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Official Publication

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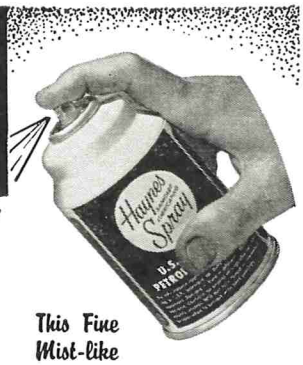

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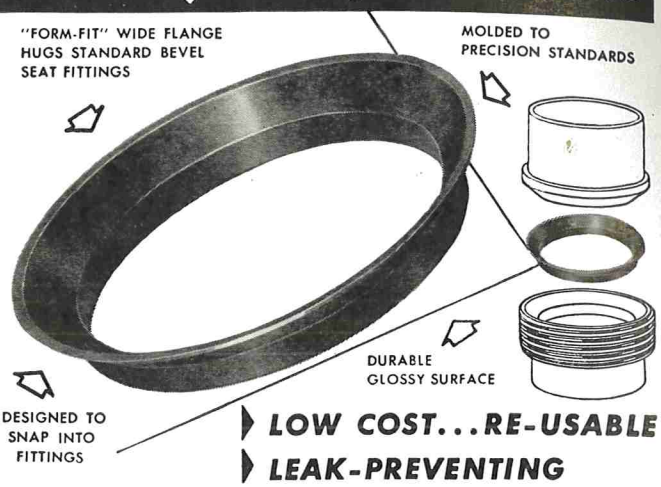

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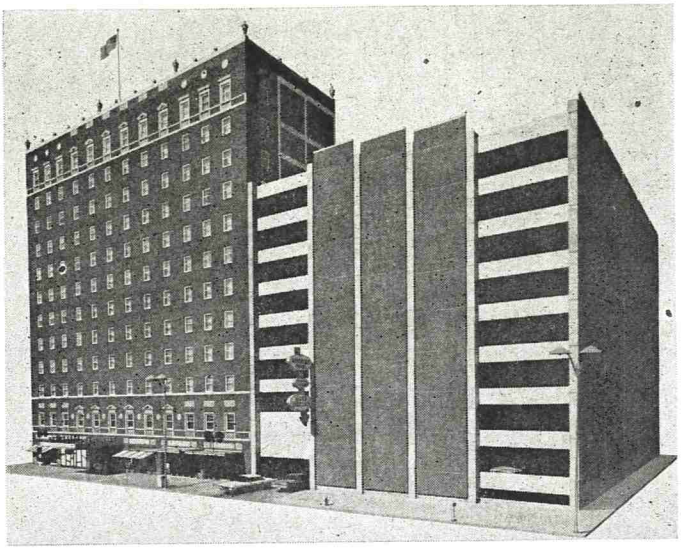

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CURRENT DEVELOPMENTS IN DETECTION OF MICROORGANISMS IN FOODS: INFLUENCE OF ENVIRONMENTAL FACTORS ON DETECTION METHODS[1]

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In this paper it will be my aim to evaluate the problem of detecting particular microorganisms in a food product from the point of view of the bacterial cell rather than a discussion of an individual or a particular procedure. In this day and age when so many of our foods are prepared or have been subjected to a processing operation, we must have knowledge of the effect of the processing operation on the bacterial cell. When the processing operation is severe enough to totally inactivate the organism we are seeking to enumerate, then there is no problem unless we are seeking to determine whether or not there has been post-processing contamination. The latter situation would imply that there was faulty technology in the overall operation. Technology per se is not our discussion topic. Technology, however, is related to our discussion in the sense that we have many food processing operations which are not designed to sterilize the food product but which are used in other functional capacities. These processing operations commonly injure the cell but do not necessarily kill or destroy it. In other words, the microbial cells which might be present in the food during processing operations will be subjected to varying degrees of stress. The degree of stress (or injury) will result in lesions to the cell. Such cells must be able to repair themselves before they can multiply and divide. We cannot enumerate them unless they are capable of division. The conditions under which such cells can repair themselves varies with strain and species. In one sense the conditions for repair are more restrictive than they are for growth and multiplication but in another sense one might say that they are less restrictive. This will be clarified later in the present paper.

The Food Microbiologist is commonly required to develop detailed quantitation. He is expected to develop data which tells others how many of a given organism are present in the sample. Yes, he is even expected to tell others when there are zero or no organisms present. I do not intend to get involved in the statistical evaluation of zero but I think that the mere mention of this unrealistic problem should make us realize that when we seek to enumerate the numbers of a microorganism present, then we must judiously select procedures which will permit us to determine all the organisms present; we should be able to detect cells that are stressed or injured but still viable as well as cells which are in a more normal physiological state.

I would like to briefly describe the effect of a common food processing operation which, before it kills or inactivates the cell, produces lesions from which the cell is able to recover by repairing itself. This discussion will be confined to describing the effect of a sublethal heat treatment. I will also include some of our data which could be considered to reflect environmental conditions in a food after the processing operation has been completed.

THERMAL INJURY OF *Staphylococcus Aureus*

When cells of *Staphylococcus aureus* are subjected to a sublethal heat treatment, the treatment produces a variety of repairable lesions. We have used a plating procedure which serves to give us a measure of the degree of injury inflicted and likewise provides us with a means of following the recovery of repair process. Injury is determined by following the difference in plate count between that obtained when the suspension is plated on a rich medium, Trypticase Soy Agar (TSA), and when it is plated on Trypticase Soy Agar containing an added 7.0% NaCl (TSAS). The injured or stressed cells are sensitive to the added salt and are unable to develop into a colony when plated out on such a medium.

When a suspension of late log cells in phosphate buffer is heated at 52 C and at intervals aliquots of the heated cell suspension are plated on the two media, the productivity of the two media is reflected in the data which is graphically represented in Fig. 1. It is apparent that the two media vary as to their productivity as the heating time is extended. The difference between the two counts is a measure of the injury inflicted on the cells by the thermal treatment.

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[1]Presented at a Round Table on Current Developments in Detection of Microorganisms in Foods at the 69th Annual Meeting of the American Society for Microbiology, Miami Beach, Florida, May 4-9, 1969.

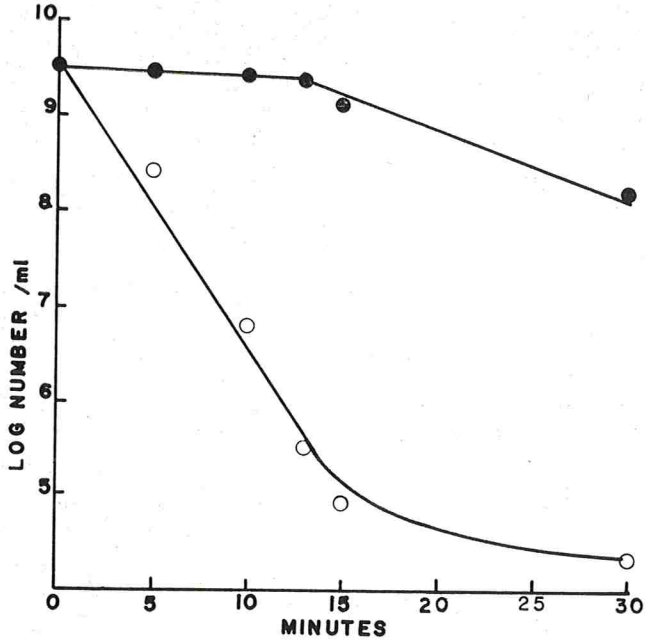

Figure 1. The effect of NaCl in the plating medium on the apparent survival of cells of *Staphylococcus aureus* MF 31. The cells were heated in 100 mM phosphate buffer at pH 7.2 at 55 C. At the indicated intervals, aliquots of the suspension were plated on Trypticase Soy Agar (TSA —●) and on Trypticase Soy Agar containing added 7.0% NaCl (TSAS —○). The difference in count between the two media is a measure of the injury impossed on the cells by the heat treatment.

When cells which have been heated for 15 min at 52 C are transferred to Trypticase Soy Broth (TSB), we can compare the growth response of such thermally injured cells to that of uninjured cells. This is graphically represented in Fig. 2. With the uninjured cell inoculum, the lag time is short and the productivity of the two plating media (TSA and TSAS) is equal. The salt-containing medium (TSAS) does not suppress colony formation by these cells. In contrast, when the inoculum is thermally stressed cells we note, *(a)* the lag time is extended and *(b)* there is a marked difference as to the count on the two media. The maximum difference in count between TSA and TSAS is evident immediately after inoculation into TSB. As incubation continues, the difference is reduced until shortly before the end of the lag phase. The productivity of the two media is then again comparable. This implies that the injured cells have returned to a physiological state comparable to that of the unheated culture. The rate of repair is linear and as the TSA count is equal to the total microscopic count, we are not measuring multiplication. We interpret these results to mean that during the thermally induced lag period, cells are recovering from the sublethal heat treatment to which they were subjected. The thermally induced lesions must be repaired before the cell can multiply and divide.

We have tried to characterize some of the lesions that are produced by this thermal treatment. Our information may be summarized as follows:

(a) The thermal treatment damages or impairs the cytoplasmic membrane. This results in the leakage of cytoplasmic constituents out of the cell into the suspending solution.

(b) The metabolic capabilities of the cell are altered. There is a selective thermal inactivation of cellular enzymes and a partial denaturation of cellular protein. Injured cells are not as efficient in utilizing an energy source as are uninjured cells.

(c) A prominent lesion is the degradation of the ribosomal RNA (rRNA). This degradation results from the thermal activation of en-

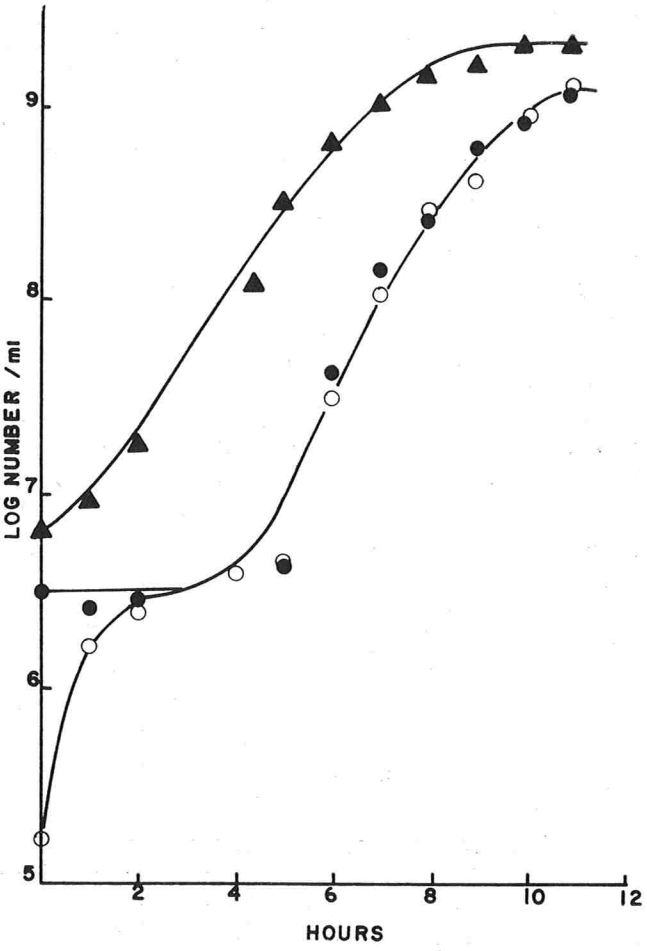

Figure 2. The growth of *S. aureus* MF 31 in Trypticase Soy Broth (TSB) before and after sublethal heat treatment. The cells were heated in 100 mM phosphate buffer at pH 7.2 for 15 min at 55 C. The unheated control (▲) represents average counts from Trypticase Soy Agar (TSA) and TSA with an added 7.0% NaCl (TSAS). The heated cells plated on TSA are represented by the closed circles (●) and the heated cells plated on TSAS by the open circles (○).

