of the succession of flavors which occur during defrosting. In general, defrosting for a single time period, at any of the temperatures used, brought about an intensification of desirable flavors. This was usually characterized as stronger chicken flavors although criticism was voiced that there was a feathers aroma and flavor present. Following this, there was a progressive loss of desirable flavor until a nearly bland or flavorless product was reached. This stage was then followed by a rapid onset of undesirable defrost flavors which rendered the product inedible.

Table I summarizes the bacteriological data on low count chicken pies when these pies were subject to defrost at 5°C. The pies were sampled at 24-hour intervals. The mesophile count, as determined by incubating TS plates at 30°C, progressed from a mean value of 4400 (about the same at the beginning of detectable spoilage) to a high of 510,000 bacteria per gram. The psychrophile count behaved similarly. Staphylococci failed to multiply.

Table II shows the microbiological data for the low count pies incubated at 20°C. The mesophile count rose slowly through the defrost of the first 24 hours, but then the count rose very quickly to an astronomical level at 48 hours. The psychrophile count behaved similarly. Staphylococci multiplied very slowly through the first 42 hours, and even at 48 hours the *Staphylococcus* count was not significant. The total counts were very low at incipient spoilage at 8 hours and high at organoleptic unacceptability at 42 hours.

TABLE I

BACTERIOLOGICAL EXAMINATION OF LOW INITIAL COUNT CHICKEN POT PIES
HELD AT 5°C. TOTAL NUMBER[a] OF ORGANISMS AT 30°C AND 5°C AND OF
THE NUMBER OF STAPHYLOCOCCI

Time at 5°C	Total ($\times 10^3$)		Staphylococci
	30°C	5°C	
0 Days	1.4–7[b]	0.14–4.5	<10–40
	4.4[c]	0.89	20
	4.8[d]	0.33	20
1 Day incipient spoilage	0.95–8.2	0.56–3.0	<10–40
	4.3	1.4	15
	4.2	1.4	<10
2 Days	1.8–15	0.51–3.0	<10–15
	5.1	1.3	10
	4.2	0.99	<10
3 Days	1.3–19	0.26–5.3	<10–35
	7.3	1.4	10
	7.1	1.5	<10
4 Days	0.67–40	0.66–6.1	<10–35
	8.8	2.4	15
	6.3	1.8	<10
5 Days	3.2–170	2.2–16	<10–15
	31.4	6.5	<10
	8.6	7.1	<10
6 Days	7.7–4,600	31–5,800	<10–170
	510	1,000	28
	100	370	<10
7 Days	38–1,400	42–2,200	<10–60
	340	500	17
	290	300	10

[a] Number of organisms per gram of sample.
[b] Range value in each case.
[c] Mean value in each case.
[d] Median value in each case.

TABLE II

BACTERIOLOGICAL EXAMINATION OF LOW INITIAL COUNT CHICKEN POT PIES HELD AT 20°C. TOTAL NUMBER[a] OF ORGANISMS AT 30°C AND 5°C AND OF THE NUMBER OF STAPHYLOCOCCI

Time at 20°C	Total ($\times 10^3$)		Staphylococci
	30°C	5°C	
0 Hours	1.3–14[b]	0.23–7.3	<10–75
	5.6[c]	2.1	28
	4.2[d]	0.85	20
8 Hours Incipient Spoilage	3–22	0.1–4.0	<10–100
	7.6	1.3	25
	6.6	0.62	15
16 Hours	2.1–400	0.44–9.3	<10–80
	62	3.3	35
	17	3.1	25
24 Hours	62–1,700	9.7–460	<10–190
	490	220	86
	320	200	70
30 Hours	420–110,000	310–>10,000	<10–690
	26,000	4,700	240
	18,000	8,600	190
36 Hours	2,900–>1,000,000	800–45,000	70–4,700
	410,000	15,000	2,100
	110,000	6,100	2,300
42 Hours Organo. Unaccep.	270,000–1,700,000	85,000–380,000	<10–6,400
	890,000	280,000	2,400
	810,000	320,000	2,800
48 Hours	570,000–2,300,000	310,000–2,100,000	500–57,000
	1,500,000	1,100,000	25,000
	1,400,000	910,000	21,000

[a] Number of organisms per gram of sample.
[b] Range value in each case.
[c] Mean value in each case.
[d] Median value in each case.

At 37°C, the mesophile count began to climb sharply at 6 hours (incipient spoilage), as shown in Table III. At 12 hours very high numbers of organisms were reached. In contrast, the psychrophile count climbed more slowly and failed to reach the populations observed at lower defrost temperatures. Staphylococci multiplied somewhat, but even at the termination of the experiment had not reached levels which might have been suspected as those associated with staphylococcal food poisoning. The total counts were high at organoleptic spoilage at 15 hours.

Table IV presents the microbiological results for the examination of high count chicken pies defrosted at 5°C. The total bacterial counts were moderate in numbers at 24 hours where incipient spoilage began. The mesophile count rose rapidly at this temperature and very appreciable numbers of organisms were reached beginning with the third day at 5°C. In contrast, the psychrophile count rose more slowly and never reached the levels achieved by the mesophiles. Staphylococci, of course, failed to grow.

At 20°C (Table V), high mesophile and psychrophile counts were reached in 24 hours. After 16 hours at the beginning of detectable spoilage, the counts were moderate. At 30 hours at organoleptic spoilage, the total counts were quite high. The psychrophile counts failed to reach the same levels that the mesophilic bacterial counts did. Staphylococci also failed to multiply at this temperature.

At 37°C (Table VI), the mesophilic bacterial count rose very sharply and very high numbers were obtained after 6 hours (incipient organoleptic spoilage). Very high bacterial numbers were found at organoleptic spoilage at 15 hours. The psychrophile count rose more slowly and did not attain the same numerical level as the mesophiles. Staphylococci grew very slowly and failed to achieve appreciable numbers.

From the voluminous number of photographs made in this study, pictures have been selected which show a nondefrosted control pie, a pie at incipient organoleptic spoilage (90% acceptability) and a pie at organoleptic spoilage (80% unacceptability). Because black and white photographs do not give an adequate representation, only pictures for a single temperature are shown. Pictures of pies defrosted at 20°C were selected because room temperature may be the temperature at which most mishandling occurs. Higher temperatures are encountered on hot summer days and in improperly operating freezers.

Figure 3 shows the unbaked appearance of the crusts of the pie after defrost at 20°C. There was a progression of color in the crusts from the dead white of the control to a yellow and finally to a gray yellow. Sinking of the crust and sogginess are characteristics of defrost, as is the collec-

TABLE III

BACTERIOLOGICAL EXAMINATION OF LOW INITIAL COUNT CHICKEN POT PIES
HELD AT 37°C. TOTAL NUMBER[a] OF ORGANISMS AT 30°C AND 5°C AND OF THE
NUMBER OF STAPHYLOCOCCI

Time at 37°C	Total ($\times 10^3$)		Staphylococci
	30°C	5°C	
0 Hours	3.1–7.8[b]	0.03–3.3	< .010–.085
	4.8[c]	.78	.023
	4.5[d]	.40	.010
3 Hours	2.2–11	0.45–3.2	< .010–.18
	6.6	1.2	.027
	5.4	1.0	< .010
6 Hours	10–4,300	0.95–>1,000	< .010–59
Incipient	340	140	.13
Spoilage	40	8.9	.055
9 Hours	420–>50,000	420–>10,000	< .010–1.
	15,000	2,900	.25
	4,700	1,800	.10
12 Hours	32,000–540,000	480–>100,000	0.14–>30
	150,000	26,000	7.5
	140,000	16,000	1.6
15 Hours	290,000–1,800,000	29,000–470,000	10–550
Organo.	750,000	140,000	110
Unaccep.	660,000	92,000	37
18 Hours	520,000–1,700,000	42,000–780,000	8–810
	980,000	260,000	230
	820,000	61,000	90
21 Hours	370,000–1,300,000	4,000–620,000	<10–10,000
	780,000	240,000	3,200
	790,000	250,000	2,300

[a] Number of organisms per gram of sample.
[b] Range value in each case.
[c] Mean value in each case.
[d] Median value in each case.

TABLE IV

BACTERIOLOGICAL EXAMINATION OF HIGH INITIAL COUNT CHICKEN POT PIES
HELD AT 5°C. TOTAL NUMBER[a] OF ORGANISMS AT 30°C AND 5°C AND OF
THE NUMBER OF STAPHYLOCOCCI

Time at 5°C	Total ($\times 10^3$)		Staphylococci
	30°C	5°C	
0 Days	1.1–4,200[b]	0.14–3,900	<10–120
	640[c]	560	32
	4[d]	.78	<10
1 Day	0.6–51,000	0.38–>5,000	<10–20
Incipient	11,000	1,700	<10
Spoilage	18	6.5	<10
2 Days	2.5–13,000	0.43–>5,000	<10
	1,400	770	<10
	5.1	1.9	<10
3 Days	3.7–59,000	0.35–>10,000	<10–55
	11,000	3,300	22
	13	6.8	<10
4 Days	3.0–>500,000	0.75–>10,000	<10–90
	110,000	3,300	27
	8.3	4.8	<10
5 Days	3.5–290,000	7.2–>100,000	<10–70
	77,000	34,000	32
	510	>500	30
6 Days	55.0–>1,000,000	110–>100,000	<10–200
	120,000	22,000	81
	720	780	100
7 Days	30.0–>1,000,000	32–>100,000	<10–180
	150,000	34,000	95
	2,000	>1,000	100

[a] Number of organisms per gram of sample.
[b] Range value in each case.
[c] Mean value in each case.
[d] Median value in each case.

TABLE V

BACTERIOLOGICAL EXAMINATION OF HIGH INITIAL COUNT CHICKEN POT PIES
HELD AT 20°C. TOTAL NUMBER[a] OF ORGANISMS AT 30°C AND 5°C AND OF THE
NUMBER OF STAPHYLOCOCCI

Time at 20°C	Total (×10³)		Staphylococci
	30°C	5°C	
O Hours	3.4–33[b]	0.19–16	<10–100
	16[c]	6.3	43
	12[d]	4.6	25
8 Hours	8.8–190	2.8–310	<10–<100
	55	48	—
	37	8.2	<10
16 Hours	10.0–11,000	4.0–>5,000	<10–3,500
Incipient	4,100	1,500	430
Spoilage	1,800	1,100	50
24 Hours	200–160,000	<10–>10,000	<10
	41,000	31,000	<10
	26,000	7,400	<10
30 Hours	2,000–1,100,000	<100–500,000	<10–30
Organo.	250,000	77,000	17
Unaccep.	76,000	36,000	10
36 Hours	<100–1,100,000	<10–>100,000	<10–1,700
	320,000	260,000	860
	210,000	4,700	<1,000
42 Hours	<1,000–960,000	<100–>500,000	<10–1,700
	550,000	130,000	860
	890,000	8,700	<1,000
48 Hours	<1,000–1,200,000	<100–>500,000	<10–2,900
	390,000	79,000	1,100
	250,000	4,900	<1,000

[a] Number of organisms per gram of sample.
[b] Range value in each case.
[c] Mean value in each case.
[d] Median value in each case.

TABLE VI

BACTERIOLOGICAL EXAMINATION OF HIGH INITIAL COUNT CHICKEN POT PIES
HELD AT 37°C. TOTAL NUMBER[a] OF ORGANISMS AT 30°C AND 5°C AND OF THE
NUMBER OF STAPHYLOCOCCI

Time at 37°C	Total ($\times 10^3$)		Staphylococci
	30°C	5°C	
0 Hours	1.2–860[b]	0.1–470	<10–190
	210[c]	110	66
	110[d]	1.2	15
3 Hours	0.9–15,000	0.45–7,900	<10–120
	2,700	1,500	26
	15	11	<10
6 Hours	13.–43,000	5.1–29,000	<10–2,000
Incipient	6,700	4,400	320
Spoilage	3,400	1,200	<10
9 Hours	800–320,000	3,100–>100,000	<100–150,000
	130,000	37,000	20,000
	80,000	23,000	750
12 Hours	3,800–800,000	1,200–>500,000	<1,000–20,000
	380,000	140,000	7,500
	280,000	190,000	5,000
15 Hours	330,000–1,800,000	22,000–620,000	2,000–220,000
Organo.	990,000	260,000	37,000
Unaccep.	850,000	330,000	10,000
18 Hours	610,000–2,100,000	31,000–410,000	1,000–730,000
	1,400,000	270,000	20,000
	1,100,000	370,000	20,000
21 Hours	630,000–2,600,000	29,000–1,200,000	<1,000–2,200,000
	1,100,000	360,000	800,000
	1,300,000	300,000	10,000