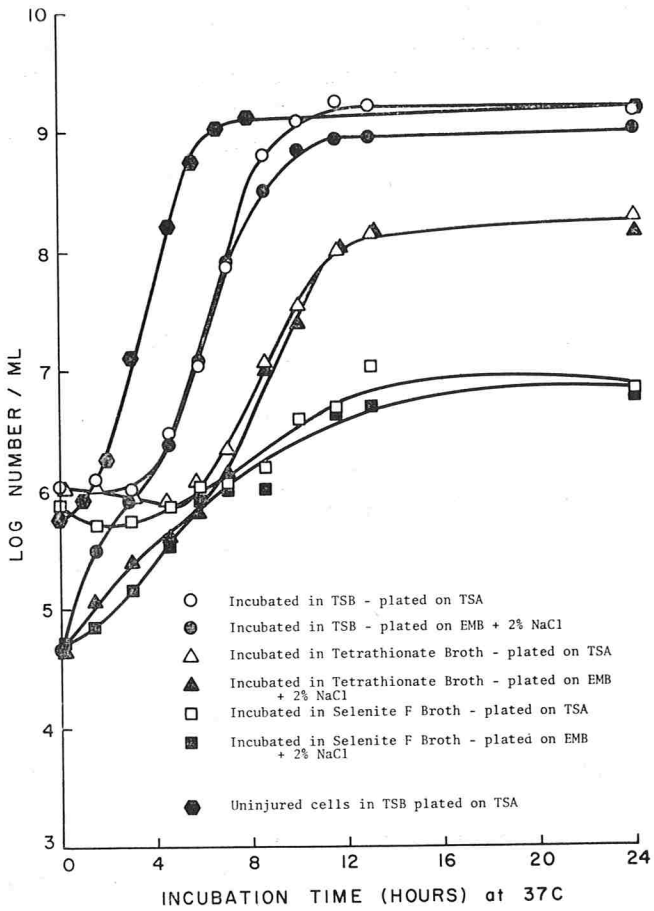

Figure 3. Recovery of growth of thermally injured cells of *Salmonella typhimurium* 7136. Washed cells of *Salmonella typhimurium* 7136 were heated in 0.1M phosphate buffer, pH 6.0, at 48 C for 30 min. The thermally injured cells were then inoculated into the indicated media in order to follow recovery and subsequent growth. Trypticase Soy Agar (TSA) was used to measure total viable cells and Levine Eosin Methylene Blue Agar (EMB) + 2% NaCl was used to demonstrate thermal injury and recovery; ○, Trypticase Soy Broth, plated on TSA; ●, Trypticase Soy Broth, plated on EMB; △, Tetrathionate Broth, plated on TSA; ▲, Tetrathionate Broth, plated on EMB; □, Selenite F Broth, plated on TSA; ■, Selenite F Broth, plated on EMB; ⬣, Normal cells grown in TSB, plated on TSA.

zymes which degrade the rRNA. The enzymes most likely involved are a ribonuclease and a polynucleotide phosphorylase. The relative importance of each enzyme is probably related to the particular environment of the cell during the thermal treatment.

Our information on the requirements for recovery or repair is also useful in characterizing the overall effect. It may be summarized as follows:

(a) The nutrient requirements for thermally stressed cells of S. *aureus* to recover are, an energy source (glucose), amino acids, and inorganic phosphate. Stressed cells will recover in a medium which will not support growth or multiplication. In this instance the nutrient requirements for repair are therefore not as rigid as they are for growth and multiplication.

(b) Stressed cells will recover in a medium containing chloramphenicol or 5 methyl-trytophan. As these compounds are inhibitors of protein synthesis, the data imply that protein synthesis is not an important aspect of the recovery process.

(c) The degraded rRNA is reformed or resynthesized during the lag period. This occurs before cell division can take place.

(d) The damage or injury to the cytoplasmic membrane is, in all probability, rapidly repaired after the injured cells are placed in a suitable recovery medium.

We have carried on some studies to determine the response of stressed cells to conditions which might be representative of foods after processing. Again using S. *aureus* as our test organism, we have determined the ability of thermally stressed cells to grow out in the presence of salt (NaCl) and other possible food preservatives. With the aid of a linear thermal gradient incubator (polytherm), we have determined the outgrowth capabilities over the whole growth temperature range of this organism.

The procedure used was to compare the outgrowth (lag time, slope of the growth curve, and maximum population level) of thermally stressed cells with those of normal cells. The recovery medium used was TSB containing 4% added NaCl (total 4.5%) adjusted to pH 6.0. This pH value was selected in order to provide more appropriate pH conditions for some of the preservatives tested, such as nitrite and benzoate. The polytherm incubator permitted us to follow the growth pattern at 16 different temperatures in the range of 8 C to 48 C using a single inoculum source for the thermally stressed cells or for the uninjured cells. The outgrowth was followed during a 5-day incubation period by recording the optical density (OD) of the growing culture on a B & L Spectronic 20 colorimeter and recording the result on a B & L VOM recorder. The most striking results were first, the extension of the lag time when stressed cells were the inoculum and secondly, the reduced temperature range in which the stressed cells were able to repair themselves and then to multiply. As expected once the stressed cells had recovered from the thermally induced lesions they were able to multiply as normal cells. The slope of the growth curves and the maximum population reached were comparable to those obtained when normal (uninjured) cells were used as an inoculum. Data on the

TABLE 1. TEMPERATURE GROWTH RANGE FOR NORMAL AND THERMALLY STRESSED[1] CELLS OF *Staphylococcus aureus* IN THE PRESENCE OF SELECTED PRESERVATIVES

Preservative (mg/l)	Temperature (°C)			
	Normal cells		Injured cells	
	Minimum	Maximum	Minimum	Maximum
None	21	45	21	45
$NaNO_2$ + $NaNO_3$ (150)	25[2]	45	28[2]	39[2]
Methyl p-hydroxy-benzoate (600)	21	45	24[2]	42[2]
Na_2SO_3 (200)	21	45	27[2]	45
Boric acid (300)	21	45	21	45
K Sorbate (400)	21	45	24[2]	45
EDTA (300)	21	45	36[2]	36[2]
Nisin (7.5)	24[2]	45	27[2]	36[2]

[1]Cells heated at 52 C for 15 min.
[2]Conditions which altered minimum or maximum growth temperature.

TABLE 2. PRODUCTIVITY OF VARIOUS SELECTIVE MEDIA FOR THE ENUMERATION OF NORMAL AND THERMALLY[1] INJURED CELLS OF *Salmonella typhimurium* 7136

Selective agar medium	Per cent of TSA[2] count	
	Normal cells	Thermally injured cells
MacConkey	100	83
Endo	100	81
Bismuth sulfite	100	80
Desoxycholate	100	77
Brilliant green	41	69
Eosin methylene blue (Levine)	97	39
Salmonella-Shigella	86	36
Levine EMB + 2% NaCl	100	2
Desoxycholate citrate	26	2

[1]Cells heated for 30 min at 48 C in 0.1M phosphate buffer, pH 6.0.
[2]Trypticase Soy Agar.

effect of the growth range is presented in Table 1. When normal cells were used as the inoculum only, the antibiotic, nisin, and the nitrite-nitrate combination increased the minimum growth temperature. None of the preservatives in the concentration used reduced the maximum growth temperature of a normal cell inoculum. In contrast all the preservatives, with the exception of boric acid increased the minimum temperature at which growth and multiplication of stressed cells could occur. Four out of seven also reduced the maximum growth temperature. When EDTA was used (300 mg/l), stressed cells were only able to grow out at one incubation temperature. A repeat experiment confirmed these results. In similar experiments in which the concentration of EDTA was altered, a concentration of 400 mg/l EDTA prevented outgrowth at all incubation temperatures, whereas when the concentration of EDTA was reduced to 100 mg/l the injured cells were able to ultimately out grow at the same incubation temperatures as normal or uninjured cells. It would therefore appear that the chelating properties of EDTA was making cations unavailable that were essential for recovery and repair of injured cells.

The lag time at all temperatures was always longer for the stressed cells than for the normal cells. In general, the lag time for the stressed cells was two to three times as long as for the normal (uninjured cells).

This data has been presented to demonstrate that the viability and the recoverability of stressed cells may be dependent on the environmental conditions which a product (food) is subjected to immediately following the processing procedure; the procedure that was responsible for stressing or injuring the contaminating cells.

THERMAL INJURY OF SALMONELLAE

In our studies on the effect of a sublethal heat treatment on gram-negative organisms, we have used as our test organisms *Salmonella senftenberg* 775W and *Salmonella typhimurium* 7136. In our search for a suitable medium by which we could assay for the injury produced, we decided to evaluate a variety of selective media for this purpose. Data on the productivity of these media for S. *typhimurium* are presented in Table 2. The results we obtained with S. *senftenberg* showed a similar but somewhat different pattern. However, for both organisms the optimum medium for assaying thermal injury was Levine's eosin methylene blue agar (EMB) with added 2% NaCl for S. *typhimurium* 7136 and 4.5% NaCl for S. *senftenberg* 775W. The data demonstrates a reduced and variable productivity for stressed cells.

Various pre-enrichment and enrichment media were evaluated for their ability to support the recovery and growth of thermally stressed cells. For S. *typhimurium* 7136 TSB, Nutrient Broth, Lactose Broth and Lauryl Trytose Broth were comparable. However, thermally stressed cells of S. *senftenberg* 775W recovered more rapidly in TSB and in Lauryl Tryptose Broth than they did in Nutrient Broth or in Lactose Broth.

The recovery and growth pattern of thermally stressed cells inoculated into Tetrathionate Broth and Selenite F enrichment media is compared to that occurring in TSB. The results are presented in Fig. 3.

Neither enrichment medium supports as rapid a recovery as does TSB, Tetrathionate being superior to Selenite F. Similar results were obtained with S. *senftenberg* except that the drop in viable count

(TSA) during the recovery period was more dramatic. These data serve to emphasize the value of the pre-enrichment step in the enumeration of *Salmonella* from a processed material.

Experimental work that has been summarized as well as the specific data which have been presented emphasize the importance of understanding the effect of processing stress on microbial cells that are present in a product. This is particularly pertinent when we seek to make a quantitative enumeration of the microorganisms present.

The presence of inhibitors or other selective agents in a medium may markedly reduce its productivity. Yet we frequently rely on selective media to determine the presence of a particular organism. I have described the effect of NaCl on staphylococci but other selective agents such as tellurite cause a similar reduction in productivity when used in the selective medium.

Information such as has been presented could, in part, explain some of the descrepancies which occur between laboratories when they use a processed product to establish the reproducibility of results obtained with a medium or a procedure. If we use staphylococci as an example, the temperature of holding the product, the time the sample is in the diluent, as well as the temperature of the diluent could readily affect the number of cells that are able to grow out and form colonies on the selective medium that is being used. Likewise the ability of stressed cells to survive after processing is dependent on the environmental conditions of the product after the processing step and during storage and distribution operations.

The limited data that I have presented on *Salmonella*, partially describes the thermally induced lesions. In all probability, the types of lesions ocurring are in principle similar to those produced in gram-positive organisms. On a practical basis, these data emphasize the importance of using an adequate pre-enrichment medium in the enumeration of *Salmonella* from a processed product. Stressed cells must have an opportunity to recover or repair themselves if they are to respond in the expected manner on a selective medium.

This discussion has been concerned primarily with the stress produced by a sub-lethal heat treatment. Our scientific literature is continuing to provide us with detailed information on the effect of cold shock, freezing, freeze-drying, dehydration, irradiation, and osmotic shock on the lesions produced in the cell. All these environmental conditions will affect the ability of stressed cells to recover, multiply, and divide in a particular medium.

REPORT OF THE COMMITTEE ON FROZEN FOOD SANITATION, 1968-1969

The Committee in its 1968 Report recommended that all interested agencies participate in the development of microbiological guidelines which may be used as indices in determining the sanitary quality of frozen food products. The AFDOUS Ad Hoc Committee on the Microbiology of Frozen Foods has published a report entitled, *Recommended Bacterial Limits for Frozen Precooked Beef and Chicken Pot Pies.*

There appear to be differing views concerning "guidelines" and "legal standards" or "legal limits". This Committee may wish to explore these differing viewpoints in the future.

There appears to be some increase in interest of both industry and governmental agencies in employee training. A number of new training aids have been developed by both groups.

Local health agencies receive numerous inquiries from retail and consumer groups regarding storage time and refreezing various food products. A summary of current information on this subject would provide a ready reference for these agencies.

There are several new publications pertaining to frozen food.