[a] Number of organisms per gram of sample.
[b] Range value in each case.
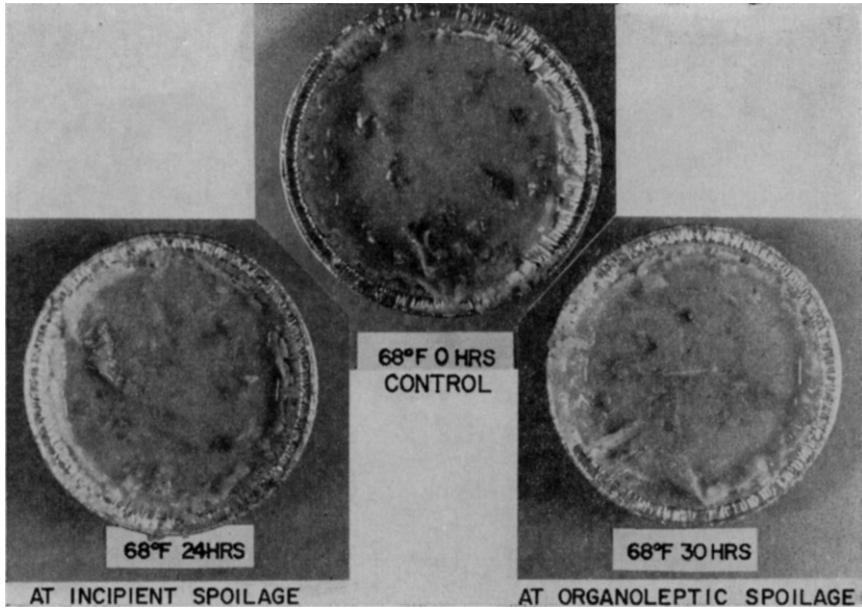[c] Mean value in each case.
[d] Median value in each case.
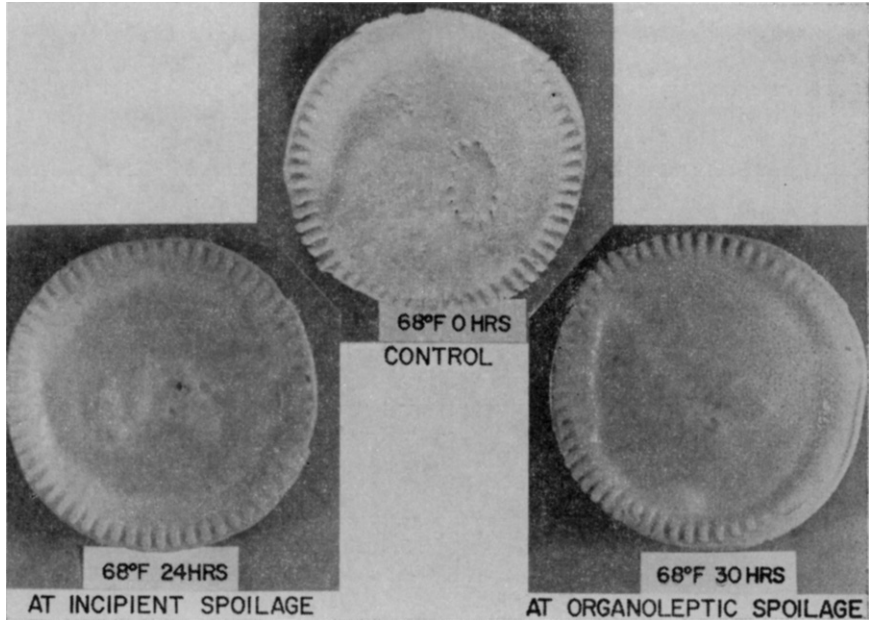
FIG. 3.   External appearance of a raw, control nondefrosted chicken pie, one at incipient spoilage and one at spoilage at 20°C.

tion of liquid and gravy contents which leaks through the top crust. Figure 4 shows the baked appearance of the same pies and the development of the layered, translucent, and shiny or greasy appearance of the crust. Defrost pies in general did not brown as nicely or evenly as the nondefrosted pies and local overbrowning was frequent. Figure 5 shows the interior view of these pies after defrosting at 20°C. The control pie shows typical, even opacity, and sparkle to the gravy. The color of the gravy is likewise bright. The progressive thinning of the gravy due to defrost, loss of color in the gravy, fat separation, loss of true color by the vegetables, and the chewed up or scrappy appearance of the meat can be observed. Frequently, the dark chicken meat assumed quite a pink color.

Pies defrosted at 5°C did not show the same extent of damage due to defrost as those defrosted at higher temperatures. The destructive effects, particularly on the pie contents, however, were out of proportion to the microbial population present. The catalytic effect of temperature changes on the rate of destruction due to defrost is shown by the fact that, at a defrost temperature of 5°C, organoleptic unacceptability was reached at 7 days plus. At 20°C defrost, organoleptic unacceptability was reached at about 36 hours and at 15 hours at 37°C. Defrost time for individual
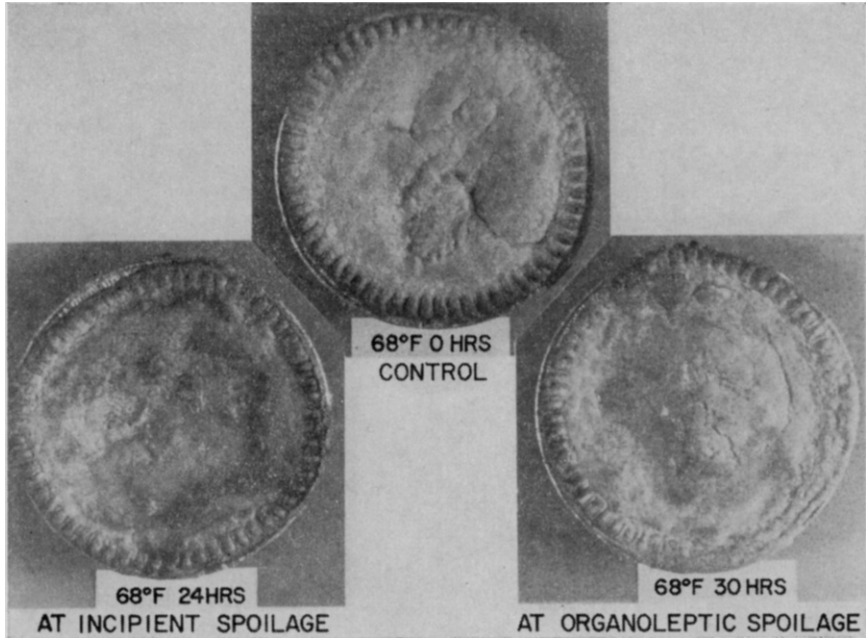
FIG. 4. External appearance of a baked control nondefrosted chicken pie, one at incipient spoilage and one at spoilage after defrost at 20°C.

chicken pies is about 24 hours at 5°C and about 50 hours are required to reach the ambient temperature. At 20°C, the defrost time was about 6 hours and at 37°C about 90 minutes are required to defrost the chicken pies. Thus, the first observations were made on the samples just as defrost had occurred at 5°C, somewhat after defrost at 20°C, and when the sample was well defrosted at 37°C.

The defrost effects, of course, were greatly magnified at 37°C. The appearance of the pie at organoleptic unacceptability was described by the panel as layered, greasy, soggy, sunken crust with boilout and was ruled unacceptable. The gravy was thin, watery, curdled, with fat separation. The vegetables had lost their fresh colors and the meat was chewed up and off-color. The odor was described as a loss of all wholesome, appetizing, chicken smell and with a strong off-odor which was described as putrid, acid, and haylike and was nonacceptable. The flavor of the pie was described as very bad, putrid, bitter, sour (acid), metallic, salty, rancid fat, gutty, and pasty. Defrosted pies invariably stuck to the pie dish after baking, usually severely enough so that the pie could not be turned out without completely disrupting the pie structure.

No known cases of food poisoning occurred among the personnel of

FIG. 5. Internal appearance of a baked control nondefrosted chicken pie, one at incipient spoilage and one at spoilage after defrost at 20°C.

either of the taste panels as a result of eating or tasting dozens of samples of the products involved. Several panel members objected to taste-testing such pies before lunch, and a visitor to our laboratory during a taste panel session said the name of this study should be the "limits of endurance!" It is obvious that at low defrost temperatures, the possible edible life of the product was unexpectedly long. At high temperatures, the edible life of the product was unexpectedly short. Loss of desirable characteristics and flavor begins to take place very shortly after defrost has occurred. The higher the defrost temperature, the more rapid the loss of desirable organoleptic qualities. Obviously, chicken pot pies which have been thawed for only a few hours (depending on the defrost temperature) can be refrozen without serious loss of desirable characteristics. In fact, the taste panels reported that slight defrost produced an increased chicken flavor, but this procedure cannot be recommended to the general public. Likewise, the amount of defrost which can be tolerated is sharply limited and chicken pot pies could not be repeatedly thawed and refrozen or handled as anything other than a perishable commodity. It also seems obvious that they are not public health menaces under defrost conditions, due to the competitive inhibition of staphylococcal growth by saprophytic organisms. The need for keeping frozen foods at 0°F or below remains as

acute as before and this study in no way changes that. This material is part of a continuing study which is presently investigating other products. These results for chicken pot pies should not under any conditions be extrapolated to other frozen foods.

Summary

(1) The desirable original organoleptic qualities of frozen chicken pot pies were very markedly impaired by defrost at room temperatures and above. Defrosting and refreezing of pies under these conditions could not be recommended to the public.

(2) Chicken pies could be defrosted for periods up to 7 days at 5°C and still be edible, although many of the desirable characteristics of the product are lacking. Such products could not be considered good or desirable and sensitive consumers probably would reject them. Organoleptic evaluation panels were always able to detect pie which had been defrosted in any degree.

(3) At 20°C, organoleptic spoilage was reached in 30 hours from the hard frozen state and incipient spoilage began at 16 hours.

(4) At 37°C, incipient spoilage was reached in 6 hours and organoleptic unacceptability was reached in 15 hours.

(5) The initial total count did not greatly affect the time necessary to reach organoleptic unacceptability. Some small differences in time were noted of the order of 6 hours at 20°C defrost temperature.

(6) Refreezing after defrost had no effect in improving or worsening the organoleptic character of the defrosted pie, but definitely did not remedy the damage already done to the product by defrost.

(7) At the time of organoleptic unacceptability, the total microbial flora ranged from five million to 100 million bacteria per gram, depending on the defrost temperature, but staphylococci failed to grow appreciably, due to competition by saprophytic microorganisms.

REFERENCES

1. A. C. Peterson, J. J. Black, and M. F. Gunderson. Staphylococci in competition. I. Growth of naturally occurring mixed populations in precooked frozen foods during defrost. *Appl. Microbiol.* 10, 16–22 (1962).

2. M. J. Fanelli and M. F. Gunderson. Defrost of frozen foods. I. Defrost temperatures of frozen meat pies, fruit pies and soups. *Food Technol.* **15,** 419–422 (1961).

3. A. C. Peterson and M. F. Gunderson. Role of psychrophilic bacteria in frozen food spoilage. *Food Technol.* **14,** 413–417 (1960).

4. G. J. Hucker and E. R. David. The effect of alternate freezing and thawing on the total flora of frozen chicken pies. *Food Technol.* **11,** 354–356 (1957).

5. G. J. Hucker and E. R. David. The effect of alternate freezing and thawing on the total flora of frozen vegetables. *Food Technol.* **11,** 381–383 (1957).

Microbiological Spoilage Problems

of Dehydrated Foods

J. H. B. CHRISTIAN

*Commonwealth Scientific and Industrial Research Organization,
Division of Food Preservation, Ryde, N.S.W., Australia*

The drying of foods is possibly man's oldest method of food preservation. Yet, until very recently its exploitation in the western world was largely confined to military catering. Most of the published microbiological studies on dried foods have been made on products prepared to these specifications. Dehydration has recently entered a new era as a supplier of an increasing range of convenience foods. The most prominent of these are probably potato flakes and granules, instant beverages, and whole meals. No comprehensive microbiological studies of these products have come to the attention of this author, but the problems of microbial spoilage are doubtless similar in principle to those which confronted the industry in its less spectacular days. In this paper we will discuss spoilage problems of dehydrated foods in general, illustrating them with data drawn from a variety of foods and processes. For convenience the production of dehydrated foods will be divided into three phases. These are predrying, drying, and postdrying.

Predrying. The preparation of many foods for drying is identical with the preparation for freezing, and to this extent the microbiological problems which arise will also be identical. They will be discussed only briefly, taking vegetable processing as the main example.

Vegetables will arrive at the plant heavily contaminated with microorganisms, a condition likely to be aggravated by unsuitable storage and handling. The processes of washing, peeling, dicing, etc., may effect either an increase or a decrease in this load, depending upon the standard of sanitation within the plant. It is the blanching or cooking treatment which is applied to many products that is the only step capable of effecting a sharp decrease in the microbial count. For example, Vaughn (*1*) showed

that an efficient blanching, one which inactivated tissue enzymes, reduced the viable count on a range of vegetables by more than 99.9%. Virtually the only organisms surviving such a treatment will be bacterial spores. Unfortunately all the products which may carry high initial loads of microorganisms do not lend themselves to blanching, e.g., onions.

It is in the period following a heat treatment that most of the organisms found in dehydrated foods probably gain access. It is also here that, should any extended delay occur, growth of the surviving and contaminating microorganisms may commence. Shewan (2) observed in fish dehydration trials that bacterial counts increased during mincing and spreading on trays after cooking the fish. Not only organisms of spoilage potential may proliferate here, for toxin-producing bacteria also present a hazard.

In the drying of fruit and vegetables, sulphite is added at this point to retard nonenzymic browning. This sulphite may also serve as a potent inhibitor of microbial growth.

Drying. The effect of the actual drying process upon the microbiological status of a food depends upon both the method used and its efficiency. The conventional method of tunnel-drying employs finishing temperatures which, for vegetables, generally range from 140° to 160°F. However, during the early stages of drying, evaporative cooling keeps the surface temperature close to the wet bulb temperature of the air stream, usually 110° to 120°F. When the drier is unevenly loaded or overloaded, or when the air temperature or air circulation are uneven, pockets of even lower temperature may occur and persist. Under such conditions, a product may undergo microbiological spoilage, usually bacterial, during the actual drying process. On the other hand, drying under properly controlled conditions can result in some reduction in viable counts, especially for products with a high finishing temperature.

In contrast, spray- and roller-drying, which have been used extensively in the preparation of dried milk and eggs, employ much higher temperatures for very short periods. Little opportunity is afforded here for microbial growth, but the preheating stage, which should destroy the bulk of the organisms prior to drying, may constitute a hazard if inadequately controlled. In freeze-drying, the drop in temperature should be sufficiently rapid to prevent growth prior to drying. The reduction in viable count during freeze dehydration is likely to be very small.

Postdrying. The water content of the dried food must be low enough to prevent the growth of molds, yeasts, or bacteria. It is now generally accepted that in most substrates the water requirements of microorganisms are best defined in terms of the activity of the water in the environ-

ment. The water activity (a_w) is given by the ratio of the water vapor pressure of the food to the vapor pressure of pure water at the same temperature, i.e. $a_w = p/p_0$. The relative humidity of the atmosphere in equilibrium with the food is defined similarly, but is generally expressed as a percentage, so that R.H. $= 100 \times a_w$. The relationship that exists between the water content of a food and its water activity (or equilibrium relative humidity) is given by the water sorption isotherm. This is a sigmoid curve, rising from zero water content at 0.00 a_w to infinity at 1.00 a_w. The equilibrium water contents of most foods are about 10% of the dry weight at 0.50 a_w and generally increase steeply as a_w is increased. At higher a_w, the water content is related clearly to the composition of the food, being greatest in foods rich in low molecular weight solutes.

The a_w of most fresh foods are above 0.99. The bacteria commonly implicated in spoilage during drying, reviewed by Vaughn (1), will grow slowly once the a_w is reduced to about 0.97. Because of the steep nature of the water sorption isotherm of most foods at high a_w, this small change in a_w will require the removal of more than two-thirds of the initial water content.