Supplemental Issue 1969—Association of Food and Drug Officials of the United States Quarterly Bulletin *Recommended Bacterial Limits for Frozen Precooked Beef and Chicken Pot Pies,* edited and published by the editorial committee. Copies of this report may be obtained from Evan Wright, Secretary-Treasurer, Post Office Box 1494, Topeka, Kansas. The cost of this report is $2.00.

The Freezing Preservations of Foods, Volume 3, Commercial Freezing of Fresh Food by Tressler, Van Arsdel, and Copley, fourth edition was recently published.

The time temperature story of frozen food will soon be available in a book to be published by the AVI Publishing Company, Incorporated, Westport, Connecticut.

EUGENE C. VIETS, *Chairman,* Chief—Food Sanitation, Bureau of Milk, Food, and Drugs, Missouri Division of Health, Jefferson City, Missouri 65101.

LEONARD FENN, National Association of Frozen Food Packers 919 18th Street, N.W., Washington, D. C. 20006.

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EATON E. SMITH, Food Division, Department of Consumer Protection, State Office Building, Hartford, Connecticut 06115.

E. R. WOLFORD, Fruit and Vegetable Products Lab., Western Washington Research and Extension Center, U. S. Department of Agriculture, Puyallup, Washington 98371.

PROBLEMS OF THE ENVIRONMENT[1]

CHARLES C. JOHNSON, JR.

Consumer Protection and Environmental Health Service
Public Health Service
U. S. Department of Health, Education, and Welfare
Washington, D.C. 20204

Editor's Note: Another reorganization of the Food and Drug Administration has occurred since this paper was prepared. Even though these organizational details are no longer accurate, the paper is being published because it offers views on a wide range of topics dealing with food protection and environmental health.

As I flew over Colorado this morning and down into Denver, I was impressed by the glorious mountains, lakes and valleys of this lovely State. When we see sights such as these, we can hardly believe the urban and environmental problems we face throughout the country. But, even here in Denver, with its invigorating and rarified atmosphere. there is a growing air pollution problem. It is about such problems I would like to talk today.

The organization I have the privilege of heading, the Consumer Protection and Environmental Health Service, is charged with helping the Nation maintain an environment conducive to human health and well-being in a time of profound and accelerating change. Our task will not be an easy one, for as you know, the problems we face with respect to our deteriorating environment are critical, and at times seem insurmountable. And the problems are urgent, for the decisions we make in our time will determine the kind of world in which our children, and our children's children will live.

For you of the National Conference on Interstate Milk Shippers, it is unnecessary to state that all concern with the environment is essentially a concern for man—for his total health, happiness, and well-being. You, as State and local health officers, university and industry leaders, State and local directors of agriculture, are concerned every day with the health and well-being of man. Yet it seems to be worth stating and restating, whenever we are faced with decisions affecting the environment.

The environmental problems that plague us today are largely the result of our narrow pursuit of limited objectives—economic efficiency, fast transportation, agricultural abundance, for example—and our tendency to endow these activities with a life and purpose of their own, separate from or even superior to the needs of the human beings they were designed to serve.

The time has come when we must recognize that the various systems and subsystems which we devise to maintain ourselves on the planet—systems of economics, transportation, education, agriculture, and so on—that all these should contribute to the *total* well-being of man, the citizen and consumer.

CONSUMER PROTECTION AND ENVIRONMENTAL HEALTH SERVICE

The Consumer Protection and Environmental Health Service (CPEHS), was established last summer in the reorganization of the Public Health Service to provide a new impetus to our National effort to save the environment, and to provide a focus on man as part of that environment.

It includes the Food and Drug Administration, headed by Dr. Herbert L. Ley, Jr.; the National Air Pollution Control Admisinstration, headed by Dr. John T. Middleton, and the Environmental Control Administration headed by Assistant Surgeon General Chris A. Hansen. For the first time in the Department of Health, Education, and Welfare, we have brought all these organizations, dealing with protecting human beings from environmental hazards, together in a situation where they can be mutually supportive. We are finding that we are now able to take a more coordinated approach to environmental problems, and we are moving ahead as rapidly as possible to create a program which will have a real and lasting impact on these problems.

When any reorganization is carried out within the Government, there is always interest, and even concern, among those who are obliged to deal with the agency or agencies involved. Certainly, the creation of CPEHS has been no exception. What does it mean? How will it affect me? These are natural questions to ask—and they have been asked, I assure you.

Food protection

Some of you have made known to me your own interest in this subject, particularly with regard to the consolidation within the Food and Drug Administration (FDA) of food protection programs which have been located elsewhere in the Public Health Service. One of these is, of course, the milk program

[1]Presented at the National Conference on Interstate Milk Shippers, Denver, Colorado, May 26, 1969.

with which this Conference is so intimately involved.

Let me say first, that I am much more interested in program functions than I am in the layout of organizational charts. Food protection programs are being brought together within FDA, not to "neaten up" these management charts, but to provide unified planning and support for these functions. Broadening the base of scientific support for these programs is particularly important in the light of the complex technology which is part of today's environment.

Milk program

Having said that, let me add that the milk program is not going to change in purpose or direction. Some members of the dairy industry have expressed concern about being brought within the jurisdiction of a regulatory agency. I can only tell them that they have been within the jurisdiction of the FDA—and for many years.

The FDA has the same statutory responsibility to insure the wholesomeness of milk in interstate commerce as it does for other food products. I suspect that milk producers have been less aware of this than some other elements of the food industry simply because State, local, and voluntary programs have been so effective in giving the consumer high-quality milk.

The National Conference on Interstate Milk Shipments has contributed greatly to this achievement since its first meeting in St. Louis 19 years ago. This is an outstanding example of what can be attained through Federal, State, and industry cooperation.

Over these two decades, you have improved the sanitary quality of milk shipped in interstate commerce. Greater uniformity has been achieved in applying sanitary standards. Laboratory methodology has been advanced. There has been progress in eliminating wasteful, multiple inspections. All of the participants in this Conference can be proud of the positive results produced by this cooperative effort. And I use the word "effort" deliberately because I know, as you do, that it has taken far more than good intentions and pretty phrases to solve some of these difficult problems.

There are some problems, of course, that still await solutions. The assignments you have given your Task Forces reflect your awareness of the job still to be done. We in CPEHS are ready to work with you in every way that we can to ensure a sound milk supply. And we will continue to encourage broader participation in this voluntary program.

The Food and Drug Administration, as those of you who have worked with the Agency already know, is no stranger to cooperative undertakings with State agencies and private industry. Some of you may have participated in the voluntary educational program initiated by FDA two years ago to combat the problem of salmonella contamination in instant non-fat dry milk. The Agency also has expanded its voluntary compliance program among the many other industry groups whose products come within the scope of the Food, Drug, and Cosmetic Act and other consumer-protection laws. The pilot self-certification agreements in effect with two major food manufacturers also reflect our intention to utilize cooperative, voluntary approaches wherever possible. The State of Minnesota, incidentally, is a partner in one of these self-certification programs.

CPEHS and FDA are concerned with many environmental factors, and some of these also must be of special concern to you. The use of pesticides, for example, can and has affected our milk supply in certain places from time to time. The use of antibiotics in food animals also can present a health problem when residues of these drugs turn up in milk or in meat or eggs. The lesson, it seems to me, is that rigid sanitary practices are not enough in themselves to assure high-quality milk.

OTHER ENVIRONMENTAL PROBLEMS

If I may, I would like to tell you about some of the other environmental problems which face our Nation today. Some of them may appear, at first glance, to have little to do with the principal concerns of this Conference. But I think we are all coming to realize more and more clearly that no aspect of our environmental health effort can, in truth, be separated from the others. In fact, one weakness that has characterized our past efforts to deal with these complex problems has been a degree of fragmentation that was clearly undesirable—which grew, quite naturally, out of our desire to break the problem down into some sort of "manageable pieces". But we have found that man does not live in "manageable pieces" of the environment. Likewise, there are few "single causes" of disease. In assessing the effect of the environment on man's health, we have come to see that it is the combined, the multiple, the synergistic impacts that reach him from every part of his surroundings that make the difference between sickness and health.

We may tolerate a certain amount of chemical, or bacteriological, or radiological contamination of milk or other food products without serious health damage. But what if similar or interacting agents are reaching us through the water we drink, the air we breathe, or the therapeutic drugs prescribed by our physician? This is an entirely different story. We find that we cannot fully understand any one of these single environmental problems fully except within the con-

text of the whole.

This is why the Consumer Protection and Environmental Health Service was formed, and this is why we are regrouping some of our programs—to provide a deeper insight into these complex and overlapping relationships between man and his *total* environment. With this broader view, we feel we are at last in a position to cope with the many environmental pressures of today's world.

Food free from contamination

You are well aware, for instance, that maintaining uncontaminated food is a continuing—and indeed a growing problem. It is estimated that over two million Americans are stricken with illness each year from microbiological contamination of food—chiefly salmonellosis.

I am told that many States have food acts patterned after the original Pure Food Act of 1906, without any of the more modern provisions requiring the preclearance for safety of food additives, pesticide chemicals, and color additives. Surely this is a matter of concern for all of us.

The value, and the hazards, connected with pesticide use are, I am sure, thoroughly appreciated by this organization. But Federal regulatory authority in this area covers, as you know, only interstate shipments and we are faced with the fact that much of the food produced on farms never crosses State lines. Effective State surveillance is a practical necessity, and yet the truth is that most States are not doing enough to protect their consumers against ingesting toxic pesticide residues contained in food or milk.

The use of DDT and other pesticides has augmented spectacularly the growth of food crops since World War II and has, in addition, played a major role in public health efforts to control disease-bearing insects. But, the side effects of the use of pesticides have received scientific attention only in recent years. As you know, some pesticide chemicals, among them DDT, tend to persist in the environment for many years.

Only a month ago, Secretary of Health, Education and Welfare Finch announced the appointment of a Secretary's Commission on Pesticides and Their Relationship to Environmental Health to explore the field of environmental pollution and its consequent risks to the health of our citizens. The Commission is to report back with specific suggestions for action in six months.

At the moment, the Food and Drug Administration's surveillance of pesticides includes collecting data on pesticide residues in the average diet, including, of course, milk. An adequate State pesticide program requires laboratories, crop analysis and inspection, control or permit systems to deal with major spraying and dusting operations, and an informational and educational program to increase voluntary compliance. There is no question that there is much to be done in all parts of the Nation, before we will have adequate control over this problem.

Solid waste disposal

Let's move to another problem, solid waste disposal, which is closely related to the dairy and food industries. The wastes from feed lots and packing houses pose a difficult problem, and when they are discharged into waterways, which are also employed as sources of drinking water, the problem has been shifted, not solved. Nationally, the solid waste disposal problem consists of 1.5 billion tons of animal wastes, 550 million tons of agricultural waste and crop residues, over 1.1 billion tons of mineral wastes, 110 million tons of industrial wastes, and 250 million tons of household commercial and municipal wastes—a total of 3.5 billion tons of wastes per year which must be disposed of in a manner that is not injurious to health. This environmental problem may well prove to be the most difficult and serious of all.

Each year, we discard more than 190 million tons of garbage, trash, cans, bottles, and other refuse. Nonreturnable bottles, aluminum cans, and new types of disposable paper products complicate the problem.

Nationwide collection and disposal of garbage and other solid waste—and this, of course, does not include agricultural and industrial wastes—cost an estimated $3.5 billion in 1967, and yet the methods used are little improved over those of 25 years ago. A colleague of mine in New York liked to point out that the only real improvement we had made in waste disposal in the last 50 years was putting an engine instead of the horse in front of the garbage truck.

Yesterday's city dump is now in today's suburb, so that most cities in the country are destroying out-of-the-way areas of natural beauty, and polluting land, air, and water, in an effort to get rid of mountains of refuse. Our Federal program is funding research and demonstration projects designed to develop alternative methods of dealing with the problem, including composting and recycling.

Under properly controlled conditions, use of solid waste as landfill material can restore certain areas to useful purposes. The problem of sanitary landfill as a disposal method, of curse, is that many cities no longer have accessible areas where this is appropriate.

Quality of drinking water

The water pollution problem which I mentioned briefly in connection with poor solid waste disposal practices, brings us to another environmental concern

which is growing in seriousness with each year that passes. I refer to the quality of drinking water. Most of the community water supply systems in this country were initially constructed over 30 years ago and were designed to serve population densities that were 20 to 40% less than today's. Despite efforts to modernize and increase capacities, many systems have fallen behind and are failing, in many respects, to meet today's needs.

These systems were designed to treat a high quality of raw water for removal of bacteria, with little or no capability for removing toxic chemical or virus contaminants. Today, both ground and surface water supplies have deteriorated.

All over the country, I believe we are rapidly approaching a crisis stage with regard to drinking water. The time has come when communities are going to have to allocate substantial resources to modernizing their treatment plants and to improve their distribution systems or continue to court serious health hazards from contamination.

Air pollution

We are all aware of the growing threat to air pollution. At the present time, toxic matter is being released into the air over the United States at a rate of more than 142 million tons a year, or three-quarters of a ton for every American. And what does this do to people? In the first place, there is no doubt that polluted air is a major contributor to emphysema, chronic bronchitis, and lung cancer—some of the major "diseases of civilization," which are on the increase.

We also pay a tremendous economic price for air pollution. The annual cost to U.S. citizens has to be computed in billions of dollars. In figures that are more easily understandable, it is estimated to cost each of the 200 million American citizens $65 per year; for those who live in highly polluted areas, the cost per person, including higher medical bills, household maintenance, and other expenses, can be more than $200 per year. The cost throughout the United States in damage to agricultural crops alone is more than $500 million every year.

Other problems

Even though I have discussed at some length the many serious environmental problems with which we must cope in the coming years, the list is far from complete. I have not mentioned the growing problems of noise, radiation, and many others deriving from the increasingly crowded and stressful conditions of life which are characteristic of our complex urban society.

Our world, after all, is a "closed life system", with a limited supply of air, water, and other resources. All elements in this system are related and interdependent. Yet, for most of man's time on earth, he has used these resources as though he could heedlessly exploit, contaminate, and alter the world about him without endangering the stability and harmony of the whole system.

We are coming to realize that this is not so. Human population has soared. Science and technology have given us a new and awesome power to alter—or even destroy—the environment on which our lives, and the lives of generations yet unborn, depend. Distress in one part of the ecological system, like a pebble dropped into a pond, makes ripples that are felt throughout the whole system.

Here in our Nation—the most "advanced" in the world—streams and lakes are dying before their time. Birds, fish, and other wildlife are threatened with extinction. We are damaging our rural as well as our urban environment. Human health is already affected by the barrage of microbiological, chemical, physiological, and psychological insults which we have injected into our environment. And, everywhere, there is anxious scientific speculation about what kind of a world we are building for the future.

Our direct efforts to maintain the purity of our milk supply, no matter how vigorous and dedicated, will surely fail if we do not succeed in reversing the present environmental trend. A rural environment, essentially free from man-made contamination, has always been the fundamental requirement for a safe and wholesome food and milk supply. Unless we maintain this, no amount of man-made manipulation of agricultural products can assure their purity.

SOURCES OF *SALMONELLA* CONTAMINATION OF MEAT FOLLOWING APPROVED LIVESTOCK SLAUGHTERING PROCEDURES[1]

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Abstract

Tissues from animals slaughtered at the university abattoir were sampled to determine the spread of bacterial contamination during slaughter. Salmonellae and coliform organisms were cultured from swabs taken of the equipment before and during slaughter and from various viscera sites during slaughter.

Since salmonellae isolations were expected to be low, coliforms were used as an index of contamination. The study indicated that equipment was satisfactorily sanitized before slaughter and minimal contamination occurred during slaughter except for the viscera pans. Contamination of the viscera and carcass of cattle, swine, and sheep was very high. It was found that the bung-dropping operation is less of a contaminating factor than is generally thought and that washing the carcass after evisceration is probably responsible for the greatest spread of contamination.

Salmonellae were isolated from only two swine. The patter of contamination spread was similar to that of coliforms.

Slaughtering and dressing meat animals afford many opportunities for microbiological contamination of the meat. Ayres in his review of the microbiological implication in handling, slaughtering, and dressing meat animals pointed out that nearly all stages of the slaughtering process provide a source of microbiological contamination *(1)*. Contamination of the carcass with microorganisms begins at the time of slaughter and continues during processing and handling.

Microbiological contamination becomes very serious in some operations including sticking, hide removal from cattle and sheep, scalding and dehairing swine, head skinning and removal, brisket opening, bung dropping, and evisceration. Washing contributes to the problem by spreading microorganisms from contaminated to uncontaminated areas on the dressed carcass. Corlett showed the microflora on the carcass in the cooler to include *Pseudomonadaceae*, *Achromobacteraceae*, *Enterobacteriaceae*, *Micrococcaceae*, and *Bacillaceae (2)*.

A number of studies have been made on salmonellae in meat animals. These studies were made on the farm, at the market, at the slaughtering plant, and in the various stages of meat processing. Salmonellae have been identified in wildlife and in meat producing animals on the farm *(7)*. Galton et al. *(4)* showed a seven-fold difference in the proportion of infected hogs on the farm and in the abattoir. A high percentage (45%) of salmonellae isolated from the bovine rumen at slaughter probably resulted from a massive exposure at the abattoir *(5)*.

Galton et al. *(4)* and Shotts et al. *(8)* showed that salmonellae were a post-slaughter contaminant of animal carcasses. Their work traced salmonellae from the farm, through the auction market, and to the abattoir. The animals were checked for salmonellae at the abbattoir before slaughter and after scalding, dehairing, singeing, washing, and evisceration. Plant equipment also was checked to determine its role in spreading salmonellae.

Other studies have shown the presence of salmonellae in prepared food products *(3, 6, 10)*. Salmonellae on the carcasses in the cooler could be expected to spread as the meat is further processed. Perhaps the biggest danger is that salmonellae-contaminated meat would contaminate the entire batch of product being prepared. "A little leaven leaveneth the whole lump." Thus, the manufacture of hamburger, fresh pork sausage, dried sausage, and other products could produce salmonellae-contaminated products not receiving sufficient heat treatment to kill the microorganisms.

Few studies on microbiological contamination of meat animals have included a step-by-step microbiological examination of the slaughtering and dressing procedures. This research was designed to study the slaughtering and dressing operations in an early attempt to determine which operations result in contamination of the dressed carcass. Salmonellae were the primary microorganisms of interest. But in those animals whose intestinal tract did not contain salmonellae, coliform organisms were used as an additional guide for measuring the possibility of fecal contamination *(9)*.

Experimental Methods

Animals slaughtered at the Meats Laboratory, Department of Animal Science, Texas A&M University were selected for sampling. Samples consisted of two swabs taken simultaneously from each site from 12 cattle, 14 swine, 6 sheep, and selected equipment. Before and during slaughter, swabs

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TABLE 1. COLIFORM ISOLATIONS FROM 16 EQUIPMENT SAMPLINGS BOTH BEFORE AND DURING SLAUGHTER

Equipment swabbed	Before slaughter	During slaughter
Head wash cabinet[a, b]	—	—
Head inspection rack[b]	—	1
Sterilizer	—	1
Carcass splitting saw[b]	—	4
Viscera pan	—	12
Worker's knife	—	3
Worker's steel	—	2

[a]Not sampled when swine were slaughtered
[b]Not sampled the three times sheep were slaughtered

TABLE 2. COLIFORM ISOLATIONS FROM 12 CATTLE, 14 SWINE, AND 6 SHEEP AT THE TIME OF SLAUGHTER. TWO ANIMALS WERE SWABBED DURING EACH SLAUGHTER PERIOD.

Area swabbed	Cattle	Swine	Sheep
Rectum	12	14	6
Brisket	4	9	3
Midline	4	10	3
Thoracic viscera	5	7	4
Stomach and intestines	6	10	4
Liver	7	6	3
Cheek meat	5	2	2
Pelvic fat	3	7	3
Diaphragm	3	7	1
Lumbar vertebrae	4	8	1
Cervical vertebrae	5	11	—
Neck meat	9	9	4

TABLE 3. SALMONELLAE ISOLATIONS FROM 16 EQUIPMENT SAMPLINGS DURING SLAUGHTER AND FROM 14 SWINE CARCASSES. EQUIPMENT BEFORE SLAUGHTER AND 12 CATTLE AND 6 SHEEP CARCASSES WERE NEGATIVE FOR SALMONELLAE.

Location	Number of salmonellae isolations
Viscera pan	1
Rectum	2
Brisket	1
Midline	2
Thoracic viscera	1

were taken of the head wash cabinet, head inspection rack, sterilizer, saw, viscera pans, and the knife and steel of a worker. Rectal swabs were taken from each animal before slaughter and the brisket, midline, thoracic viscera, stomach and intestines, liver, cheek meat, pelvic fat, diaphragm, lumbar vertebrae, cervical vertebrae, and neck meat were swabbed during the slaughtering process.

The brisket, midline, thoracic viscera, stomach and intestines, and liver were sampled immediately after evisceration. The cheek meat was sampled after the head had been washed, trimmed, and inspected. The pelvic fat, diaphragm, lumbar vertebrae, cervical vertebrae, and neck meat were sampled after the carcass was sawed and washed. The sawed surfaces of the thoracic and cervical vertebrae were swabbed.

Swabs taken at slaughter were used to initiate cultures in lactose broth; violet red bile agar and tetrathionate broth. One swab was washed in 1 ml of sterile distilled water then placed into lactose broth as a pre-enrichment medium. The distilled water was used to prepare a pour plate of violet red bile agar. After 24 hr of incubation in lactose broth; swabs were placed in tetrathionate broth.

The second swab was used to inoculate tetrathionate broth directly. All of the tetrathionate broth was incubated 18-24 hr and from this salmonellae-shigella agar, brilliant green agar, and eosin-methylene blue agar were inoculated. To screen for salmonellae, lactose-negative isolates were transferred to triple-sugar iron agar and all those which gave typical salmonellae reactions were further identified by biochemical methods. To identify salmonellae, dextrose, maltose, lactose, sucrose, and mannitol, urea agar, indole, methyl red, Voges-Proskauer, and citrate reactions were determined. Lysine iron agar also was used in identification.

Salmonellae polyvalent antisera were used on those isolates found to have biochemical reactions consistent with salmonellae. Coliform contamination was determined using violet red bile agar and the eosin-methylene blue agar.

RESULTS AND DISCUSSIONS

Since this abattoir slaughters only 8-14 head of livestock per day at a speed of 2-4 per hour, most of the equipment, such as the viscera pans and the head rack, is used only once each day. Thus, there was no opportunity to study the effectiveness of equipment cleansing and sanitizing between usages on a given day. However, the results of this study indicate that day-to-day clean-up was satisfactory since samples taken before slaughter, with one exception, were negative.

The results of equipment sanitation, as shown in Table 1, indicate that the viscera pans were most often contaminated (75%) during the slaughtering operations. Further studies of larger operations would be desirable to determine if the pans are satisfactorily sanitized after each use. The knife and/or steel of a worker were positive in 31% of the samples taken during slaughter. In two samples the knife was negative but the steel was positive, indicating improper sanitizing of the steel after use.

Fifty-three per cent of the samples from the sawed portion of the carcass (lumbar and cervical vertebrae and neck) were positive but only 30% of the splitting saw samples were positive. This would indicate that washing the carcass presents a greater source of spreading contamination than does sawing. The head inspection rack was positive in one instance while the head wash cabinet was negative in all instances. The one positive sample from the sterilizer may have resulted from an improperly heated sterilizer or from contamination of the swab during handling since coliforms do not normally survive a temperature of 180 F.

The carcasses and viscera were contaminated in 30 of 32 cases. In many instances most of the surfaces sampled were contaminated. Amounts of con-

tamination included brisket and midline 52%, viscera 54%, pelvic fat 41%, and vertebrae and neck 53%. These figures should call for a realistic re-evaluation of the so-called "sanitary dressing procedures" used in meat packing plants today. This study was conducted in a state-inspected plant under conditions equal to most state and federally inspected slaughtering operations and superior to some. Though some contamination of carcasses is to be expected, these percentages of contamination are far too high for microorganisms of gastro-intestinal origin. As indicated previously, washing the carcass was incriminated as the major mechanism of spreading contamination, since the splitting saw was positive in fewer instances (30%) than were the sawed surfaces (vertebrae and neck) after washing the carcass (53%).

Many believe that bung-dropping is one of the primary sources of contamination during evisceration. However, this study indicated that the pelvic fat in the bung area was contaminated in fewer instances (41%) than other parts of the carcass following evisceration and washing. Possibly the care exercised in detaching and tying the bung reduces contamination of the pelvic areas. However, when the detached, tied bung is dropped into the abdominal cavity and then removed with the viscera, it contaminates much of the viscera and carcass.