What then are the water requirements for growth of microorganisms, expressed in terms of the water activity of the substrate? Bacteria have not been observed to proliferate at a_w below about 0.75. This is the a_w of saturated sodium chloride, and the bacteria involved are obligate halophiles with a substantial requirement for sodium chloride. Thus only highly salted products, such as fish, are likely to be spoiled in the dry state by bacteria. The halophiles possess high optimum temperatures which increase with increase in salt concentration (3). These properties make them a significant problem in the sun-drying of salted fish under tropical conditions. Bacteria have not been observed growing in nonsaline environments at a_w very much below 0.85, which is evidence of a relatively high requirement for water.

Osmophilic yeasts, on the other hand, are well known as spoilage agents in concentrated juices and syrups, and most studies of their water requirements have been performed in these substrates. The lowest requirement reported is for *Saccharomyces rouxii* (*Z. barkeri*) which grew slowly in syrups of 0.62 a_w at pH 4.5 and 30°C (4). However, as Scott (5) has pointed out, growth of yeasts on solid substrates has not been reported at a_w below 0.75, so that their importance as spoilage agents of dehydrated foods is less than the results quoted might suggest.

Among molds, those most commonly cited as growing in very dry habitats are members of the *Aspergillus glaucus* group. Germination of spores of these species at a_w between 0.75 and 0.70 have been reported

frequently, while Snow (6) observed germination of an occasional spore of *Asp. echinulatus* after one to two years at 0.62 a_w. Very slow growth of *Xeromyces bisporus* at 0.62 a_w on laboratory media was recorded by Scott (5).

The water requirements of xerophilic molds, like those of osmophilic yeasts, are sensitive to pH and temperature. Von Schelhorn (4) recorded that the minimum a_w supporting growth of molds was lower at pH 7 than at pH 5 or 3. This contrasts with the optimum pH for yeast growth at low a_w of between 4 and 5.

Molds are thus the microorganisms most likely to spoil dehydrated foods. The a_w range of greatest interest is from 0.8 to 0.6. Above 0.8 a_w, most products spoil very rapidly at favorable temperatures. Below 0.6 a_w, no growth is likely. In between, there are marked differences in growth rates, depending upon a_w, temperature, atmosphere, and substrate. An inverse relationship exists between a_w and the logarithm of the time for mold growth to appear on foods, and this relationship is approximately linear at a_w below 0.80. The substrate is important in respect of its nutritional and inhibitory constituents and its pH. At a given a_w, the growth rate will be decreased when any of the other factors is suboptimal.

Therefore, it is not possible to lay down a precise level of a_w preventing spoilage unless all these conditions, including time of storage, are known. Limits that have been suggested range from 0.75 to 0.65. Snow *et al.* (7), working with seed and grain products, recommended as maximum a_w 0.72 for 3 months and 0.65 for 2 to 3 years storage. Another specific recommendation was that of Hicks (8) for fruit cake, which kept for 12 months at 25° and 30°C when the a_w was 0.72 to 0.73.

The moisture contents to which dehydrated foods are normally dried are aimed at obtaining a product that is not only safe from microbial spoilage but also susceptible to a minimum of chemical change.

For dehydrated protein foods such as meat, fish, and dairy products, the maximum acceptable water content is 3% or less while for most vegetables it approximates 5%. At these moisture contents the a_w of the majority of these foods fall in the range 0.05 to 0.25. Obviously there is no possibility of microbial spoilage under these conditions. Retention of these conditions during storage is, of course, a problem in packaging technology.

As dehydrated foods are not normally produced as sterile products, the fate of their microbial populations after drying is of some interest. Higginbottom (9) noted that in milk powder, the number of surviving bacteria increased as the a_w was reduced below 0.80, with maximum survival at about 0.10 a_w. She confirmed with pure cultures that the optimum a_w for survival was in the range 0.05 to 0.15 a_w. It is not surprising that the

moisture levels which minimize the chemical changes in foods should also minimize the chemical changes in microorganisms which may lead to death.

When dehydrated foods are packed in large containers, considerable temperature fluctuations and gradients are possible. These give rise to gradients in vapor pressure, and hence to the migration of water. It is possible in this way to produce zones of sufficiently high a_w to permit microbial growth in a food whose average water content would normally be considered safe.

A final consideration in the microbiology of these foods relates to rehydration. A product which possessed a high microbial population postdrying and which was stored under conditions maintaining the viability of cells may be much more perishable when rehydrated than in its unprocessed form.

Thus the major microbiological spoilage problems of dehydrated foods are not concerned with the growth in the adequately dried product of microorganisms with low water requirements. Such strains are a problem in fruit concentrates and syrups. Spoilage of dehydrated foods is generally the result of microbial growth in the product prior to or during removal of water. Control of such spoilage requires not only careful sanitation but also consideration of the relationship between time, temperature, and a_w at all stages of manufacture.

REFERENCES

1. R. H. Vaughn. "The Microbiology of Dehydrated Vegetables." *Food Res.* 16, 429–438 (1951).
2. J. M. Shewan. "The Bacteriology of Dehydrated Fish. III. Observations and Experiments Made during Small-Scale Commercial Production." *J. Hyg.* 52, 247–252 (1954).
3. M. Ingram. "Microorganisms Resisting High Concentrations of Sugars or Salts." *Symp. Soc. Gen. Microbiol.* 7, 90–113 (1957).
4. M. von Schelhorn. "Control of Microorganisms Causing Spoilage in Fruit and Vegetables." *Advan. Food Res.* 3, 429–482 (1951).
5. W. J. Scott. "Water Relations of Food Spoilage Microorganisms." *Advan. Food Res.* 7, 83–127 (1957).
6. D. Snow. "The Germination of Mold Spores at Controlled Humidities." *Ann. Appl. Biol.* 36, 1–13 (1949).
7. D. Snow, M. H. G. Crichton, and N. C. Wright. "Mold Deterioration of Feeding Stuffs in Relation to Humidity of Storage." *Ann. Appl. Biol.* 31, 102–110, 111–116 (1944).
8. E. W. Hicks. "Mold Wastage in Foods Stored in the Tropics. Preservation of Grains in Storage." *FAO Agricultural Studies* No. 2, pp. 126–128 (1948).

9. C. Higginbottom. "Effect of Storage at Different Relative Humidities on the Survival of Microorganisms in Milk Powder and in Pure Cultures Dried in Milk." *J. Dairy Res.* **20**, 65–75 (1953).

Open Discussion

DR. MALLMANN: Does anyone have any comments or discussion they would like to bring up with regard to bacterial standards or bacterial spoilage of food?

DR. LOY: I might point out there is another method of dehydration being used principally on meat products that wasn't mentioned, which was vacuum dehydration at an elevated temperature. In this case there is a very real problem of spoilage during dehydration which can only be controlled by controlling your initial count.

DR. INGRAM: I would like to say at the risk of offending everybody that I have been rather disappointed in this afternoon's proceedings. I would have been very interested, for example, to hear Dr. Bohrer talk about the new type of canned products we are getting which are not sterilized which pose some extremely unpleasant questions about the types of microorganisms we are likely to find. Before such an audience as this, I would have thought this would have been quite an interesting topic. Similarly, talking about dehydration, I would have been more interested in the type of problem this gentleman has mentioned. This is just a general comment, sir. It seems to me that we neglected the most interesting parts of this subject.

Research Needs in Food Microbiology —

Food Spoilage

M. INGRAM

Low Temperature Research Station, Cambridge, England

Because aspects related to public health and processing are covered by other papers in this symposium, this paper will be confined, as far as possible, to the causes and nature of the microbial spoilage of food.

The aim of the microbiologist is to prevent such spoilage, by stopping the source of the causative microorganisms, by killing them or otherwise preventing their growth in food, or by blocking or diverting the course of their spoilage activities. These three alternatives call respectively for a (necessarily brief) consideration of the detection and identification of the spoilage organisms, the means of preventing or predicting their development in a food, and the nature of the spoilage alterations which they cause.

Identification of Spoilage Organisms

Before a source of spoilage organisms can be stopped it must be located, which requires the ability to detect and identify any spoilage organisms in it. Furthermore, the same requirements arise, at once, in any attempt to pin down any organism as a causative agent of the spoilage.

We badly need better means of identifying the organisms concerned. Consider, for example, the well-known *Pseudomonas-Achromobacter* association in which, according to the literature, *Pseudomonas geniculata* is the dominant organism in spoilage of chilled meats. Niven's strain is not the same as Sulzbacher's in our tests; and both differ from the A.T.C.C. strain which is distinctly green, the species description having been altered in the last edition of Bergey's Manual to accommodate that fact. The situation is even worse with *Achromobacter*: most of the organisms to which this name is commonly applied do not resemble the original descriptions of the genus. Associated genera are equally confused. In such cir-

cumstances, it is scarcely surprising that it is at present impossible to describe any significant difference in the spoilage floras, for example, of chilled beef, pork, and poultry; that different workers disagree as to whether the spoilage of chilled eviscerated poultry is by pigmented or non-pigmented pseudomonads; and that there should be difficulty in deciding whether these organisms come from soil on the bird, or wash water in the plant, or the ice in the chilling tanks. Similarly, work in my laboratory on the osmophilic yeasts, most of which are at present "lumped" in one or two species, aims to make it easier to identify particular strains with particular sources and particular foods.

In these situations, the food microbiologist sadly lacks, in order to select particular systematic groups, the array of selective media which (thanks to past generations of medical bacteriologists) are available for bacteria important in public health. The lack is particularly severe with the yeasts and fungi, where the investigator is driven to test-by-test identification of every individual isolate: the situation in which the public health bacteriologist would be, if he were obliged to do coliform counts by running down every colony following the tests in Bergey's Manual. The development of selective media for spoilage organisms is an outstanding need, and time devoted to it would be well spent.

Selective media are however not enough. For instance, there is already a wide choice for fecal streptococci, some of the more recent ones make possible the immediate recognition of *Streptococcus faecalis* and, although the *faecium*-intermediate-*durans* group is still confused, the selective procedures establish the identity of the organisms sufficiently well to make one reasonably confident, for example, that the presence of "fecal streptococci" in a fruit juice does not necessarily indicate fecal pollution. Unfortunately, such identification does not reach the point where one can say with certainty that a fecal streptococcus in a plant organ is the same as that found in a juice, and different from those in an animal gut.

In a comparable situation with salmonellae or staphylococci, the public health bacteriologist resorts to serological or phage typing to attain an altogether higher order of identification. A serious attempt is now being made to develop an internationally uniform system of serological typing for the fecal streptococci, with the object (so far as my laboratory is concerned) of clarifying their distribution between animals and animal foods. Phage typing has been profitably applied to characterise animal-pathogenic *Pseudomonas aeruginosa*, and is being tried for plant-pathogenic pseudomonads; a similar approach might be helpful in the fluorescent and nonfluorescent groups of spoilage pseudomonads. Another group of spoilage bacteria for which such tests might be helpful are the

lactobacilli: many of them fall in the *casei-plantarum* section of *Lactobacillus*, but further identification is scarcely possible at present. If such developments could be linked with a fluorescent antibody technique, they would provide a highly specific and direct means of seeking for the origins of particular organisms.

Prevention of Spoilage

Next for consideration are procedures for killing, or for preventing the development of, any spoilage organisms admitted to the food. With present techniques, except freezing, it is seldom possible to prevent growth of spoilage organisms completely, without causing too much loss of quality in the food, so that a compromise is usually struck, in which some spoilage has to be accepted. Within these limits, there are so many ways of preserving foods, ranging from the prehistoric drying to the new-fangled irradiation, that there is clearly not time to consider them all. It must suffice here to indicate that food microbiologists have still much to learn about the old procedures, and have scarcely begun to grapple seriously with the new ones.

One might think that we should by now know enough about a traditional process like salting, but questions still remain to be answered. For instance, it has been reported that in practical trials the preservative effect of salt is increased by reduction of temperature, whereas work with individual species indicates less lethal or inhibitory action at lower temperatures—why this discrepancy? Again, the presence of salt often raises the maximum and optimum temperatures for growth; does it also raise the minimum temperature—if so, could this be a significant factor in the control of undesirable organisms? How much salt is needed, for example to prevent toxin formation by staphylococci which are well able to grow on salted foods? Does salt have any particular effect on for example putrefactive activity, distinct from any action on cell multiplication? There is evidently much still to be learned, even at the elementary level, about the action of salt in preventing spoilage. The position is similar with other traditional processes: for instance, virtually nothing is known about the basis of the preservative action of smoking.

When one turns to the new process of inhibiting multiplication of spoilage organisms with antibiotics, the activity of food microbiologists appears to be lacking in vision. For two decades, broadly speaking, it has not extended beyond the rule-of-thumb testing of antibiotics recommended primarily for therapeutic use. So far as I know, there are only two antibiotics, nisin and tylosin, which have been developed specifically as food preservatives, and neither has been widely investigated. There has been

little attempt to explore the antagonisms which undoubtedly exist between the microbes occurring in foods, such as the antibacterial activity exhibited by Niniivaara's sausage-curing *Micrococcus* M53. These antagonisms surely suggest the existence of antibiotic substances of potential interest; indeed, the discovery of nisin sprang from an observation of this kind. Despite the large volume of work on antibiotics as food preservatives, the subject still seems to be in its infancy.

In trying to understand the results of vacuum-packing the microbiologist is hampered, not only by shortage of data on the gaseous exchanges of the foods themselves, but also by an almost total absence of quantitative measurements of the respiration and growth responses to oxygen and carbon dioxide concentration by the microorganisms involved. Further, it is clear that the concentrations of these gases affect the metabolic capabilities of spoilage organisms, though we can seldom describe precisely how. Such information as is available usually refers to organisms not important in food microbiology, observed under conditions quite unlike those in foods.

A rather similar hiatus exists in research on the microbiology of irradiated foods, where most of the fundamental information is obtained by radiation biologists working with organisms not concerned in food spoilage. Thus, for example, the proposition that irradiated bacteria recover better on poor media, based entirely on work with a radiation-sensitive *Escherichia coli* strain, has now been shown by the workers at Wantage and elsewhere to be quite incorrect for organisms and media relevant in food microbiology; similarly, my colleagues have been unable to confirm Hollaender's suggestion, also based on work with *E. coli*, that recovery is much improved by incubation at specific temperatures. There is much basic work still to be done on the irradiation of spoilage microorganisms.

Perhaps the most interesting thing about research on irradiation preservation is, for a food microbiologist, the way in which it raises problems which reveal the weakness of our knowledge of food microbiology in general. It discloses, of course, organisms previously quite unknown, like *Micrococcus radiodurans* (which may not be a *Micrococcus* at all) or the *Bacillus*-like lactobacilli described by Thornley & Sharpe. It obliges us to examine the basis of our attitude to spoilage processes, as I shall mention shortly. It raises, in exaggerated form, the possibility of selection by and of adaption to the preservative agent. It requires us, again, to know how rapidly microorganisms in food consume oxygen. It asks, like thermobacteriology, fundamental questions about the nature of resistance in the spore. It leads us to seek the factors which decide whether the few organ-

isms surviving irradiation, or a similar process, will be able to germinate or grow. It presses us to justify, on woefully vague grounds, the requirement for a 10^{-12} inactivation of *Clostridium botulinum* in food processing; and it poses equally embarrassing questions about our attitude to other matters of food hygiene; this discussion must however be restricted to the subject of spoilage.

Prediction of Spoilage

To make avoiding action possible, it is obviously desirable to be able to predict the occurrence of spoilage; and this is also fundamental to the question of microbiological standards because, where they relate to spoilage, the standards are in effect an attempt to predict its onset.

The essential groundwork is the establishment of the relations between initial numbers of spoilage organisms, factors like temperature and humidity, and the time to reach cell populations capable of causing spoilage. Notable work of this kind has been done in recent years by Ayres with chilled meats, and there are similar investigations with fish. The range of foods and conditions so far covered is however lamentably small, and each requires separate consideration: it is obvious, for example, that the relations for minced meat are quite different from those for the same meat unminced. Moreover, sufficient change in temperature (or other conditions) may select an altogether different kind of spoilage organism and thus change the whole course and nature of the spoilage. There is, unfortunately, little systematic information about spoilage at temperatures other than those of the refrigerator; the need for this information is probably less in the United States than in other parts of the world.

The type of approach via enumeration of viable organisms is often too time consuming for practical use, because of the labor involved. Hence there is a great demand for accelerated tests like dye-reduction. The development of satisfactory tests of this kind would be a great step forward; unfortunately, few of them are wholly satisfactory.

A root cause of many of the difficulties, in the above approaches to the problem, is that neither the viable counting methods nor the biochemical tests usually employed are sufficiently specific, to that limited proportion of the microbial flora which is going to cause the spoilage and to the metabolic activities involved in it. The development of more specific procedures is evidently necessary. An ingenious approach to this problem was made by Mossel, in proposing model systems, where essential features are the choice of the right kind of organism, and of a biochemical reaction related to the nature of the spoilage. These are certainly the correct prin-

ciples to follow, principles too little regarded in proposals for microbiological standards relating to food spoilage.

Nature of Spoilage

However, in order to be confident in regarding particular organisms as the cause of particular kinds of spoilage, we need a better characterization of the nature of the spoilage, in relation to the activities of well-identified species. To mention an outstanding case, the nature of the various rots of eggs has yet to be satisfactorily correlated with the metabolic activities of the different rotting bacteria, and it seems possible that conditions like "hay-taint" might represent preliminary stages of particular kinds of rots. The situation is little better with meats and similar foods, where the variety but characteristic nature of the off-odors under diverse circumstances, is a continual reminder of our ignorance of the details of the spoilage processes.

We have to decide, in spoilage, whether it is the organisms which are objectionable or their metabolic products. The question is well illustrated by a paradox: on one hand are those who recommend cells of microorganisms as food, on the basis that they contain excellent protein and fat and are replete with vitamins; on the other, there are suggestions that a food carrying many microorganisms should be rejected on aesthetic grounds because it contains a significant proportion of alien material in the shape of microbial cells. We could resolve the paradox better if we knew what is contained in the cells of the spoilage microorganisms. This question must be faced with any procedure which, for its preservative effect, relies less on suppression of the spoilage flora than a diversion of its metabolism; which is apparently the situation in proposals to extend the storage life of uneviscerated poultry (in British practice) by feeding antibiotics just before death, when the effect of the antibiotic appears mainly to be inhibition of the production of H_2S by the bacteria of the gut.

In most cases, it is probably the products of metabolism which are objectionable. This is suggested by examples like the fecal streptococci, which are suspected of sometimes causing food poisoning when grown in meats but never in milk products, presumably because different breakdown products arise on the different substrates. We now recognize that several organisms are liable to cause more or less mild kinds of poisoning, called "unspecific" because we cannot identify the substances responsible. It is at least clear from some of these unspecific poisonings that they are not necessarily associated with putrefaction.

In trying to relate the spoilage changes to the activities of the microorganisms involved, one is hampered by inadequate knowledge of their

metabolism. Though there is a large amount of information about the breakdown of carbohydrates and of proteins by microorganisms, it still requires assembling in systematic form, in the manner attempted for carbohydrate metabolism by de Ley. Unfortunately, relatively little work has been done on the metabolic biochemistry of typical food spoilage organisms and, of that little, much was with organisms which were (inevitably) not well identified. There is, by comparison, a dearth of knowledge about the breakdown of fats and related compounds. For example, the undoubted lipolytic activities of yeasts and fungi are largely disregarded save in dairy microbiology; it has only recently been discovered that lipase activity is much more specific than has been generally supposed; and little is known in detail about the products of such breakdown. This dearth of knowledge is unfortunate when it begins to appear that characteristic odors and flavors, e.g. in cheeses or cured products, may be the result of attack upon the fats in the food.

We need, as a background for such studies, much more detailed knowledge of the substrates available to the microorganisms in foods. It would help, too, to have better methods to indicate which substrates the microorganisms have actually been attacking. Where spoilage is caused by an association or a succession of several dissimilar species, as in many cured products, straightforward analysis does not properly reveal the individual activities of the species present.

Problems regarding the nature of spoilage become acute immediately when one is presented with a type of spoilage different from normal; immediately, that is to say, when one cannot bury one's head in the sand of tradition. What view should be taken of an irradiated chicken carrying 500 million per gram of *Achromobacter* but showing little obvious alteration, when a normal bird developing an equal load of *Pseudomonas* would be rejected without question as putrid? Almost nothing is known about the metabolic activities of *Achromobacter* on food. And, indeed, upon scrutiny even the idea of putrefaction is ill defined: to some, especially medical writers, putrefaction is caused only by putrefactive anaerobes and is essentially dangerous; others (as I have just done) include types of spoilage which occur under conditions precluding participation of clostridia, and which are not necessarily harmful. What basis of distinction is there; why is it acceptable to hang game till it stinks, but not carcase meat which can however be "aged"? Ignorance of such matters leads the food microbiologist into positions quite indefensible in logic, for many cured or fermented products are prepared through microbial actions which in other contexts would be condemned as spoilage. As a specific case in point, a succession of fecal streptococci and lactobacilli is regarded as

essential to the organoleptic quality of certain kinds of salami, whereas the same bacteria (or, at least, bacteria at present not distinguishable from them) are supposed to cause the spoilage of vacuum-packed bacon. Wherein lies the difference? Only detailed scrutiny of the alterations produced is likely to tell us; and the development of chromatographic methods, most recently of vapour chromatography, opens revolutionary possibilities for investigation in this area.

Conclusion

Food microbiology is a young subject. It has been able (not always wisely) to borrow its ideas about food hygiene ready-made from public health bacteriology. It has, however, been necessary to begin from the beginning in the study of food spoilage, and hence a great deal of the work hitherto has consisted essentially of fact finding and description. In this hasty survey of research on spoilage it has appeared, repeatedly, that closer consideration brings us face-to-face with lack of knowledge about the nature of the spoilage organisms and their activities, which leads to this general conclusion. The stage has been reached when we might, with advantage, devote less time to *ad hoc* research and more to investigation of the identity, nature, behavior, and activities of the spoilage micro-organisms themselves.

Research Needs in Food Microbiology —

Food Processing

CARL S. PEDERSON

New York State Agricultural Experiment Station,
Cornell University, Geneva, New York

The microbiologist has played an important role in the development of the food industry. This role has been in large part that of determining and correcting causes of losses that have occurred.

The microbiological problems of the food processing industry are numerous, varied, and, in many cases, extremely complex. Men well trained in fundamental microbiology and related sciences will find in the food industry ample outlets for application of their knowledge. The application of this knowledge is dependent upon a clear understanding of the various operations of a food processing plant and the many factors which influence microbial growth.

In actuality, the food industry has been operating with only a limited knowledge of the fundamental physiology of microorganisms. Foods in general are preserved either by applying enough heat to kill all organisms present or by preparing or storing the foods in such a manner that organisms which may be detrimental to the food cannot develop. The latter type of preservation includes drying, fermentation, freezing, use of chemicals, and, of great importance, preservation by use of controlled concentrations of the various natural food ingredients such as salt, acid, sugar, and water. All of these methods concern the physiology of the organisms in their relationship to the food. It is important to learn more about these relationships.

It is impressive to note the great changes which have occurred in the food industry. Although there has been an ever-increasing interest in food quality, even more noteworthy is the large number of new products and the variety of ways in which food is presented to us. Of course frozen foods are the outstanding example. In order to remain competitive, there-

fore, the food processer must continue to improve his products and develop new products. New ideas, new concepts, and new improvements are always in order. It is interesting to note the number of small industries which after developing new products, have been absorbed, to their profit, by larger companies.

With new developments, new problems present themselves. The need for well-trained scientists becomes increasingly important. It is surprising how similar many problems are to those met with on previous occasions. This emphasizes the value of experienced microbiologists. The past must be projected to the present and future.

Some of the needs today concern increasing knowledge of the use of natural food ingredients as preservatives, of the changes that occur during food fermentation, and greater application of previous experiences to the present. All of this must be predicated on a greater knowledge of the physiological and biochemical changes produced by microorganisms.

Natural food substances such as acid, salt, and sugar can be used in some food products to inhibit growth of spore-forming organisms or even all organisms. Minimum heat processes may be sufficient to preserve such products. Some foods are not acceptable with excessive use of such ingredients.

Among the natural food ingredients, the effect of acids is more fully understood than that of other ingredients. Since spore-forming organisms cannot grow in highly acid products, the heat processing of pickles, relishes, and kraut need only be sufficient to kill the low heat-resistant vegetative cells. Since so little is known about the effect of factors such as salts, sugars, fats, water concentration, and other ingredients of foods, either alone or in conjunction with others, no general applications can be made. Improvement in preparation of many food items must take into consideration greater knowledge of the combined effects of the above inhibitives with other as yet unknown influences either to lower the heat processing to a minimum or to dispense entirely with a heat process.

Catsup, chili sauce, and similar products remain safe for considerable lengths of time after their containers are opened. In general, since these products are acid they would be subject to spoilage only by lactic acid bacteria, yeasts, and molds. Moreover, the combination of salt and sugar with the acid intensifies the inhibitive effect on microbial growth. Similarly, the combined effect of acid and sugar in jellies and preserves and the acid and salt in pickles and olives is inhibitive to growth of microorganisms. Unfortunately there is too little knowledge available in regard to the levels of these natural food ingredients required in food and of their effect upon pasteurization temperature. Yeasts will grow on the surfaces

of some of such foods when exposed to the air. In some instances chemical preservatives in trace amounts far below the levels ordinarily recommended are effective microbial inhibitors in such foods. For example 0.01% sorbic acid as potassium sorbate in some pickle and olive brines is effective in producing absolute inhibition of yeast growth. This chemical and benzoic acid when used at 0.015% levels are also effective not only in inhibiting yeast growth in grape and other fruit juices stored at 24° to 28°F, but also in causing a slow death of yeasts present in the juices.

Tomato juice of high pH is subject to spoilage by aerobic spore-forming flat-sour bacteria. Heated spores of the organisms fail to germinate in juice if the hydrogen-ion concentration is below pH 4.30. The effect of buffers and acid ions other than the citrate ion have not been clearly elucidated. However, lowering of pH by addition of citric acid is effective in control of the spore-former. The pH relationships should be of value in processing other food products. For example if a pineapple-rice pudding is processed at temperatures high enough to kill spore-forming bacteria, the pudding caramelizes. If the pH is adjusted it can be processed at temperatures low enough to retain the pineapple flavor. Bread baked in cans is now a reality as are various canned cakes and puddings. Spore-forming bacteria cannot develop in such products if the pH and water content are adjusted to the proper level. Certain cheese spreads are processed at low temperatures. The various salad dressings are not heat processed. The natural inhibition of growth of organisms in well-aged cheese suggests the production during cheese aging of some type of natural preservative. During fermentation and processing of sausages such as summer sausage, inhibitive products other than acids are undoubtedly also produced. If more information were available as to their nature, improvement in quality could be effected.

The entire field of inhibition of microorganisms by means of heat or cold in conjunction with the addition of natural, digestable ingredients, sometimes with minimal chemical preservatives is a field of research of great possibilities. The necessity for knowledge concerning conditions for growth of the various organisms in a particular food is obvious. A chemist once attributed spoilage of a thousand island dressing to the organisms which he assumed came from the chili sauce. Had he known basic bacterial physiology, he would have known that this could not be true. The spore-forming bacterium which caused the spoilage could not have grown in the chili sauce. It was shown to have originated with the mustard and peppers and could not have developed in a stable emulsion with the proper acidity.

Fermented foods are an important part of the food industry. Knowledge

of the changes that occur in such foods during fermentation is inadequate. The carbohydrate metabolism has been studied more intensely than the metabolism of proteins, fats and other ingredients. It is quite well known that some fermentations are produced by a sequence of growth of several types of lactic acid bacteria which may be succeeded by growth of species of other microorganisms. Temperature, salt content, and other factors are known to influence the development of various species.

Recently it has been observed that lipids of vegetables are hydrolyzed by lactic acid bacteria during fermentation. This accounts for the presence of acids other than lactic and acetic in these products. It further demonstrates the possibility that choline, present in acetylcholine, and lactylcholine, always present in these foods, could have originated from the hydrolysis of the phospholipids. The mechanism by which the fatty acids are formed and the species of lactic acid bacteria which are responsible for hydrolysis are not known. The presence of the lower molecular weight fatty acids in some samples is not explained. Why does the proportion of fatty acids in the fermented product differ from the proportion of fatty acids of the lipids in the raw product? Does some degradation process occur or is a type of synthesis involved? Is it this type of change that occurs in cheese so that well aged cheese is inhibitive to microbial growth? Is the tangy flavor of fermented sausage due in part to such fermentations and do the lactic acid bacteria in sour dough bread impart certain desirable characteristics to bread?