The animals sampled were raised under better conditions than are generally found, so the level of *Salmonella* contamination was expected to be low. Therefore, coliforms were used in this study as an indicator of contamination. *Salmonella* was isolated from two swine slaughtered on the same day. The viscera pan was contaminated during slaughter. *Salmonella* was isolated from the rectum, brisket, and midline of one carcass and from the rectum, midline, and thoracic viscera of the other. Though *Salmonella* was isolated from only two animals, the pattern of its spread and the pattern of coliform spread on all animals sampled indicated that the potential for contamination of carcasses with enteric pathogens is great.

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INHIBITION OF *PSEUDOMONAS* SPECIES BY HYDROGEN PEROXIDE PRODUCING LACTOBACILLI[1]

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Abstract

Eighty-one microbial species isolated from seafoods and other marine sources were examined to determine the extent of interactions among these species. Spot-plates, cross-plates, and concurrent growth experiments at 7, 15, 20, and 30 C indicated that *Lactobacillus* species were capable of inhibiting other microorganisms. *Lactobacillus* species isolated from oysters and identified as *Lactobacillus plantarum* produced a substance inhibitory to *Pseudomonas, Bacillus,* and *Proteus* species, the most sensitive being *Pseudomonas.* The inhibitory substance accumulated in *Lactobacillus* culture media, reaching maximum concentrations in 4 to 5 days at 30 C. The active substance was dialyzable, heat labile, and inactivated by catalase. Inhibitor production paralleled H_2O_2 formation in *Lactobacillus* cultures, further indicating that the observed inhibition resulted from H_2O_2 produced by lactobacilli. These findings may explain the abnormal shifts in microbial flora observed in foods where *Lactobacillus* species have overgrown the natural flora.

Microorganisms from food products have been studied to determine the extent and importance of microbial interactions taking place in these products. Studies on the effect of microorganisms on the growth of staphylococci have shown that various microorganisms belonging to *Pseudomonas, Achromobacter, Proteus, Escherichia, Aerobacter, Klebsiella, Flavobacterium, Alcaligenes, Streptococcus, Lactobacillus,* and *Leuconostoc* species can inhibit *Staphylococcus aureus (6, 8, 24).* Others have shown that *Pseudomonas* species isolated from dairy products repressed *Achromobacter* growth *(33),* and that streptococci inhibited *Pseudomonas fragi (22).*

Most investigations on microbial interactions, however, focused on the lactic acid bacteria. Some lactic streptococci in this group produce the well characterized antibiotics nisin and diplococcin *(2, 15, 21).* Antibiotics produced by lactobacilli also have been reported. They include acidophilin and lactocidin from *Lactobacillus acidophilus (32, 34),* lactolin from *Lactobacillus plantarum (9, 10),* and lactobacillin, which was later identified as hydrogen peroxide, from *Lactobacillus lactis (35, 36).* Other workers, however, have claimed that lactic acid *(8, 31),* hydrogen peroxide *(5, 36),* or unidentified heat labile substances *(23)* were responsible for the inhibitory effects associated with lactobacilli.

Microbial population shifts in food products during storage eventually result in one or two dominant microbial genera *(4, 12).* Studies on changes in microbial flora during spoilage of Pacific oysters *(Crassostrea gigas)* indicated that lactobacilli increased from 55 to 80% of the flora while *Pseudomonas* species decreased from 35 to 20% after two days storage at 7 C *(27).* Since seafood spoilage is normally associated with *Pseudomonas* species *(13),* possible suppression of *Pseudomonas* by *Lactobacillus* was suspected. In this study, *Lactobacillus* species from Pacific oysters and *Pseudomonas* species from marine sources were investigated to determine whether such interactions could have been responsible for the flora shifts, and to determine the nature of such interactions.

Materials and Methods

Microorganisms

Lactobacillus species L-1, L-2, and L-3 were isolated from Pacific oysters, and identified as *L. plantarum* according to the classification of Sharpe, Fryer, and Smith *(25).* The lactobacilli produced acid but no gas from raffinose, lactose, sucrose, and glucose, grew at 15 C, and did not produce NH_3 from arginine. Stock cultures were maintained in Elliker broth (Difco) and transferred weekly.

Pseudomonas strains PI-950, PI-1005, PII-970, and PIII-985, classified according to the scheme of Shewan *(26),* were isolated from ocean perch *(Sebastodes alutus)* by Lee et al. *(12). Pseudomonas* strains PI-153, PI-406, PII-133, PIII-320, PIII-225, PIII-322, and PIV-130 were obtained from the National Collection of Marine Bacteria, Torry Research Station, Aberdeen, Scotland. All other microorganisms were from stock culture collections of this laboratory. Cultures were maintained on tryptone-peptone-yeast extract-NaCl agar (TPN) *(14)* and transferred monthly. For interaction studies, cultures were grown in 1% peptone broth (Difco), transferred twice, and the final cell concentrations adjusted to an absorbancy of 0.1 at 530 nm with sterile 1% peptone prior to inoculation. Absorbancy readings were made on a Bausch and Lomb Spectronic 20 spectrophotometer.

Interaction assays

Preliminary assays were conducted in 1% peptone broth. Duplicate tubes were inoculated with a 1:1 ratio of *Lactobacillus* species to the test microorganism, and identical tubes inoculated with each organism alone served as controls. Growth after 24 hr at 30 C was determined by measuring absorbancy at 530 nm, and the difference in absorbancy between test and controls indicated possible interaction. A total of 36 pairs of microbial species were examined in this manner. They were *Lactobacillus* species L-1, L-2, L-3, and *Lactobacillus casei* against *Pseudomonas* species PI-153, PI-

[1]Technical Paper No. 2669, Oregon Agricultural Experiment Station.

950, PI-1005, PII-970, PII-133, and PIII-985, *S. aureus*, *Escherichia coli* B, and *Salmonella typhimurium*. Initial screenings were made at 7, 15, 20, and 30 C. Since incubation at 30 C more readily demonstrated interaction between *Lactobacillus* species and test microorganisms, unless otherwise noted, 30 C was selected for interaction studies with *Lactobacillus*.

Spot-plate and cross-plate tests also were conducted with some pairs of microbial species on TPN and Elliker agar. All plates were incubated aerobically at 30 C and observed for four days.

Sterile cell-free spent media were obtained from lactobacilli which produced inhibitory substances. The lactobacilli were grown in spinner flasks (Bellco) that consist of two chambers separated by a PH semipermeable membrane (Millipore Filter Corp.). Each chamber contains magnetic stirring bars to enhance diffusion and mixing rate studies with methylene blue indicated that 100% exchange across the membrane occurred in 12 hr. The apparatus was incubated in a water bath over two magnetic stirrers, and a Lauda K-2/R (Brinkmann) constant temperature circulator was used to regulate water bath temperature. To obtain cell-free filtrates, one flask was inoculated with 5.0 ml of a 24 hr culture of lactobacilli, and the metabolic products liberated in the medium were collected from the other flask. This technique eliminated the need to centrifuge to obtain cell-free filtrates and provide a simple means of following the build up of metabolic products in the growth medium.

Antimicrobial activity of lactobacilli metabolites was determined by inoculating duplicate 10 ml samples of cell-free spent medium with 0.3 ml of optically adjusted bacterial suspensions. Growth was followed by measuring absorbancy at 530 nm and compared to growth in 1% peptone. Possible depletion of nutrients in the spent medium was compensated for by adding sterile concentrated peptone.

Viable counts were made on TPN and Elliker agar by spread plating. Differential counts of *Pseudomonas* and *Lactobacillus* were conducted by plating the mixed culture on TPN and Elliker agar. The former was incubated at 20 C for 24 hr and the latter at 30 C for 24 hr in a candle jar. When necessary, dilutions were made in sterile 1% peptone.

Identification of inhibitory substances

The effect of heat on the inhibitory substances in lactobacilli culture filtrates was determined at various pH values as follows. Filtrates were adjusted to predetermined pH values with 1 N HCl or 1 N NaOH, heated at 100 C for 30 sec, cooled rapidly to 30 C, and readjusted to pH 6.3 with 1 N HCl or 1 N NaOH. Values of pH were determined with a Zeromatic II (Beckman) pH meter. For autoclaving, samples were heated at 121 C under 15 lb steam pressure for 15 min, and then cooled rapidly to 30 C. Perchloric acid (PCA) treatments were performed according to the method of Cogan, Gilliland, and Speck *(3)*. Cell-free filtrates and reconstituted lyophilized filtrates were dialyzed against distilled water at 0 C for 36 hr. Nutrient levels of the dialyzed solutions were readjusted by adding sterile concentrated peptone. The effect of catalase or inhibitory activity was determined by adding 3,000 I. U. bovine liver catalase (H_2O_2: H_2O_2 oxidoreductase, EC 1.11.1.6) (Calbiochem) to 10 ml of culture filtrate. The inhibitory activity of *Lactobacillus* L-3 culture filtrate subjected to the above treatments was measured against *Pseudomonas* PIII-322. Untreated filtrate and 1% peptone served as inhibitor and growth controls.

Hydrogen peroxide assays

Hydrogen peroxide concentrations in culture media were determined by a modification of the A.O.C.S. method *(1)*. A solution containing 5 ml cell-free spent medium, 0.5 ml saturated KI, and 0.5 ml of 0.001 M ammonium molybdate in 1 N sulfuric acid was allowed to stand with occasional shaking for 1 min at room temperature. The solution was then titrated with 0.001 N sodium thiosulfate until the yellow color almost disappeared. Five-tenth ml of 1% soluble starch (B & A reagent) in distilled water was added and the titration continued until the blue color just disappeared. Titrations of less than 0.5 ml were repeated using 0.0001 N sodium thiosulfate. Titrations were made in duplicate and results were recorded as ml of 0.001 N sodium thiosulfate. Known concentrations of H_2O_2 in 1% peptone were assayed along with the spent media, and μg H_2O_2/ml medium determined from the H_2O_2 standards. This method proved accurate for H_2O_2 concentrations from 1 to 40 μg/ml.

RESULTS AND DISCUSSION

Screening for interaction

Concurrent growth experiments with *Lactobacillus* and *Pseudomonas* species at 7, 15, 20, and 30 C indicated that growth in mixed cultures was greater than in controls. The 1% peptone provided sub-optimal growth media for the test microorganisms, but amplified the degree of interaction. Figure 1 shows the concurrent growth of *Lactobacillus* L-3 and *Pseudomonas* PIII-985 at 30 C. Similar results were obtained from *Lactobacillus* L-1, L-2, L-3, and *L. casei* in combination with other *Pseudomonas* species, but neither stimulation nor inhibition was observed for lactobacilli or pseudomonads in combination with *S. aureus*, *E. coli* B, or *S. typhimurium*. Via-

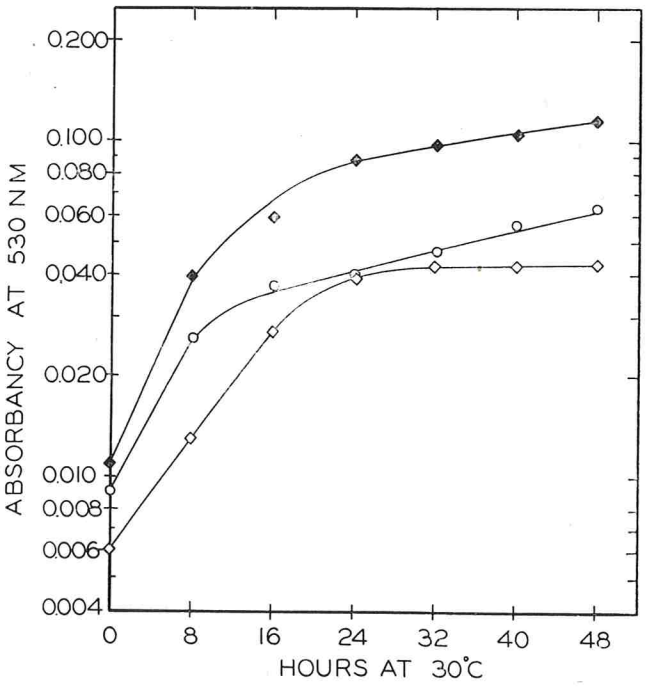

Figure 1a. Concurrent growth of *Lactobacillus* L-3 and *Pseudomonas* PIII-985 in 1% peptone broth at 30 C (Absorbancy). Symbols: ◇, *Lactobacillus* L-3 control; O, *Pseudomonas* PIII-985 control; ◈, mixed culture.

ble counts (Fig. 1b), measured by differential plating, indicated that the increased growth in mixed cultures resulted from increased growth by *Lactobacillus* while the counts for *Pseudomonas* decreased steadily. Within 48 hr, *Pseudomonas* PIII-985 was no longer detectable and the remaining population consisted solely of *Lactobacillus* L-3.

Cross-plates and spot-plates employing *Lactobacillus* L-1, L-2, L-3, and *L. casei* against five *Pseudomonas* species, five *Flavobacterium* species, *E. coli* B, 25 *Achromobacter* species, and 33 unidentified microbial isolates from Pacific oysters and Pacific hake *(Merluccius productus)*, however, did not show zones of inhibition. Inability to demonstrate interaction on solid media resulted from poor growth of *Lactobacillus* species on TPN and Elliker agar, and overgrowth by test organisms.

TABLE 1. INHIBITORY ACTIVITY OF SPENT MEDIUM FROM A 96 HOUR CULTURE OF *Lactobacillus* L-3

Test microorganism[1]	Inhibitor-induced lag (hr)
Pseudomonas PI-406	2
Pseudomonas PII-320	7
Pseudomonas PIII-322	6
S. aureus	0
E. coli B	1
E. coli 24	1
Bacillus cereus	2
Bacillus megatherium	4
Micrococcus sp.	1
Aerobacter aerogenes	1
Proteus vulgaris	5
Vibrio sp.	1

[1]Inoculum standardized to an absorbancy of 0.1 at 530 nm.

TABLE 2. INHIBITORY ACTIVITY OF *Lactobacillus* L-3 CULTURE FILTRATE SUBJECTED TO VARIOUS TREATMENTS.

Treatments	pH	Residual activity[1] (%)
Heating 100 C, 30 sec.	2.0	60
Heating 100 C, 30 sec.	4.0	80
Heating 100 C, 30 sec.	6.0	60
Heating 100 C, 30 sec.	7.0	70
Heating 100 C, 30 sec.	8.0	60
Heating 100 C, 30 sec.	10.0	40
Heating 100 C, 30 sec.	12.0	40
Autoclaving	6.3	0
PCA treatment	6.3	0
Lyophilization	6.3	40
Dialysis	6.3	0
Catalase (300 U./ml)	6.3	0

[1]Activity measured against *Pseudomonas* PIII-322

Inhibitory substances produced by lactobacilli were readily demonstrated in culture filtrates, and the inhibitory effect was characterized by increased lag periods of test microorganisms. The length of inhibitor-induced lag, therefore, could be used to compare the relative sensitivities of microorganisms. Data in Table 1 show the inhibitory activity of spent medium against several indicator microorganisms. The inhibitor-induced lag represents the difference between lag in spent medium and in 1% peptone broth. *Pseudomonas* PII-320 and PIII-322 showed the greatest sensitivity to the inhibitory substance in this survey, whereas *S. aureus* appeared resistant. When the inoculum level was reduced, however, the filtrate became bacteriostatic to *S. aureus*, and proportionally increased the lag for other sensitive organisms. Thus, the inhibitory activity appeared dependent on the relative concentrations of cells and inhibitor.

Characterization of inhibitor

Several investigators have proposed that lactic acid or the reduced pH in the medium was responsible for the bacteriostatic activity of lactobacilli cultures *(8, 31)*. *Lactobacillus* L-3 lowered the pH of 1% peptone broth from 6.7 to 6.3 in four days. The growth of *Pseudomonas* PIII-985, however, was not affected in 1% peptone adjusted from pH 5.6 to 7.0 with lactic acid. Since *Lactobacillus* L-3 was unable to lower the pH of 1% peptone to the level inhibitory to *Pseudomonas* PIII-985 in four days, the role of lactic acid or lowered pH did not appear responsible for the observed inhibitory effect.

Data indicating the stability of the inhibitory substance in spent medium from a four day *Lactobacillus* L-3 culture, subjected to various treatments, are summarized in Table 2. The inhibitory substance was dialyzable and inactivated by heat. Furthermore, 3,000 I.U. of catalase in 10 ml of culture filtrate completely inactivated the inhibitor. The substance retained 40% activity after lyophilization, and the ability of H_2O_2 to remain active after lyophilization was confirmed by reconstituting a freeze dried solution of H_2O_2 and by the loss of activity when catalase was added to reconstituted lyophilized filtrate. Thus, the inhibitory compound was identified as H_2O_2,

Accumulation of H_2O_2 in culture medium during growth of *Lactobacillus* L-3 is shown in Fig. 2. Hydrogen peroxide values are averages of duplicate determinations made on cell-free samples withdrawn from the uninoculated chamber of a spinner flask. Hydrogen peroxide appears to be a metabolic product released during growth of *Lactobacillus* L-3, and maximum accumulation occurs after 4 to 5 days incubation at 30 C. Concentrations of H_2O_2 in the culture medium decreased slowly after five days to a level of 10 to 12 μg/ml at the tenth day. The two stage curve noted for H_2O_2 production appears to be characteristic of this microorganism and parallels

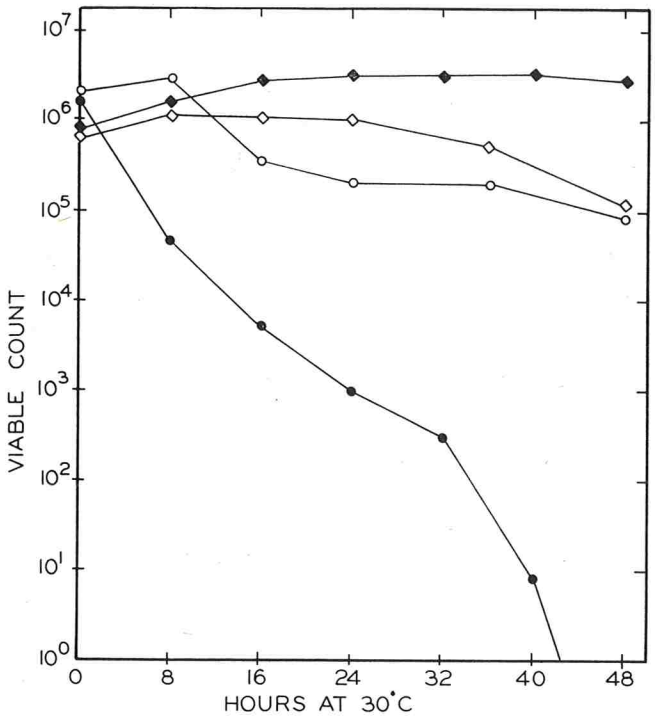

Figure 1b. Concurrent growth of *Lactobacillus* L-3 and Pseudomonas PIII-985 in 1% peptone broth at 30 C (Viable count). Symbols: ◇, L-3 control; ◆, L-3 in mixed culture; O, PIII-985 control; ●, PIII-985 in mixed culture.

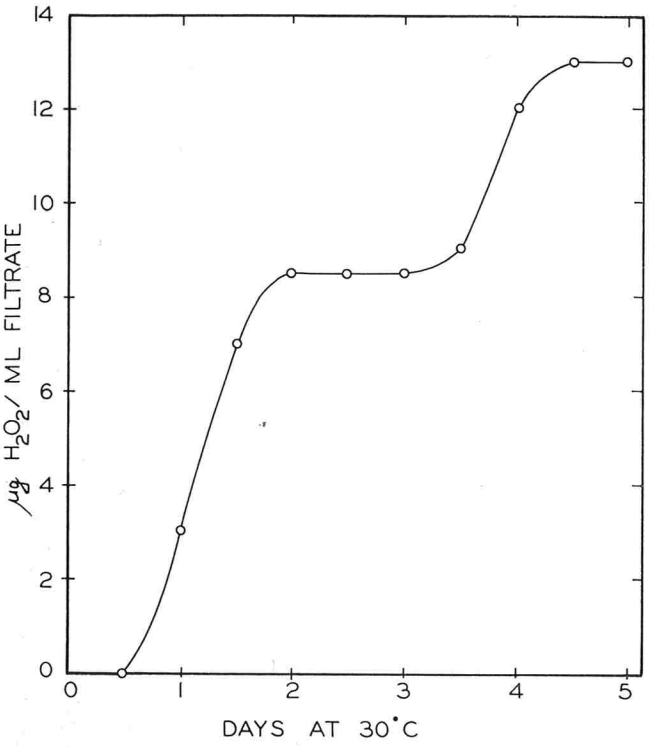

Figure 2. Accumulation of H_2O_2 in 1% peptone broth during growth of *Lactobacillus* L-3 at 30 C.

the production of inhibitory substance by *Lactobacillus* L-3 measured against *Pseudomonas* PIII-322 and PIII-985 (Fig. 3). All three assays showed a pla-

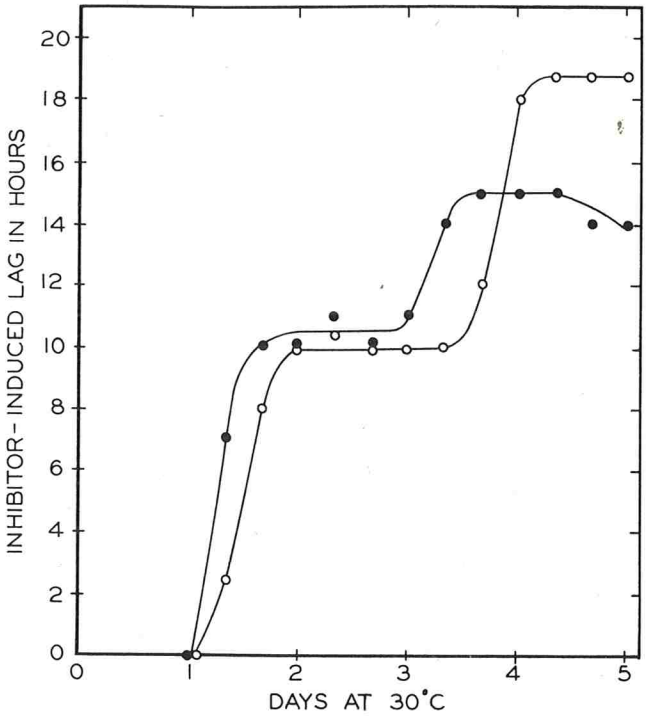

Figure 3. Inhibitor-induced lag in 1% peptone during growth of *Lactobacillus* L-3 at 30 C. Symbols: ●, *Pseudomonas* PIII-985; O, *Pseudomonas* PIII-322.

teau between 2 and 3 days growth and maximum accumulation at 4 to 5 days growth. The close parallel between H_2O_2 production and inhibitory substance production by *Lactobacillus* L-3 further suggests the identity of the inhibitory compound to be H_2O_2.

The effect of H_2O_2 on the length of the lag for *Pseudomonas* cultures is best illustrated in log-log plot shown in Fig. 4. Values for inhibitor-induced lag are differences between the lag in 1% peptone controls and in 1% peptone with added H_2O_2. Minimum bacteriostatic concentrations of H_2O_2 under the conditions of our assay were from 2 to 8 μg/ml, and this increased the lag periods for *Pseudomonas* from 1 to 7 hr. When *Pseudomonas* species were subjected to 25 to 40 μg H_2O_2/ml, the lag period became infinite. These findings indicated that H_2O_2 produced by lactobacilli would inhibit *Pseudomonas* species and might play a significant role in selecting microbial populations in foods.