Research on cheese manufacture conducted in various institutions has demonstrated the complexity of these fermentations. The characteristic flavors and textures cannot be artificially duplicated by known manufacturing methods.

Spoilage of foods and quality deterioration continue to be problems of the food industry. In many cases, similar types of spoilage or deterioration have occurred previously, possibly in some other type of food product. Experiences of the past, therefore, are invaluable in the solution of present and future problems. Wooden equipment, foam, corks, and other factors interfere with normal transmission of heat or cold. This repetition of contamination problems occurs because of lack of knowledge.

Concepts of size, rate of growth, and activity of microorganisms are foreign to many of the personnel of the industry. Similarly, these concepts in relation to equipment, pores, crevices, foams, and exudates are equally foreign to many well-trained bacteriologists. Before the advent of stainless steel equipment, wooden containers were commonly used. Although these were apparently clean, the possible contamination of food by organisms impregnated in the pores and crevices of wood was seldom considered.

It may be recalled, however, that the common form of inoculation of foods such as milk, cheese, such as Limburger and Roquefort, sausage, bread, and other fermented foods was brought about by contact with such surfaces.

Foam upon the surface of foods is an effective barrier to the transfer of sufficient heat to kill microorganisms and to the cooling of foods to delay growth. It was observed years ago in the milk industry that foam was a deterrent to effective pasteurization. Furthermore, in some cases bacteria actually developed in the foam. More recently foam on the surface of juices in heating kettles was found to harbor living yeasts and molds that were then transferred to bottles or carboys. Still more recently yeasts were found growing in the foam on the surface of pasteurized grape juice being cooled for storage at 24° to 28°F. Years ago, foam and condensate on the surface of tomato purees in 5-gallon containers were found to harbor lactic acid bacteria which developed when the purees were cooled. The flat-sour spoilage of tomato juice, caused by spore formers, has been traced directly to growth of the organism in the foam in cans, filler bowls, and holding vats.

Cork is similar to foam in its insulating properties. Numerous spoilages attributable to cork closure have been observed. In grape juice stored in 5-gallon carboys with cork stoppers, paraffining the bottom half of the corks was effective in reducing mold and yeast growths.

For centuries the wooden surfaces of vats have been sources of desirable inoculation in fermentation of pickles, sauerkraut, beers, wines, and other products. At other times these surfaces have furnished undesirable organisms. The lactic acid bacteria sometimes caused souring of beers or wines. Certain molds spoil sauerkraut and pickles in vast areas adjacent to the wood. Wooden surfaces in the past contributed to other losses. The high mold counts experienced in tomato products during the early days of mold count control were often attributed to the moldy wooden surfaces of equipment. Spoilage by lactic acid bacteria in products such as catsup and chili sauce has been attributed to organisms lodged in the pores and crevices of wooden holding tanks. Wooden containers have harbored yeasts which have fermented grape juice and other fruit juices stored at 24° to 28°F. Cheeses such as Limburger are inoculated with *Bacterium linens* by contact with wooden shelves.

In the frozen food industries growth of microorganisms has been detected in exudates and residues on belt lines and other equipment. This growth provides a continuous source of contamination for food passing over these surfaces. This illustrates particularly the need for better sanitary control of equipment.

The organisms present in the air are often sources of inoculation and contamination. Cheeses such as Roquefort were apparently first inoculated with mold spores present in the air of storage caves. Tanks of fruit juice held at 24° to 28°F are contaminated by yeasts from the air in storage rooms.

The problems of heat transfer are equally important. Rates of heat transfer in starchy or viscous products such as corn are much slower than in more fluid products. Also even today freezers of many food items fail to realize that when products are placed in cartons and then in cases, cooling is so slow that the products are subject to spoilage. Numerous other incidents of contamination or inoculation might be cited, all emphasizing the value of applying past experience to present problems.

The factors which influence growth or death of microorganisms are of great concern to those who prepare and process foods. These include the concepts of size of microorganisms in relation to their surroundings, growth as well as biochemical characteristics of organisms in relation to a particular food, the selectivity of the various species, the inhibition of growth in some conditions and stimulation of the desired types of growth in others.

Biochemical changes in certain food products are at times extremely significant. This may apply to the production of undesirable toxic substances in some foods or the desirable changes that occur in fermented foods. Also of importance are problems of heat or cold transfer in relation to microbial growth or death.

Men with vision, well trained in the fundamental aspects of the physiology and biochemistry of microorganisms as they may be related to foods, will find ample opportunities in the food industry for application of such knowledge. They must realize that few men in the industry have an adequate concept of microbiology and its related sciences. The scientist therefore must take the initiative.

Research Needs in Food Microbiology — Industry

W. L. BROWN AND C. VINTON

John Morrell & Co., Ottumwa, Iowa

Important advances in food microbiology have been made in the last decade. New media have been developed that greatly enhance our ability to distinguish between the various groups of organisms. Techniques for identification of organisms have been refined and improved.

Since most of my experience has been with the meat industry, I will confine my remarks to the problems associated with the production of meat and attempt to point out the areas in which additional research is needed. The meat industry has the responsibility of producing quality meat and meat products for the consumer. Methods have been devised to control microorganisms in the plants and on the products that are produced commercially. Refrigeration to retard growth of organisms and heat to pasteurize or sterilize are the main methods of bacterial control.

Knowledge of the numbers and types of organisms as the meat enters the plant until it reaches the consumer is essential if we are to understand the problems of this industry. Our discussion then will start with the bacterial flora of the animals and follow the meat through processing to the consumer.

Bacterial Flora of the Skin

Empey and Scott (1) Jepsen (2) Haines (3) and Jensen and Hess (4) have made detailed studies of the bacterial flora of meat animals. They report the normal skin flora composed of various species of cocci. When the skin contacted dirt and manure the flora increased in numbers and types of microorganisms. In the slaughter of hogs the skin is not removed from the carcass but is cleaned by scalding, scraping, and singeing. Jensen and Hess (4) found bacterial numbers from 10,000 to 1,000,000 per square cen-

timeter of skin surface. In our laboratory we have found the range of 1,000 to 1,000,000 organisms per square inch.

Studies have been made in the Morrell Laboratory to compare the flora of the skin before and after slaughter. Two sections of skin from thirteen hogs were removed from animals on the bleeding rail. The skin was examined as removed from the animals, after shaving, after shaving and washing. The washing and shaving removed many of the contaminants. The majority of the organisms were aerobic spore formers with small numbers of clostridia present.

Bacterial Flora of the Scalding Tub

After killing and bleeding the hog carcasses are immersed in a scalding tub. The temperature of the water in the scalding tub is sufficiently high to reduce the vegetative population of organisms. Samples of water were taken from the scalding tub at different times and on different days for a period of several months. Data collected in 1960 and 1961 from this study are shown in Table I. This work indicated a low number of anaerobic spores and agrees with the findings of Jepsen (2).

TABLE I

ORGANISMS PER MILLILITER OF SCALDING TUB WATER

Samples	High	Low	Average
Total count	6,000,000	3,000	61,000
Aerobic spores	8,700	<10	246
Anaerobic spores	30	0	6

Total counts were made on tryptone glucose extract agar and the plates were incubated for 48 hours at a temperature of 35°C. Aerobic spore counts were determined after pasteurizing for 5 minutes at 100°C. Anaerobic spores were determined by using Halvorson's most probable number technique and Eugon agar after pasteurizing as above.

Since aerobic and anaerobic spores were present on the incoming animal and not removed in the scalding tub the next problem was to determine their location on the skin. Portions of skin and connective tissue were removed from the carcass. The skin was separated with sterile knives from the fat and connective tissue. Ten grams of each sample were weighed and placed in sterile physiological saline, pasteurized and used for spore counts. The fat and connective tissue did not show the presence of spores. The skin after pasteurizing had approximately 300 total spores per gram

skin and microscopic examination of colonies indicated 2 anaerobic spores/gram. Counts were made to determine the number of organisms on the skin surface. An examination of this data indicates almost complete removal of the organisms from the surface of the skin. Most of the organisms left were obviously embedded in the pores of the skin.

Jepsen (2) indicated a reduction in his counts after scalding, scraping, and singeing to less than 1% of the initial load.

Bacterial Flora of Equipment

At the time this work was in progress an interest was developed in the anaerobic spore load since the work of Harriman et al. (5) and Burke and Steinkraus (6) indicated that putrefactive spores are a part of the normal flora of meat and meat products. Preliminary surveys indicated that it was possible to transfer spores from the skin to the surface of the meat. The Townsend skinner, a piece of equipment used to skin hams, shoulders, and bellies, was selected for study. Scrapings were taken from one square-inch surface of the skinning blade while the machine was in operation, after use and after clean-up. Counts were made using the procedure indicated in this paper.

The above information indicates that it is possible to transmit spores from the skin to the meat using standard equipment of the industry. This information stimulated a survey of the bacterial load of skin from animals slaughtered at twenty-four other packing plants in the midwest. Data are presented in Table II.

TABLE II

THE BACTERIAL LOAD OF ANIMAL SKIN FROM SOME SLAUGHTERHOUSES IN THE MIDWEST

Plant location	Average total count	Spores (Organisms per gram)	
		Aerobic	Anaerobic[a]
Iowa	26,000	<10	0.9
Illinois	20,700	<10	3.0
Missouri	2,200	<10	0.1
Kansas	6,000	<10	1.0

[a] From 95 skins examined, 73 of the skins contained anaerobic spores.

These data indicate that spores embedded in the skin of the pork carcass can be transferred to the surface of the meat by equipment used

on the cutting floor. Obviously there are many other opportunities for the meat to come in contact with bacterial spores.

Bacterial Flora of Product

Since refrigeration of the meat product does not permit growth or multiplication of the spores the next problem was to determine the number and types of spores in the raw materials for canning. For these studies meat was obtained from production lines in the Ottumwa plant before processing. The cans were opened aseptically and a sterile alemite gun filled with meat from the center of the can. Twelve tubes (10 $\times$ 75 millimeters) were filled with approximately 1 gram of meat and sealed with a blast lamp. After a 5-minute preheat at 79°C the tubes were processed in an oil bath maintained at ±0.1°C. After the heating schedule all tubes were immersed in water at 21°C to cool. Six of the tubes were opened after heat treatment and subcultured into glucose brain broth. The presence or absence of growth was determined after 48 hours incubation at 35°C. The results of several years data are shown in Table III.

TABLE III

SURVIVAL OF SPORES AFTER PROCESSING AT VARIOUS LEVELS

Number of samples	% Samples positive at processing levels of F_0: (minutes at 121°C)			
	0.05	0.2	0.6	1.0
607	70	39	21	19

The normal process for luncheon meat ranges between a process level of F_0 0.2 and F_0 0.6. These data indicate that 21 to 39% of the commercially sterile cans contain viable spores. Microscopic examination of the subcultures indicated that aerobic spore-formers were important in the spoilage of thermally processed luncheon meat. The spores were present and viable in luncheon meat for many months.

The average bacterial load on products made in the sausage department is of particular importance to the packer since the higher the initial level the shorter the storage life at the retail level. Table IV shows total counts on all meat frankfurters made over a period of several years. The franks were sampled after packaging and immediately prior to shipment. The entire contents of a package were comminuted in a sterile Waring Blendor to make these counts.

TABLE IV

TOTAL COUNTS ON FRANKFURTERS

Number of samples	Bacteria per gram	Per cent
7	<10	2.8
61	<100	24.0
108	<1,000	42.5
67	<10,000	26.3
10	<100,000	3.9
1	<1,000,000	0.4
254		100.0

Approximately 75% of all product contained less than 1000 bacteria per gram.

Another product sold in a 1-pound package is sliced bacon. Typical counts for this product are given in Table V. These counts indicate that most of this product contains less than 10,000 organisms per gram of meat.

TABLE V

TOTAL COUNTS ON SLICED BACON

Number of samples	Bacteria per gram	Per cent
6	<10	0.8
47	<100	6.2
236	<1,000	31.2
239	<10,000	31.6
156	<100,000	20.6
57	<1,000,000	7.5
16	>1,000,000	2.1
757		100.0

Bacterial Flora of Additives

One of the problems of the food industry at the present time is the bacterial control of additives used in industry. A typical example of the range in counts of additives for meat is shown in Table VI.

TABLE VI

TOTAL COUNTS ON ADDITIVES USED IN MEATS[a]

| Additive | Number of determinations | Bacteria per gram | |
		Lowest count	Highest count
Dry skimmed milk	5	600	20,000
Gelatin	10	<10	100,000
Spices	12	<10	50,000,000
String	3	100	20,000
Paper	40	<10	40,000
Olives	6	300	100,000
Extenders	6	400	1,500

[a] It has been our experience that the additives can contain heavy spore loads and markedly change processing schedules or product storage life. Additives have also been studied by Jensen et al. (7).

Bacterial Inhibition

One of the most significant needs of the meat industry is to understand the relationship between types of organisms, natural inhibitors, curing salt inhibition, temperature, and antibiotics. Inhibition by the addition of antibiotics to canned foods has been studied by Anderson (8), Campbell and Sniff (9), Denny et al. (10), and Greenberg and Silliker (11). They found various degrees of inhibition by combining the antibiotics with heat processing in different canned food items. Tylosin appears to have desirable inhibitory qualities against spores after germination of food spoilage organisms.

Delayed germination of spores in a canned meat product was demonstrated in our laboratory by filling 266 cans (208 × 109) with luncheon meat emulsion and processing the cans at a level calculated to destroy all vegetative organisms. Half of the cans were placed in storage at 5°–10°C. The remainder of cans were held at a temperature of 21°–23°C. After 15 months no evidence of spoilage was found in the cans stored at 5°–7°C so they were transferred to an incubator at 35°C. Nine months after transfer spoilage started and after two years 57% of the can spoiled. At 21°C, 45% of the cans spoiled within 3 years. Only 0.4% spoilage was observed during the first year. All spoiled cans were checked and in each case the spoilage was due to the aerobic spores present in the meat. Anaerobic spores were present and could be detected viable for a 2-year period from the date of processing.