Production of H_2O_2 by lactobacilli

Hydrogen peroxide was implicated as the inhibitory product of *L. lactis* isolated from Gruyére cheese by Wheater et al., although they were unable to demonstrate the presence of H_2O_2 in the culture medium *(35, 36)*. Dahiya and Speck, working with lactobacilli from yogurt starter cultures, identified H_2O_2 as the compound in *L. lactis* and *L. bulgaricus* culture filtrates that inhibited *S. aureus (5)*. Heat

labile antimicrobial agents from *L. acidophilus* and *L. plantarum* also have been reported, but were not identified *(9, 10, 23, 34)*. Since H_2O_2 remained after lyophilization, and is infinitely soluble in water *(11)*,

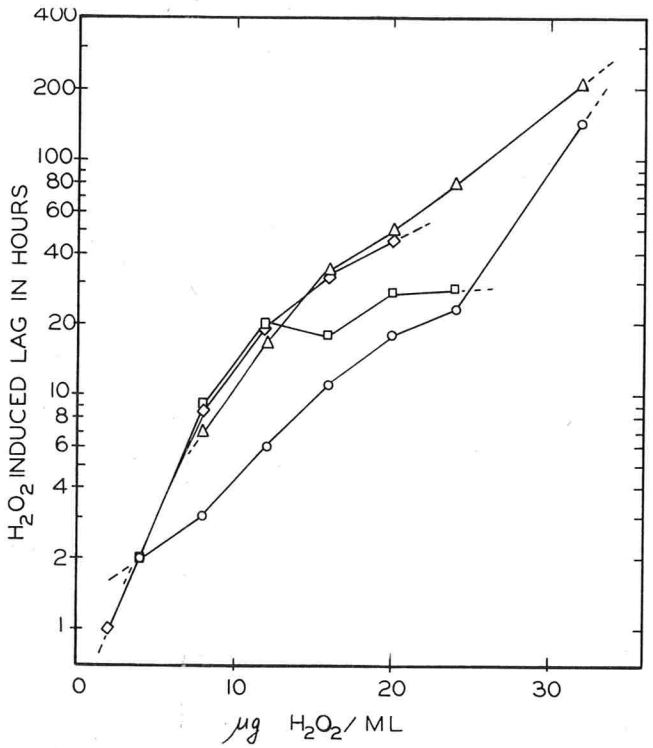

Figure 4. Inhibitory effect of hydrogen peroxide on *Pseudomonas* in 1% peptone broth at 30 C. Symbols: ○, *Pseudomonas* PI-406; ◇, *Pseudomonas* PII-320; □, *Pseudomonas* PIII-332; △, *Pseudomonas* PIII-985.

the low temperature extraction procedure used by Kodama to obtain crude extracts of the antibiotic lactolin from *L. plantarum* cultures *(10)*, could have also concentrated H_2O_2.

Dahiya and Speck have speculated that H_2O_2 produced by lactobacilli may be an important factor in the repression of certain undesirable bacteria in food *(5)*. In a similar manner, H_2O_2 production by lactobacilli might have caused repressed *Pseudomonas* growth in oysters *(27)*. Some strains of lactobacilli oxidize glycerol, which is found in high concentrations in oysters *(16)*, with high rates of oxygen consumption *(29)*. Although the pathways of these oxidations are not clearly understood, it is believed that flavin components, instead of heme components, are involved *(29, 30)*. Since H_2O_2 accumulation is suggestive of flavoprotein oxidase activity *(29, 37)*, it is possible that a similar system is present in oyster lactobacilli. Thus the oxidation of glycerol by lactobacilli would result in the formation and accumulation of H_2O_2.

Meyer and Wurtz have proposed that *L. plantarum* produces an unidentified substance that interferes with electron transport in *Pseudomonas fluorescens (17)*. The compound specifically inhibits or interacts with a reducing substance "RH_2", preventing it from taking part in the reduction of cytochrome "c" *(7, 17, 18)*. The inhibitory substance was not identified nor were assays for H_2O_2 conducted. Since *L. plantarum* cultures in our laboratory actively produced H_2O_2 under conditions similar to those employed by Meyer and Wurtz, The unidentified inhibitory substance could have been H_2O_2.

Significance of H_2O_2 in foods

The formation and accumulation of H_2O_2 by lactobacilli in food products may have either beneficial or deleterious effects. Kao and Frazier have suggested that the killing of staphylococci by lactobacilli may make viable counts an unreliable indication of endotoxin levels in food products *(8)*. Lactobacilli dominate the microbial flora of irradiated oysters *(27)* and vacuum packed fish *(20)*. In irradiated products, the microbial counts may reach 100 times that of non-irradiated controls before spoilage can be detected *(28)*, and the odors associated with spoilage of these products often lacks the characteristic putrid smell found in non-irradiated products *(19)*. In these instances, lactobacilli may be inhibiting microorganisms responsible for the organoleptic changes normally associated with spoilage, and thus invalidating many spoilage criteria used. Lactobacilli can be beneficial in some food products, since the ability to produce H_2O_2 may enable them to repress the growth of *S. aureus, E. coli, C. botulinum*, and other undesirable microorganisms *(5, 20 36)*.

Although the ability of lactobacilli to produce and accumulate H_2O_2 in culture media and the inhibitory activity of H_2O_2 against various microorganisms has been demonstrated, the significance of these events in food products has to be determined systematically with various foods. Part of such an investigation is now in progress.

ACKNOWLEDGMENTS

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TYPES AND POPULATIONS OF MICROORGANISMS IN THE AIR OF FLUID MILK PLANTS

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Abstract

Airborne viable particle counts in the milk processing areas of 10 dairy plants averaged 14 molds and 32 non-molds/10 liters. The bacteria isolated were primarily micrococci, Gram-negative rods (excluding coliform), bacilli, and corynebacteria. Twenty-five per cent of the isolates grew at 10 C in 5 days. These were principally bacilli and Gram-negative rods (excluding coliform). There was no apparent relationship between airborne microbial populations and keeping quality of the packaged milk.

Present day processing of fluid dairy products requires that all possible sources of post-pasteurization contamination be eliminated if the products are to have adequate shelf life. Airborne microorganisms have been implicated as a cause of shortened shelf life in Cottage cheese by Angevine *(3)* and Cannon *(4)*. Whitehead and Hunter *(13)* have demonstrated airborne infection of starter cultures with bacteriophage. The role of airborne microorganisms in post-pasteurization contamination of fluid dairy products has not been adequately assessed.

Torre *(12)* reported the bacterial content of dairy plant air to be so low "that dangers of aerial contamination were considered to be very small." Other European workers, however, have reported rather high bacterial counts in dairy plant air *(5, 8)*. Perry et al. *(10)* reported *Lactobacillus* populations ranging from <1 to >65/28.32 liters (1 ft^3). However, Heldman et al. *(6)* reported much lower microorganism populations in the packaging areas of a dairy plant, with average populations of 5 bacteria, 2 yeasts, and 12 molds/28.32 liters.

The objectives of these studies were to determine the types and numbers of microorganisms in the air of representative dairy plants and to determine if any relationship existed between airborne microbial populations and keeping quality of the packaged milk.

Procedures

Ten randomly selected fluid milk plants in Alabama were used as test plants. Each plant was visited three times at intervals of approximately 7 months. At each visit viable particle populations were determined on four 56.63 liter (2 ft^3) samples of air collected during the processing day using the solid impingement method of Anderson *(2)*. Air samples were taken near the defoamer of the paper packaging machines and near the air breather cap of the pasteurized storage tanks. Microorganisms were cultured on plate count agar at 32 C for 48 hr. Following incubation, colonies were classified visually as mold or non-mold and enumerated.

Randomly distributed non-mold colonies were picked from each set of plates at the time of counting into trypticase soy broth and incubated at 32 C for 24 hr. At the end of the incubation period, Gram stains were made and each culture was transferred to a plate count agar slant for the catalase test, a lactose broth fermentation tube (incubated at 32 C for 24 hr), and to 2 tubes of trypticase soy broth (TSB). Spore formation was determined by heating one of the TSB transfers to 85 C for 10 min followed by incubation at 32 C. The other TSB transfer was incubated at 10 C for 5 days and examined for growth. Based on these tests, the cultures were classified according to the scheme of Jackson and Clegg *(7)* modified to the extent that lactose fermentation with production of acid and gas was used to differentiate coliforms from other Gram-negative rods.

Each time air was sampled, three consecutive cartons of pasteurized, homogenized milk were obtained from the filler. These samples were placed in an incubator at 7.5 ± 1 C. After 0 and 5 days incubation the standard plate count (SPC), psychrophilic count, coliform count *(1)*, and organoleptic flavor score *(9)* were determined on each sample. After 10 days incubation only the flavor score was determined. A separate carton of milk was used at each incubation period.

Statistical analyses of data were made according to Snedecor *(11)*.

Results and Discussion

Average viable particle counts obtained from the air of dairy plants in the study are shown in Table 1. The extreme variability in counts within and among plants is indicated by the high standard deviations. No significant differences ($P > .05$) in the viable particle counts were obtained among plants, by location within plants, or among trials. While the population levels obtained in this study were considerably higher than those reported by Heldman et al. *(6)*, they were within the ranges reported by Labots *(8)* and Perry et al. *(10)*.

The type and distribution of bacteria picked from air plates is given in Table 2. In all plants, the bacterial flora was made up primarily of micrococci, corynebacteria, bacilli, and Gram-negative rods other than coliforms. Only a very few lactobacilli, streptococci, and coliforms were isolated.

One-fourth of the organisms isolated from the air plates showed growth in TSB at 10 C for 5 days

TABLE 1. AIRBORNE VIABLE PARTICLE COUNTS FROM PROCESSING AREAS OF TEN DAIRY PLANTS[1]

Plant	Viable particles			
	Non-mold		Mold	
	Ave.	Std. Dev.	Ave.	Std. Dev.
	— — — — No./10 liters — — — —			
0	68	119	7	6
1	24	15	44	49
2	41	45	3	2
3	20	10	19	22
4	24	16	7	8
5	35	48	5	5
6	24	16	3	4
7	12	14	36	57
8	26	46	17	49
9	45	79	2	2
Ave.	32	53	14	24

[1]Four 56.6 liter air samples were analyzed from each plant on each of three different days.

(Table 3). Of these, 51.5% were Gram-negative rods other than coliforms and 26.1% were bacilli. Thus a relatively large portion of the airborne bacteria in the plants studied would have the potential to cause milk spoilage during refrigerated storage.

Keeping quality of the milk from the plants was assessed as shown in Table 4, to determine if there were any relationship between airborne viable particle populations and keeping quality of the milk. Plant 7 had the lowest airborne bacterial population (Table 1) and the best milk keeping quality (Table 4). The keeping quality of the milk from plant 0 was nearly equal with that from plant 7, but plant 0 had the highest average airborne bacterial population. Milk with the poorest keeping quality was obtained from plant 2, which also had a relatively high level of bacteria in the air. Other than this, no relationships between the various keeping quality tests and the air populations *per se* nor the cultural characteristics of the airborne microorganisms were evident.

TABLE 2. TYPE AND DISTRIBUTION OF BACTERIA ISOLATED FROM DAIRY PLANT AIR[1]

Plant no.	Micrococci	Streptococci	Coliform	Other Gram-negative rods	Lactobacilli	Corynebacteria	Bacilli	No growth
	— — — — — — — — — — — — — — % — — — — — — — — — — — — —							
0	28	1	0	33	0	22	11	5
1	32	0	1	25	0	11	27	5
2	23	1	0	23	3	10	31	9
3	30	1	0	34	1	13	17	3
4	25	0	0	36	0	12	21	6
5	32	2	0	17	2	14	25	8
6	31	1	3	19	0	20	16	10
7	41	3	0	23	2	16	9	8
8	18	0	1	17	1	19	33	12
9	28	0	1	16	0	26	24	6
Ave.	29	1	1	24	1	16	22	7

[1]1,757 random colonies picked from air plates.

TABLE 3. TYPE AND DISTRIBUTION OF BACTERIA ISOLATED FROM DAIRY PLANT AIR CAPABLE OF GROWTH IN TRYPTICASE SOY BROTH AT 10 C

Plant no.	Micrococci	Streptococci	Coliform	Other Gram-negative rods	Lactobacilli	Corynebacteria	Bacilli	% of total isolates
0	5	0	0	83	0	2	10	23
1	0	0	5	62	0	0	33	12
2	6	1	0	51	4	3	34	39
3	8	0	0	70	2	10	11	35
4	22	0	0	56	0	7	15	15
5	6	3	0	33	0	12	46	18
6	12	0	12	47	0	6	24	28
7	4	4	0	63	0	8	21	15
8	11	0	0	24	0	16	49	32
9	14	0	2	37	0	31	16	29
Ave.	9	1	2	52	1	10	26	25

TABLE 4. KEEPING QUALITY OF MILK AS MEASURED BY CHANGES IN BACTERIAL POPULATIONS AND FLAVOR DURING INCUBATION AT 7.5 C

Plant	Bacterial Counts[1]						Samples with flavor deterioration[2] after incubation for	
	SPC		Psychrophilic		Coliform		5 days	10 days
	Fresh	Incubated 5 days	Fresh	Incubated 5 days	Fresh	Incubated 5 days		
							— — % — —	
0	1,600	89,000	29	33,000	<1	<1	0	41.7
1	640	280,000	45	290,000	<1	27	25.0	58.3
2	4,600	30,000,000	27	17,000,000	<1	4	75.0	91.7
3	2,300	500,000	190	46,000	<1	52	0	50.0
4	3,700	150,000	110	79,000	1	14	18.2	45.5
5	430	470,000	140	460,000	1	420	18.2	100.0
6	1,800	67,000	85	7,000	1	140	0	45.5
7	1,600	16,000	23	2,100	<1	<1	0	33.3
8	870	570,000	25	690,000	3	75	16.7	58.3
9	2,500	1,300,000	290	9,100,000	<1	310	0	100.0
Ave.	910	340,000	66	170,000	1	25	15.4	64.1

[1]Logarithmic averages of 12 samples per plant picked up on three different days.
[2]Organoleptic flavor score below 36.