Spoilage in meats does not appear to depend on the spore load as much as conditions conducive to spore germination and outgrowth. The spores most likely to germinate are members of the Bacillus group and most can be classified as *B. cereus*, *B. subtilus*, and *B. megaterium*.

Curing salts delay germination and growth of spores. Excellent papers published by Bullman and Ayers (*12*), Evans and Tanner (*13*), Yesair and Cameron (*14*), Jensen (*15*) and Stumbo *et al.* (*16*) discuss this inhibition in detail. The reason for this inhibition has not been completely determined.

Resistance of meat spoilage organisms to irradiation has been studied by Anderson *et al.* (*17*), Williams and Kempe (*18*), Niven and Cheesbro (*19*), and Brown *et al.* (*20*). Most strains of meat flora are sensitive to irradiation and a strain of cocci and several spore formers are very resistant. More fundamental work needs to be done to compare heat and irradiation-resistance differences of organisms.

Summary

Data presented and references cited in this paper indicate that additional microbiological research is needed in the food industry. Knowledge of the relationship between types and numbers of microorganisms is necessary to fully evaluate present sanitation practices, processing schedules, and product temperature requirements. Additional work is needed to elucidate the differences observed between spores found as natural contaminants and spores added to meat from culture collections. Natural spore inhibitors in food products should be investigated and clarified so that we know the inhibitory effect of each product. The relationship between aerobic spores and anaerobic spores should be investigated. This information is necessary to determine the amount of heat necessary to destroy spores to a level that will insure commercial sterility.

Additives that contain high bacterial populations should be further investigated to establish the types of organisms present and the effect of these organisms on product quality and storage stability.

Another problem that concerns the food industry at the present time is the legal problem associated with the control of perishable foods after the product is shipped from the plants. The one universal problem in the food industry is the fear of food poisoning claims after our product is in the hands of consumers. At times it is extremely difficult to determine the handling conditions of perishable products before consumption. Another question that invariably arises is the consumption of other products in the same meal with the product that was supposed to have caused illness. In most cases, it is impossible to obtain samples of all the components of a meal to determine the true cause of illness.

Methodology used in microbiological control testing in industry leaves much to be desired. The recommended time of incubation for the total plate count is 48 hours. With the use of computer techniques in formulation and inventory control, the 48-hour time period presents many problems. Perishable food products may show changes in quality with the 48-hour holding period. Quick, accurate, rapid methods would be most desirable for testing the microbiological condition of raw materials.

Another problem is the inability of suppliers to improve on the bacteriological condition of their products. In closing I would like to quote a paragraph from a letter received asking a supplier to check the bacteriological condition of his product:

"For more than 30 years we have been a major supplier of this product to hundreds of packers throughout the United States and to several foreign countries. Never, before your letter of August 2, have we had the subject of bacteria brought to our attention. Therefore, it is to be understood that the opinions that we express are not based on experience or established facts from experts in that field."

REFERENCES

1. W. A. Empey and W. J. Scott. Microbial contamination acquired in the meat works. *Council Sci. Ind. Res. Bull. 126* (1939).
2. A. Jepsen. Bacteriology of Pork. Microbial contamination acquired in the meat works. *Roy. Vet. Agr. Coll. Copenhagen, Den., Yearbook* pp. 40–85 (1947).
3. R. B. Haines. Observations on the bacterial flora of some slaughter houses. *J. Hyg.* 33 (1933).
4. L. B. Jensen and W. H. Hess. Bacteriology of normal tissues of swine. *Food Res.* 7, 273 (1942).
5. L. A. Harriman, V. J. Del Quidice, and B. M. Shin. Spore formers in pork. *Ann. Meeting, Illinois Soc. Am. Bacteriologists* (1948).
6. M. V. Burke and K. H. Steinkraus. Incidence of putrefactive anaerobic spores in meat products. *Food Technol.* 4, 21–25 (1950).
7. L. B. Jensen, J. Yesair, and O. B. Williams. Microbiological content of untreated spices. *In* "Microbiology of Meats," 3rd Ed. pp. 380–382.
8. A. A. Anderson. Effect of Subtilin on spores of *Clostridium botulinum*. *J. Bacteriol.* 64, 145 (1952).
9. L. L. Campbell and E. E. Sniff. Effect of Subtilin and Nisin on spores of *Bacillus* coagulase. *J. Bacteriol.* 77, 766 (1959).
10. C. B. Denny, L. E. Sharpe, and C. W. Bohner. C. Effect of Tylosin and Nisin on canned food spoilage bacteria. *Appl. Microbiol.* 9, 108 (1961).
11. R. A. Greenberg and John H. Silliker. The action of Tylosin on spore forming bacteria. *J. Food Sci.* 27, 64 (1962).
12. C. Bullman and J. C. Ayers. Preservative effect of various concentrations of curing salts in comminuted pork. *Food Technol.* 6, 255–260 (1952).

13. F. L. Evans and F. W. Tanner. The effect of meat curing solutions on anaerobic bacteria. II and III. *Zentr. Bakteriol. Parasitenk. Abt. II* **89,** 48–54; **91,** 1–13.

14. J. Yesair and E. J. Cameron. Inhibitive effect of curing agents on anaerobic spores. *Canner* **94,** 89 (1942).

15. L. B. Jensen. "Microbiology of Meats." Garrard Press, Champaign, Illinois, 1954.

16. C. R. Stumbo, C. E. Gross, and C. Vinton. 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 (1944).

17. A. W. Anderson, H. C. Nordan, R. F. Cain, G. Parrish, and D. Duggan. Studies on a radio resistant *Micrococcus.* I. Isolation morphology culture characteristics and resistance to gamma rediation. *Food Technol.* **10,** 575 (1951).

18. N. J. Williams and L. L. Kempe. Combined effects of heat and radiation in food sterilizations. *Appl. Microbiol.* **5,** 366 (1955).

19. C. F. Niven, Jr. and W. R. Cheesbro. Antibiotics and irradiation in meat preservation. *Proc. Am. Meat Inst. 8th Conf. Res.* **47,** (1956).

20. W. L. Brown, C. Vinton, and C. E. Gross. Radiation resistance of the natural bacterial flora of cured ham. *Food Technol.* **14,** 622–625.

Research Needs in Food Microbiology —

Public Health

KEITH H. LEWIS

Division of Environmental Engineering and Food Protection,
U. S. Department of Health, Education and Welfare,
Public Health Service,
Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio

Character and Scope

Food technology has developed so rapidly in recent years that micro-biological research has not kept pace with the complex changes in production, processing, marketing, and serving of foods. Public health problems have increased greatly in scope and variety due to the introduction of new commercially prepared products. Many such items contain ingredients of world-wide origin, which may be produced under conditions that allow contamination with numbers and kinds of microorganisms not usually encountered in domestic products. Prepared foods are often blended and preserved by novel processes that do not result in sterility. They may be packaged in a variety of unconventional wrappers or containers that influence selectively the growth of molds and bacteria. The major trend is toward centralized preparation and widespread distribution of convenience foods that involve a minimum of culinary skill but require special handling to avoid development of microorganisms and their toxic products. These technological changes introduce a variety of health hazards about which more information is needed by industry and government health agencies in order to protect the public.

Additional health problems arise from deficiencies in the consumer's preparation and serving of foods. The high cost of modern equipment and skilled labor, as well as the outside employment of housewives, encourage short-cuts in the handling of perishable products. The time and skill required to prepare foods safely is not always applied in private homes,

institutions, or commercial food services. Additional knowledge is needed to develop supplemental protection measures that are less dependent on local circumstances and individual performances than are the conventional methods of food sanitation.

It is doubtful if anyone can comprehend the entire range of microbiological research required to cope with current problems of food protection, much less foresee clearly future needs. The field seems almost limitless because there are so many interrelated variables to be studied. For example, if one wishes to understand the occurrence, possible health effects, and means of controlling a group of organisms in a single category of foods such as dairy products, he will need factual information about (a) the properties of the organisms, (b) methods of studying them, (c) the associated microflora, (d) conditions of milk production, (e) effects of processing, (f) composition of the final product, (g) influences of packaging, storage, and marketing, and (h) how these products are prepared for serving. If we assume, for purposes of illustration, that there are at least ten kinds of dairy products involved and ten or more unknowns in each of the above-mentioned categories of information, then theoretically the number of specific facts required to attain the desired knowledge will be represented by the product of the pertinent variables, or, in this example, more than one billion items. Quite obviously this is an exaggeration of the situation, because there is an accumulated backlog of basic information from years of prior research, and the new information gained from one study may satisfy, in part, the needs of other areas. Furthermore, some of the combinations may have little or no significance, and the relative importance of studying any particular facet of the field will depend largely on the viewpoint of the investigator and the circumstances in which he works. Nevertheless, it is evident that effective protection of the public health in an era of changing technology will require an almost infinite number of research contributions by food microbiologists. The opportunities for interesting, useful work exceed the imagination of the most visionary forecaster.

Priorities

At any particular time, the emphasis placed upon areas of needed research within the broad field of food protection will be influenced by a variety of factors including (a) the objectives of the sponsoring organization, (b) the special competence and insight of the individual participants, (c) the available resources, (d) the adequacy of methods and background information for making worthwhile contributions to the problem at hand, (e) the degree of interest and concern exhibited by the public or affected

segments of the population (such as industrial associations, professional organizations, labor unions, and consumer groups), and (f) the leadership exercised to gain the cooperation and support of other organizations that could either participate in research or utilize the results to protect the public health.

Even with these guidelines, assignment of priorities in research requires judgment which can best be developed by planning among the senior investigators and consultation with those responsible for the broader aspects of the program. Most of the important public-health problems relating to food require a team approach; therefore, the total pattern of research should be shaped to take advantage of contributions, not only from microbiology, but also from the other biological, physical, and medical sciences.

In a recent report, the Committee on Environmental Health Problems (1), under the Chairmanship of Dr. Paul M. Gross, recommended a major expansion of food-protection activities by the Public Health Service as part of its over-all plan to develop an Environmental Health Center. In addition to greatly increasing its grants program, the Service has been urged to support university training of scientists and to develop a broad intramural research effort having as its over-all mission the improvement and protection of the public health and welfare as they may be affected by foods and beverages, alone, or in combination with other environmental stresses.

In anticipation of implementing the Committee's recommendations, the Division of Environmental Engineering and Food Protection has sought the advice of a Food Protection Task Group, which includes the members of the "Gross Committee" who were responsible for the Milk and Food Section of its report. Their recommendations (2), together with the accumulated experience of the Public Health Service, suggest that the areas of food protection most in need of attention and in which microbiological research must make substantial contributions include the following. The order of presentation is not indicative of relative importance, because all have a high priority.

1. *Better detection, investigation, and reporting of foodborne diseases.* The notorious deficiencies in this area preclude an accurate appraisal of the impact of contaminated food on health in the United States. Bouts of gastroenteritis are a commonplace fact of life which, though admitted privately, are seldom reported officially unless the victim seeks medical attention. About 10,000 cases are reported annually to the Public Health Service, but conservative estimates indicate that the incidence may be 100 times this figure.

A third or more of the outbreaks are consistently reported by one state; the majority of states admit to no more than one or two outbreaks per year. These discrepancies are not due to major differences in incidence of foodborne disease but to the varied emphasis placed upon the problem by the health agencies involved. We are much in need of more efficient techniques for finding cases, identifying the causative agents, determining sources of contamination, and preventing recurrence of similar outbreaks.

Although staphylococci and salmonellae are frequently associated with foodborne gastroenteritis, the preponderance of outbreaks are of undetermined etiology. Either no laboratory work is done, or the methods prove inadequate to identify the causative agent. Occasional outbreaks of botulism, typhoid fever, trichinosis, dysentery, or other well-known foodborne diseases are recognized, but more and more often the epidemiologist's finger of suspicion is pointed at other bacteria, viruses, fungi, or protozoa as possible causes of so-called food poisoning. Carefully controlled studies are needed to determine what role, if any, is played by various aerobic and anaerobic spore-forming bacteria, pseudomonads, fecal streptococci, and certain coliform organisms. Transmission of viral and rickettsial agents through food has scarcely been explored, despite the convincing epidemiological data linking infectious hepatitis to the consumption of contaminated shellfish and potato salad. Possible involvement of infectious or toxigenic fungi has also been suggested. Quite obviously microbiological research must supply definitive answers to these problems before the full extent and changing character of foodborne disease problems can be defined.

2. *Cooperative surveillance of contaminants in market products.* Examination of foods being offered to the public is a common practice of industry and governmental agencies. Tests vary from simple microscopic observations or plate counts to elaborate procedures for the quantitative estimation of specific pathogens or toxic chemicals. The results are often used as a basis for product standardization, grading, specifications for purchase, indicating sanitary quality, compliance with established criteria, comparison with other products, or evidence that legal requirements have been violated.

Such data are usually considered confidential for fear they will be misused, and are relegated to closed files after serving the particular needs of the organization that collected them. This attitude deprives the organization of the broader comparison of products that could be made through exchange of information, and prevents it from learning whether its operating practices and laboratory procedures are more or less effective than those employed by others.

Of far greater importance from the standpoint of consumer protection, is the fact that these sources of technical information could serve as important means of recognizing potential or actual health problems and guiding research toward their solution, thus stimulating the application of preventive measures. Considerable skepticism is to be expected regarding any venture that requires the contribution of information from multiple sources for evaluation and coded distribution to the participants. On the other hand, because the cost of acquiring such information is prohibitive for any one organization the fullest possible use should be made of available data from all sources.

Development of this concept will require mutual confidence among the participants and guarantees against revealing the identity of products by brand name or source. Many questions will arise concerning the tests applicable to a given product and the interpretation to be placed on the accumulated data, especially where supporting information about the history of the product is not available. Nevertheless, the knowledge to be gained about the occurrence, significance, and sources of microbial food contaminants is worthy of extensive collaborative surveys to identify the hazards against which the food industries and the health agencies should direct preventive action.

3. *Improvement and evaluation of methodology.* Methods are the tools of the technical investigator and cannot be separated from the aims of his work. They must be continually improved and adapted to the problems at hand. Progress in research is heavily dependent upon advancements in methodology which make possible the collection of data not previously attainable. On the other hand, the repetitive examination of specimens for compliance with established criteria requires standardization of methods to yield reproducible results of known specificity and sensitivity. The methods should also be capable of uniform application by technicians in different laboratories with a minimum of cost, effort, time, and special equipment.

The methods now used in food microbiology have mainly been adapted from other fields. Those employed to detect specific pathogens in foods implicated in disease outbreaks have been derived from diagnostic laboratory procedures. Often they depend on primary isolation in a selective environment which is delicately balanced and may be upset by any one or a combination of factors related to the type of food being examined. Modifications necessary for one product may be unsuitable for another, as has been demonstrated in attempts to isolate salmonellae from such diverse specimens as eggs, meat, yeast, bone meal, and cocoanut. Procedures that detect qualitatively the presence of pathogens in high concen-

trations are frequently unreliable for the detection of low levels, especially in the presence of other more abundant microorganisms. Practical methods for the assay of staphylococcal enterotoxin have yet to be developed, and techniques for the isolation of viruses from foods have received little attention.

Methods of counting bacteria in food and of determining their sanitary significance have come, almost without modification, from the procedures for examination of water and milk. Before these can be adopted as standard methods for the examination of other products, much work remains to be done with respect to such variables as culture media, time and temperature of incubation, diluents, sample preparation, and confirmatory procedures. From several recent contributions relating to the isolation and identification of specific groups of organisms, such as fecal coliform organisms and enterococci, it appears that this well-worked field of research can still make important practical contributions to food sanitation. Over-all, the continued improvement and evaluation of methods must be regarded as an essential phase of food microbiology.

4. *Development of standards applicable to potentially hazardous foods.* Experience with bacteriological standards for water, milk, and shellfish indicates that criteria applicable to one product may or may not be suitable for another. For example, the potability of water is judged primarily on the concentration of coliform organisms; the quality of raw milk is usually determined by the plate count, and that of pasteurized milk by both the coliform concentration and the plate count. The acceptable levels are, of course, widely different in each instance.

Only by the most painstaking and exhaustive studies can the utility of any proposed standard be established for a particular food. At the outset, imperfections in sampling, inadequate laboratory methodology, and lack of knowledge correlating laboratory findings and health hazards, as well as uncertainties about the impact on industry, may loom so large that tentative criteria can only be established on an arbitrary basis supported by judgment and experience. An extended period of collaborative investigation and trial use is then necessary to reconcile technical procedures, industry practices, and public health needs. Attempts to shorten this development process by premature legislation or administrative action have generated bitter controversies and caused strong resistance to criteria that might otherwise have become as widely used as bacteriological standards for milk. Centralized processing and interstate or international distribution of commercially prepared, nonsterile foods are creating demands for microbiological standards that can be applied without the benefit of traditional plant inspections. It remains to be determined whether micro-

biological data, so obtained, can be reliably interpreted; there seems to be no doubt, however, that authorities in receiving areas will take action on food shipments found grossly contaminated with infectious agents or toxins. It is incumbent on food microbiologists to investigate the technical aspects of these complex problems in order to guide the responsible agencies toward reasonable solutions which will, in fact, protect both the consumer and the food industry. Several organizations, including the National Research Council, the Association of Food and Drug Officials of the United States, and the International Association of Microbiological Societies, have committees working in this area. Their efforts are, however, so severely handicapped by the dearth of pertinent factual information that none has yet announced agreement on firm standards.

5. *Reevaluation of food protection programs and practices.* The patterns of food sanitation were largely developed before World War II and need to be reevaluated in the light of modern food technology. In recent years, food manufacturers have been spending over $100 million per year to develop new equipment, processes, and products, all of which add to the assortment of microbiological and other problems. The food-service business has grown to approximately $18 billion dollars per year, and increasing public acceptance is being given to commercial catering and vending, as well as other novel services that did not exist 20 years ago. Government agencies have been hard-pressed to keep abreast of these developments because the resources for food protection programs have not kept pace with population growth.

To a degree, research organizations are responsible for the limited application of their findings. Many otherwise excellent studies stop short of demonstrating how the results may be used or what their effectiveness will be in practice. Considerably more effort is needed to bridge the gap between the laboratory and actual operations with respect to the control of microbiological contamination. The opportunities for applied research seem especially attractive in areas relating to equipment design and process control where elimination of manual operations or exclusion of aerosols would materially reduce the possibilities for contamination of the final product.

6. *Advancement of basic knowledge about food in relation to health.* A continuing flow of scientific information is essential for the recognition of new health hazards and the timely development of measures that will prevent unsafe foods from being offered to the public. By delving deeply into problems of microbial metabolism, genetics, virulence, and ecology, the microbiologist can contribute much to understanding the interrelated

factors that determine the fate of microorganisms in foods. Such data, when integrated with detailed knowledge about the physical and chemical properties of food products, will provide a basis for advancing food technology along lines compatible with protection of the public health.

Of particular importance are the interactions among microorganisms in nonsterile products, as affected by various methods of formulation, preservation, packaging, storage, and preparation for serving. Where pathogens are not totally excluded or destroyed by accepted procedures, control of the associated microflora may well be an important means of preventing growth and toxin production by such common food-poisoning organisms as *Staphylococcus aureus* and *Clostridium botulinum*.

Much more knowledge is also needed concerning the varied microflora of foods that influence the growth, vigor, and longevity of the consumer. From the evidence already available on germ-free or pathogen-free animals and on the effects of antibiotics or other dietary supplements that modify the intestinal flora, one may guess that supposedly innocuous foodborne microorganisms play an important role in the physiological development of man, his resistance to infection, and possibly his susceptibility to certain degenerative diseases. Perhaps research in this area may provide the basis for a new positive approach to good health through control of microorganisms in the diet.

Conclusions

The successes of food microbiology during the past 60 years have created a false impression that the major problems of foodborne disease are already solved. Nevertheless, staphylococcal food poisoning, salmonellosis, and gastroenteritis of undetermined etiology remain common foodborne diseases in the United States. In part, the difficulties of prevention and control result from failure to utilize existing knowledge, but the accelerating rate of change in food technology has also created gaps in scientific information that can only be closed by additional research.

Coordinated programs between industry and government are needed to improve technical intelligence, laboratory methods, evaluative criteria, and operational procedures for protection of the public health. Progress in these areas depends on the integration of microbiological research with investigations in the other sciences that contribute to food protection. First steps toward a more effective national program include:

1. Improved investigation and reporting of foodborne diseases.

2. Surveys of health hazards associated with market foods.

3. Improvement and evaluation of methodology for examination of foods.

4. Development of microbiological and other standards applicable to potentially hazardous foods.

5. Reevaluation and modernization of food protection activities, including the translation of research findings into practice.

6. Advancement of knowledge about the relation of food to health through research, specialized training, and public education.

REFERENCES

1. Report of the Committee on Environmental Health Problems to the Surgeon General. U. S. Department of Health, Education, and Welfare; Public Health Service Publication No. 908, U. S. Gov't. Printing Office, Washington, D. C., 1962.
2. Report of the Food Protection Task Group Meeting. Sponsored by the Division of Environmental Engineering and Food Protection, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Washington, D. C., July 5–6, 1962.

Open Discussion

DR. ORDAL: The four papers just presented are now open for discussion. Who would like to comment first?

DR. EL-BISI: Moved by Dr. Ingram's statement at the conclusion of yesterday's afternoon session, and stimulated by several viewpoints brought up during this morning's presentations on research needs and objectives, I feel compelled to punctuate and perhaps reemphasize several points with regard to the current status of development and research needs in several basic problem areas governing the technology of canning.

Dr. Bohrer presented an excellent review of the conventional canning process in its classic sense in the short time allotted to him.

This seemingly stabilized and problem-free technology is undergoing a slow evolutionary stage, facing as it progresses several major questions of which the following are but few examples.

First, the emerging, ever increasing volume of so-called "specialty" products. These are essentially products formulated from both acid and low-acid ingredients mixed together in various proportions, shapes and sizes with a final over-all pH ranging from 4.5 to 5.2, commonly referred to as the "intermediate pH range," and could be more appropriately referred to as the "grey pH zone." Examples of such products are soups, sauces, dinners, salads, etc. The thermoprocessing of this class of foods remains, as Dr. Ball once referred, "unscientific," based in most cases either upon unjustified interpretation of the acidity classification rule, or empirical trial and error techniques. The pH distribution pattern; the thermal death kinetics of major spoilage sporeforming bacteria as well as their physiologic behavior in the processed products; the heat transfer characteristics through such products—all remain unsettled basic questions.

For the past 4½ years our laboratory has been engaged in settling some of these basic questions, especially those concerned with the thermal death

kinetics. Through the preliminary stages of this work basic methodology has been and still is being developed, such as: the establishment of more effective sporogenesis environment and the effect of such environment on the thermal quality of the spore populations produced; the effect of post-harvest treatments on thermoresistance; the optimum reference buffered heating menstrua at such pH range; the optimum recovery of severely heated spores; the effect of major model food components on resistance as well as recovery, etc.

Second, the slow but certain evolution towards the high temperature-short time aseptic packing. Here, we are faced by the most rewarding yet the most challenging process in the evolution of canning technology. Although the principle dates back to the twenties, development to a sound economic commercial scale production is still far from reality. Here, we confront three major problem areas: one is concerned with the product development and its storage stability and this is the responsibility of the food technologist; the second is concerned with the unit process development and this is the responsibility of the food engineer; and the third is concerned with commercial sterility and public safety and this is our responsibility as food microbiologists. This last problem area can be further subdivided into two main subareas: one is concerned with the food sterility, namely, death kinetics of spores at extremely high temperatures and their interrelation or intercrossing with the kinetics of chemical, biochemical, and physical deteriorative activities; and the second is concerned with the container sterility. This latter problem demands the establishment of new reference thermal death kinetics in different dry hot atmospheres or superheated steam. Preliminary findings on this subject suggest a different pattern of comparative resistances and possibly different kinetics as contrasted with the conventional moist-heat death kinetics.

Most important with regard to container sterility, is the desperate need for alternative cold sterilization techniques for handling containers susceptible to excessive heat shock or heat damage such as glass and plastics. Such methods must be industrially compatible and feasible, and economically competitive. In our laboratory over the past three years, we have been engaged in a research project exploring and establishing the sterilization potential of *chemicals in the vapor-phase. This field, although it dates back in practice to* ancient times, remains an empirical art with very little known about the basic laws and mode of activity. In our work we attempted to develop specialized equipment and instrumentation which enables one to provide, control, and monitor such reactive atmospheres with all the parameters which govern their biocidal activity. Our experimental approach has been directed along two main avenues of activities progressing simultaneously. One is "exploratory," namely, the survey of microbiocidal activity of orderly selected chemicals in the vapor phase under standard test conditions. The other is "determinative," namely, the establishment of the detailed kinetics of activity and hopefully the mode of action. From such basic quantitative information specific applications can be extended to specific problems within their operational limits.

Third, the whole area of canning pressurized or squeezetype foods. Their development, handling, and thermoprocessing require many years of problem solving.

Fourth, the area of canned semiperishable meat and fish products, products which may harbour residual spoilage and/or intoxicating populations, yet

popular and salable. Here, the complex phenomena of spore dormancy and bacterial ecology are still in the stone age.

Last and perhaps the most significant, is the very recent emergence of basic studies on the cytological, cytochemical, and physiological properties of bacterial endospores. Such studies as those conducted on the biochemistry of sporogenesis, spore metabolism, biochemistry of spore germination and the mode of spore resistance to moist heat—will ultimately render the enemy more vulnerable and the canning technology of tomorrow, hopefully, more economical and, quality-wise, far superior.

DR. HURST: I would like to add to a point made by Dr. Brown. I think there might be a solution to the *Salmonella* problem if we had a test that took 15 minutes or perhaps less. I think this is the sort of aim we ought to have for controling, for instance, meat that comes into factories which is suspected of containing salmonellae. It is very seldom much use to know that the product contains salmonellae several days after it has been sold, and this is the stumbling block. For instance, they cannot buy meat on this basis. If we had a test that could be completed in 15 minutes, it might be possible then to buy meat on the basis of whether it contains salmonellae. I know this sounds rather fantastic, but nevertheless I don't think it is any more fantastic than aiming for the moon. It seems perfectly reasonable as a concept.

DR. THATCHER: Mr. Chairman, recently I had the opportunity to discuss problems in food microbiology and this is my third opportunity to assemble with an international group such as this. The remarkable thing is the high degree of unanimity of opinions with regard to these two aspects. One, the areas of potential hazards, and two, those areas where further study is essential to give us adequate comprehension of the full interrelationship of these problems. The great weakness that we seem to be exposed to now is not in ideas but in some way to bring out the whole impact of our collective knowledge to be recognized in such a way that Dr. Lewis, myself, and others with a legal responsibility to do what they can to protect health, shall have a voice which can allow us to get the man power, to get the facilities, to tackle these very problems which for years many of us have recognized do exist. Our staff is barely able to keep up with prevailing problems and has almost no time whatsoever to study new potential hazards which we see clearly. I would urge, therefore, that all support possible be given to new programs, whether they be national or international and that all of us in the field of microbiology recognize our responsibility to make every effort to obtain such support.

DR. BUCHBINDER: I would like to mention something which I don't think has been mentioned, or if it was, only very briefly. I know I mentioned it. That is education. Most of us here perhaps rightfully so, feel that we are pretty ignorant about food, but I think we will also agree that we know a lot more than people who are not in this field, and it seems to me that we know enough to educate others. I would like to point out that in New York City, about half of our reported outbreaks are home outbreaks. Now does anybody know of a book which can be used in high schools to teach people how to take care of food at home? We have 40 educators represented here. It seems to me this is an area of transmitting knowledge to everybody of how to handle foods at home. It should be part of the education of every citizen.

DR. ORDAL: Thank you, Dr. Buchbinder. I think that you have brought out a very important point, and especially cognizant to the particular point you made is the terminal handling of food. That is undoubtedly a situation where our trouble comes from.

DR. JAY: I have heard at least two persons speak of numbers of staphylococci as related to maximum limits in foods. I would just like to suggest that additional research is desirable here in differentiating the so-called human from the so-called animal strains. I think it is generally agreed that the so-called animal strains are less important to human infection so I think the numbers should not be predicated on this basis. I personally don't think we are any better off here than on the question of enterotoxin in respect to their origin in food.

DR. SLOCUM: I would like to make a comment which I think is somewhat along the lines mentioned by Dr. Thatcher and Dr. Buchbinder but I got the thought primarily from Dr. Pederson's talk, and that is, in spite of our limited range of knowledge we do have a vast fund of information about the microorganisms in food which needs to be applied to food production at the present time. We all know that the wooden tubs that are used in the food industry will build up contamination and we know many other factors which are not appreciated in the food industry which effect microbial contamination and which can be avoided. I think we need to press the application of existing knowledge to present practices which are obviously at fault.

DR. ORDAL: Thank you, Dr. Slocum. I think this is certainly apropos. In addition to this, it would be interesting to know if they had statistics as to how many wooden tubs are still left.

DR. SLANETZ: I would like to respond to Dr. Jay's comment with reference to the differentiation of strains of *Staphylococcus aureus* isolated from human and animal sources. I feel it has been demonstrated that as far as the staphylococci causing bovine mastitis are concerned, they are essentially similar to the *S. aureus* strains isolated from human infections. In June 1962, we published a paper in the Journal of Infectious Diseases on the bacteriophage and serological types of staphylococci isolated from cases of bovine mastitis. Many of our strains were similar to types that have been reported from various human infections. Thus I am not sure staphylococci from animal sources can be distinguished from those causing infections in humans.

DR. BALL: I have one question for Dr. Brown. In regard to the spores that are recovered from cans of cured unsterilized meats that contained spores, will these organisms produce spores if innoculated back into the same type of meat from which they were isolated.

DR. BROWN: We found that when you take these spores from their natural environment, take them out and run them through a couple of generations in the laboratory and put them back, you get an entirely different picture. I think Dr. Silliker pointed that out too. You can change the spore pattern entirely by growing the spores in the laboratory and innoculating them back into the meat.

DR. WALTER: In answer to Dr. Buchbinder's question, I think one reason that many students don't get much of this in high school is that many of the teachers feel very inadequate in this area. On our campus this summer we had 34 teachers who never had any bacteriology. I had over 500 applications for the course we were giving, indicating that there are many people inter-

ested in this area. I think that is probably one way we can get some of this information into high schools. The other thing I would like to say is that many people here have mentioned that we need more people in research, more people coming into industry. The only thing I can say there is all of you have a responsibility in helping get some of these people in the colleges with an interest in this area. We can do something with them once they get there but other people have to help get them into those particular fields.

DR. GOLDBLITH: I would like to respond to Dr. Slocum's remarks by saying we should utilize existing information. Also, I think this might add something to Dr. Ingram's provocative comments yesterday. You take the case of freeze-dried seafoods. Here is an industry which is new; in fact the technology is far ahead, certainly, of the basic microbiological information. Yet here is an industry which uses raw products which on the market may have counts from over several hundred thousand to several million in the case of shrimp or crab meat. The corresponding products which are frozen precooked may have counts in the order of several hundred thousand. But by utilizing the basic techniques that we now know in terms of microbiology in food handling and sanitation, in the case of freeze-dried shrimp you will find the products on the market will have total counts where 99% of the samples are under five thousand; 95% under 1000 per gram; and better than 85% under 500 per gram. In other words, a practically sterile product. So here is a situation where a new industry, by applying the techniques we do know, has actually given in terms of public health, an improved product.

FROM THE FLOOR: I would like to direct a question to Dr. Thatcher relative to his report of yesterday. Particularly I would like to ask if the recommendations of the ad hoc committee constituted a recommendation that regulatory agencies in this country and abroad adopt these standards?

DR. THATCHER: Mr. Chairman, I had hoped to make clear that the observations I summarized yesterday were simply to express the corporative opinion of what was obtainable under present good commercial practice, and which would substantially, in our corporative opinion, contribute to improved safety in those foods that we specifically referred to. They are not intended to be precise recommendations for regulatory control, but we would think or hope that if such corporative opinion of many nations have merit, then where regulatory action is to be anticipated, that these recommendations would get due consideration and be obeyed. This is the intent.

DR. BROWN: One of our biggest concerns is of course people and we feel we have hit upon a way to improve our relationship with the local people. We have hired the local science teacher, a student advisor, for work in our plant this summer.

DR. HOBBS: I thought you might be interested in the educational program that we have in England. There are several school books in England that are applicable to home consumption of food.

DR. ORDAL: Dr. Mrak will now present a summary of the conference.

Conference Summary

E. M. MRAK

Chancellor, University of California, Davis, California

I had an opportunity to read the papers prior to the Conference but decided not to do so because I desired to seek out the thread or threads of broad concepts running through the several days of meeting, if possible. I tried to avoid thinking in terms of specific organisms or numbers.

First of all, the Conference, as I understand it, was to bring together a number of people working in the field of food microbiology and to establish the broadest type of program possible. This was done, but at the same time it is obvious that a number of areas of importance were left out.

Another purpose of the Conference was to develop ideas; useful ideas, and ideas that would lead to "useless" work that eventually would be useful. Still another purpose was to point out areas of important research needs, and to stimulate interest in this important field. I think the Conference has done this very well, and I am only hopeful that the interest and enthusiasm developed here continues in one way or another.

The Conference covered various points of view including those of industry, enforcement, public health, other government agencies, universities, and various research groups. This, of course, is important. It covered problem areas, foodborne diseases, indexes of quality, standards for foods, and above all, research needs.

In attempting to follow the thread through these various areas as they were discussed, I must admit it was severed now and then. In spite of this, I think I was able to follow it throughout the meeting. The program was limited to 2½ days and because of this, it was necessary to omit a number of areas. For example, viruses and fungi were barely mentioned, and the microfauna of the ocean and the illnesses that some of these may cause were completely omitted. Some people have wondered about algae, but these were not discussed. What about ciguatera poisoning? There wasn't much said about amoebiasis. The Conference could have gone on for several weeks if everything was to be covered thoroughly. The discussions

on many of the new foods were limited or omitted. Some that were mentioned were doughs to heat, new fish packs, vacuum packs, partially cooked foods, prepared mixes, etc. A number of new processes were barely touched on.

Then we have new processes introduced for fresh products such as hydrocooling, bulk handling, vacuum cooling of lettuce, etc. I wondered whether or not there are microbial problems in these cases.

Why was the Conference limited to 2½ days? It seems to me the answer is obvious. The field is now so large and complex it could not have been covered in breadth in a week. I don't know how long it would take to cover it in both breadth and depth. One person sitting next to me made the comment when someone referred to streptococcus, "Heavens, they can talk on that all day," and I think he was being conservative. It appears to me the problem is vast, and few people realize the extent, the complexities, and the importance. Another interesting area that came to my mind during the meeting is the relationship of food microbiology to the field of environmental health. This field of course includes water, waste disposal, occupational health, air, radiation health, and what not. Several of these areas were touched on during the discussions, and this I found extremely interesting.

Now let's take a look at the breadth and complexity of this problem. Of course, the broad areas referred to above a number of times could be classed as food infections and spoilage, but these are such simple and disarming terms. The research worker, for example, must consider the consumer. But what are the things he must keep in mind with respect to the consumer? You heard a paper yesterday on chicken pot pies, and the question was asked, "When will the consumer eat something spoiled or when won't he?" So we get into the field of acceptability and the factors that influence it, such as: microbial changes, cooking treatments, storage conditions, and so forth. We must consider another factor, and this was touched upon indirectly this morning. I refer to the matter of stability and its relation to storage, handling, distribution, and treatment in the home. People in the home are so often unaware of the dangers of handling foods improperly. So we get into the matter of educating all those handling foods.

Utility was mentioned a number of times, the ease of use by the consumer. This of course is bound to change the characteristics of the product. I recall so very well a few years ago someone was thinking of producing a dry product. They said if we can raise the moisture content, even people with false teeth can eat it. But the addition of moisture caused other problems, spoilage in particular. So a food processor must think of utility,

but likewise he must think of the hazards that may come along with it.

There is one other problem I think is extremely important. I am referring to the nutritive value of a product. If we destroy the nutritive value by processing, then one wonders what is the value of safety. You destroy the organisms but supply the consumer with a food deficient in nutrients. These two factors need to be considered for today when we are trying to increase the protein intake of people in many of the nations, we need both safe and nutritious foods. I believe this is a serious problem, for when do we take a calculated risk?

I once heard a person from South Africa ask with respect to agricultural chemicals, "Would it be better to starve to death at the age of 30 from the lack of food or live to the age of 90 and die from cancer because of the ingestion of agricultural chemicals?" I sometimes wonder if this type of thinking might not apply to some of the protein foods going into countries where they need proteins. Do we take a calculated risk and where is it? I think we need to know something about this with respect to safety and need.

Then, of course, the factor of safety is most important and is taken up after the other factors only because I want to emphasize it. Suffice it to say that this is to a large extent what the present Conference is all about.

However, the problem is broader. It is more than the things I mentioned above. We must think in terms of the complete food path. It was quite pleasing to me to know that there are many people who are thinking in terms of the food path from the time the seed is planted, or the organism is conceived, or the egg first starts to take its place in the chicken, until it gets to the consumer. There are problems all along the line and no longer can we ignore those closer to the soil. We must think of the total picture. I believe Dr. Townsend mentioned yesterday the influence of adding tomato waste to the soil. In this case, the vines and waste tomatoes are disked into the soil. It so happens that this practice increases the thermophilic load in the soil and eventually these organisms cause problems in the cannery. We must therefore have full-spectrum thinking. I might say here that such a full spectrum thread wobbled throughout the entire Conference. We must think in terms of animal feeds, the handling of these animal feeds, and then we must think in terms of what happens to the animals that eat these feeds, furthermore, we must think of what happens to the animals when they are shipped to market. We get one little pig who doesn't feel very well; maybe he'll infect all the others before they get to the stockyard. These are real problems that cannot be overlooked.

It is apparent, too, that we may get a variation from one country to

another or from one area to another, dependent on the handling. This makes it more complex, but also more important.

Another way of looking at this total picture and complexity is from the standpoint of technology and new products. Each area of preservation has its problems. This was made obvious during the discussions held here. New products, new methods of serving by use of vending machines, etc., all go back to the original packer. A great change has occurred in the handling and use of frozen foods. Refrigerated foods cause problems. A lot was said about dehydrated foods yesterday, and I was pleased when something came up on the fundamentals of moisture and microbial growth. Most certainly we need more work in this area.

More fresh products are now sold than ever before. I am certain that there are problems from the standpoint of nutritive value, but I wonder about the microbiological picture. I have no hesitation in saying that I have been genuinely shocked by some of the things I saw at the old Boston fresh market on one of their hottest June days. There must have been problems.

Another problem mentioned is that of transportation. Foods are being shipped throughout the world, and this has resulted in some difficult problems. Dried eggs, animal feeds, peanut products, frozen products, etc., are all being shipped from one country to another. What wasn't mentioned at the Conference was rice, which may have a toxic penicillum on it. Rice imported by Japan has, at times, had this toxic mold on it. We can say with certainty that foods and problems related to them know no boundaries. There is another interesting aspect to this which was touched on only briefly. I refer to the relationship of rodents to this problem. They are wonderful travelers; they do contaminate foods.

Now to look to the future. In my opinion, the problem will get worse. Some countries have reached a stage where fulfilling the wants of people seems to be more important than fulfilling their needs. In the United States, no longer are we worried about giving the people only their needs, such as calories; we are far more worried about giving them their wants, and their wants are changing continuously with respect to the type of foods desired. The food processor has disenslaved the housewife and given her foods that she can cook and prepare very rapidly. It even seems possible that the time may come when she will purchase the food, put it in an oven, then push a radar button and have it ready to eat in one minute. But we are in the age of wants and this brings about such changes as mentioned above. So we can expect more and more problems resulting from these changes.

On the other hand, in countries that are still fulfilling their needs or

attempting to do so, we also have problems. This is well illustrated by the recent peanut problem.

Another change occurring in the world is related to the expected increase in population. We can expect more and more high density living, and this will pose more problems in food handling, distribution, use, and so forth. There is another high density development occurring in this country, and I think it will occur in other countries. As we use much of our agricultural land for housing developments and six and eight lane highways, and as taxes go up, etc., the farmer has been compelled to go into what I have chosen to call "high density agriculture." The chickens are now literally raised in factories. They are crowded together in many small pens. We have feed lots where the animals spend about 100 days, but they are crowded together. We have more plant products per area. All this means problems and a greater opportunity for diseases.

I have already mentioned the change in soil flora and its importance. New varieties are being bred to increase production, and at times this results in undesirable changes in some of the characters. A good example is the tomato. Production and solids have been increased by breeding, but a decrease in acidity in some cases has accompanied the changes mentioned above. We now have tomatoes that are hard as rubber balls and will bounce when you throw them on the ground. The solids content are high, and they make good tomato paste; but one does wonder about the acidity and, of course, the susceptibility of the canned product to the growth of *Clostridium botulinum*. Truly, we are living in an age that is moving rapidly forward to what I call a full-spectrum food problem from the farm to the consumer.

But there are still other complex relationships in the total field of environmetal health. At this Conference we mentioned food, water, waste, and even occupational health. In a number of cases we mentioned the sanitation and purity of water, a factor which we cannot divorce from the food picture.

There was some mention of occupational health hazard, such as ornithosis in poultry plants. Then, again, how important are human carriers if they work in restaurants or food plants?

Considerable emphasis was placed on the problem of waste disposal. This problem will increase. It is one of the greatest problems in the food processing industry.

Finally, as I see it there are three great needs. We need a vast and broad research program from the theoretical to the applied. I have tried to list some of the areas that would fit into the full-spectrum picture. These are methodology, ecology, end products, water relations, physiology

or spore germination, chemistry of toxins, genetics of micro-organisms and also of higher plants, viruses, fungi, waste disposal, and the relationship of processing to nutritive value. Taxonomy was mentioned this morning, and this is important; so we need a vast and broad research program. We need to develop fundamental information, some of which we can draw out of the savings account in the future as useful information. We need an education program. Dr. Buchbinder's question this morning made me wonder if the book entitled the "Rutgers' Food Saver" might not be useful in telling the consumer about using stored food. It is written so the layman can understand it. Some University Extension Services have publications in this area if you can find them.

But there is a more important aspect to the training program. I think we need to train scientists. There should be training grants to increase the number of graduate students in this area. It is apparent, therefore, that we need education clear across the board.

The third great need is for more devices for the exchange of ideas and information, and also for meetings such as this. The Public Health Service is to be complimented for providing financial support for this meeting. This agency will be remiss if it doesn't do all possible to promote more meetings in the future. We must develop the philosophy of prevention as well as one of cure. There has been a definite philosophy directed toward cure, and I hope the worm is turning and that the future will see more emphasis on prevention.

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