TABLE 5. AIRBORNE VIABLE PARTICLE COUNTS AND KEEPING QUALITY OF MILK

Trial	Av. viable particle counts		Samples with flavor deterioration[1] at	
	Non-mold	Mold	5 days	10 days
	— No./10 liters —		— — — % — — —	
1	32	19	22	81
2	34	20	18	68
3	31	6	8	45

[1]Organoleptic flavor score below 36.

It is probable that contamination of milk from sources other than air was sufficient to overshadow any contamination from airborne microorganisms. This contention is further borne out by the fact that, as the study progressed through the three trials, very little change in airborne microbial populations was noted, but a steady improvement in keeping quality was evident (Table 5).

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REMOVAL OF EXTRANEOUS MATTER FROM CREAM USED FOR BUTTERMAKING

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Abstract

Butter with consistently low levels of extraneous matter can be produced from farm-separated cream only if a suitable clarifying or filtering step is included in processing. The following sequence of plant operations is proposed: *(a)* pass cream through a strong nylon bag of about 30 mesh per inch, to remove larger pieces of extraneous matter; *(b)* heat cream to 70-80 F and neutralize; *(c)* heat to 130-140 F and clarify or filter; *(d)* heat to 170-175 F to pasteurize; *(e)* cool, allow to stand overnight and churn.

A number of choices are open to plant operators respecting equipment and operating conditions. For a filtering process, choices exist respecting at least the filter size, filter material, and flow rate. Filtering and clarifying appear about equally effective in the removal of extraneous matter.

Commercial dairy products have always contained small amounts of extraneous matter. This material, in the form of finely divided particles includes a variety of plant fragments, some of manurial origin; particles of fine sand, silt, and clay; insect fragments; hairs of bovine, human, and rodent origin; cotton, wool, and other fibers; pieces of metal, paint, etc.

The existence of a relationship between sediment tests and bacterial counts has been debated *(2, 7)*. But regardless of the public health aspect, the dairy industry has an obligation to keep amounts of extraneous matter to the lowest levels possible in all dairy products. Although the particles are too small to be seen, consumers do not expect such materials to be present in their food. Governments have taken the attitude that it is their duty to ensure that consumer's interests are safeguarded and that foods offered on the market do not contain more than minimal amounts of extraneous matter.

In Canada, regulations which limit the amounts of extraneous matter in fluid milk and cheese have long been in effect. However butter, which normally contains less extraneous matter per pound than cheese *(2)*, has been subjected to close scrutiny only during the past three years. During this time the Canada Department of Agriculture has taken samples for sediment tests and has reported the results to processors as encouragement to bring about reductions in amounts of extraneous matter. In dairies which receive milk and separate their own cream there have been relatively few problems, and generally the butter produced has had acceptable sediment scores. Problems with unsatisfactory sediment scores have been limited almost entirely to plants receiving farm-separated cream, and tests in these plants revealed that the cream received was an important source of extraneous matter. Obviously it would be desirable to keep all extraneous matter out of cream, and efforts are being made to improve the handling of cream. But it is clear that the only hope immediately to bring about a reduction in amounts of extraneous matter in butter is to remove the extraneous matter from cream before churning. Even with better quality cream receipts, such removal will probably continue to be needed as long as farm-separated cream is delivered to dairies.

Australian studies on the removal of extraneous matter from cream have been reported by Crittall and co-workers *(4, 5, 6)*. They carried out a thorough investigation of filtration practices including types of filter cloths, size of filter and flow rates, temperature of filtration, and point in the process most suitable for filtering. Two reports on cream filtration have recently come from Saskatchewan *(1)* and Alberta *(3)*.

The studies reported here, involving clarification and filtration of cream, were carried out with the cooperation of local dairies. In all, tests were conducted on approximately 140 batches of cream and on the butter made from the cream.

Methods

Equipment

Clarifier tests were conducted with a Westfalia Separator Model RN 1254. Both positive-pressure and centrifugal pumps were used in the tests. Filtration tests were conducted with four sizes of filter assemblies and two filter bags. All filter assemblies consisted of perforated stainless steel frames to support the filter material and channels to direct cream through this material. The dimensions of the four filters (diameter in inches, length in inches, and area in square inches) were as follows: No. 1, 1.75, 16, 90; No. 2, 3, 35, 330; No. 3, 6.5, 20, 410; and No. 4, 10, 30, 942.

Although the Australian workers *(5, 6)* found nylon filter cloths most satisfactory, material of the quality recommended was not available, and various other filter materials were used in these tests. Those used in most of the tests were woven cotton flannels of approximately 44 mesh per inch. In a few trials with filter Number 2, non-woven fabrics[1]

[1]Distributed by Johnson and Johnson, Chicago, Illinois and Montreal, Quebec

TABLE 1. RESULTS OF TRIALS ON CLARIFYING CREAM

Temp. of cream (°F)	Flow rate (lb/hr)	Type of Pump[1]	Quantity of cream lb	Remarks
42-48	5000-6000	c	4500-5000	Sediment removal marginally satisfactory
96-98	3500-4500	pp	2000-3000	Removal satisfactory Slime deposition limited volume
120-125	3500-4500	c, pp	4500-5000	Satisfactory
145-150	5000-6000	pp	4500-5000	Satisfactory

[1]Note, c = centrifugal pump, pp = positive pressure pump.

having pore sizes of approximately 40 μ were used. In a few tests, filtering was accomplished with filter bags of approximately 280 fluid oz capacity and of 50 and 100 μ ratings made of AFCO Filter Products, Division of American Felt Company, Glenville, Connecticut[2]. One series of tests was conducted with a continuous heating and filtering device consisting of a steam-heated 12-plate heat exchanger connected to filter Number 2. In all trials, large pieces of extraneous matter were removed from the cream before they reached the clarifier or the filter described above. Usually this was accomplished by passing the cream through a coarse nylon filter bag (approximately 30 mesh per inch).

[2]Supplied by E. F. Walter Ltd., Toronto, Ontario

Procedures

Sediment tests were performed on cream samples before and after clarifying or filtering and on the butter produced from the cream. Cream sediment tests were performed on 100 ml samples (except in some of the earlier tests when 130 ml samples were used). Butter sediment tests were conducted on 45 g samples. Both cream and butter samples were filtered through a filter area of 0.25 inch diameter *(8)*. The discs were scored for quantity of sediment and frequently they were examined under a low power microscope to observe the nature of the sediment. In earlier tests, scores were assigned on the basis of a 4-point scale used by the Canada Department of Agriculture in scoring creamery butter samples *(9)*. The four points of this scale, when later rated on the Research Unit's Guide for Sediment in Cream and Butter *(8)* were considered to represent approximately 0.05, 0.10, 0.20, and 0.30 mg of sediment. Butter samples which received the two lower scores were considered acceptable with respect to sediment and those receiving the two higher scores were considered unacceptable.

In a number of instances, the extraneous matter removed from a batch of cream by filtering was washed from the filter as completely as possible with the aid of acetone and petroleum ether, collected on filter paper in a Buchner funnel, and examined visually and under a microscope.

Supplies of water, salt, and neutralizer used in the plants were also examined for extraneous matter.

RESULTS AND DISCUSSION

Raw cream samples generally contained amounts of sediment in the range of 0.20 to 0.30 mg/100 ml.

TABLE 2. RESULTS OF TRIALS ON FILTERING CREAM

Filter Used					Temeprature of cream (°F)	Flow rate (lb/hr)	Type of pump[1]	Quantity of cream (lb)	Remarks
Assembly no.	Diameter (inches)	Length (inches)	Area (square inches)	Filter material					
				woven cotton	45-60	4500–5000	pp	4000-5000	Removal unsatisfactory
1	1.75	16	90	woven cotton	96-98	3500–4500	pp	2000-3000	Satisfactory
				woven cotton	145-150	5000–6000	c, pp	4500-5500	Satisfactory
2	3	35	330	woven cotton	110	9600	c	4000-5000	Rate became unacceptably slow
				woven cotton	130-140	9600	c	4000-5000	Satisfactory
				non–woven cotton	130-140	9600	c	4000-5000	Satisfactory
3	6.5	20	410	woven cotton	48-55	9000–10,000	c	4000-5000	Removal marginally satisfactory from pasteurized cream
4	10	30	942	woven cotton	50-70	5000–6000	c	4500-5500	Removal marginally satisfactory
				woven cotton	70-90	5000–6000	c	4500-5000	Satisfactory
	Bags (50 and 100 μ pore size)				85-135	9600	c	4500-5000	Satisfactory

[1]Note, c = centrifugal pump, pp = positive pressure pump.

In almost all instances clarifying or filtering reduced this to below 0.20 mg/100 ml and permitted production of butter that was acceptable with respect to extraneous matter.

The test conditions and results are summarized in Tables 1 and 2. Both clarifying and filtering were found satisfactory and about equally effective in the removal of extraneous matter. Although marginally satisfactory results were obtained when pasteurized cream, cooled to approximately 50 F, was filtered through Assembly Number 3, it was considered that extraneous matter should be removed at the earliest possible stage of processing and that the cream should be heated to facilitate this removal. On the basis of the results observed in this study together with some viscosity measurements on raw cream, and in order to protect the cream against lipase action, it was considered that 130 to 140 F was the most suitable temperature for processing to remove extraneous matter.

All of the filtering materials used in these tests appeared to perform equally well. The non-woven fabric was the cheapest material and obviously a single use item. The woven cotton materials and the filter bags could be washed and reused, although the reuse would introduce problems of sanitation and decreased efficiency.

In most of the filtration tests a centrifugal pump was used in preference to a positive pressure pump. Although no damage to filters was noticed, it was thought that without a pressure-regulating device, the positive presure pump might cause the enlargment of some of the openings in the filters and force through some sediment that under conditions of lower pressure would have been held by the filter.

An unsatisfactory aspect of filtering exists in the failure of the filter materials used in these studies to remove all fine sand-and silt-like particles. In samples taken from vats that had stood overnight there was a higher concentration of sediment at the bottom than in the middle or top of the vat. Unless some better filtering materials are used, perhaps a type such as the AFCO Mark III cartridges *(3)*, it would appear that filtering could to advantage be supplemented with a further step to remove "settlings". Such a step might be the segregation of the first 1 or 2 gallons, and the last 5 or 6 gallons, drawn from the vat. Removal of the sediment might then be accomplished, partially at least, by diluting, settling, and decanting *(1)*. In several tests, similar high concentrations of sand- and silt-like sediment were observed in the "steaming", i.e., the last portions of cream removed by steam from cans at the dump tank. For this reason, this fraction of the cream should also be kept separate and be treated in the manner suggested for settlings.

Microscopic examination of extraneous matter collected from filters and observed on sediment discs showed the presence of the following: paint (in the color of the markings on cream cans), aluminum foil and other metallic fragments, straw, hay, partly digested vegetable matter, hairs, pieces of sponge, cloth threads, insects and insect parts, pieces of coal, and a few tar-like threads thought to be fragments of packing from a pasteurizing vat. The amount of large straw and hay pieces removed by the filters in some tests indicated that contamination of cream after separating was a major problem, and possibly the problem is entirely one of contamination after separating. Bird *(2)* has reported that cream from the separator contains little extraneous matter. It also was evident that contamination could arise from sources in the plant, particularly by paint and other materials falling from cans into the dump tank.

Both neutralizer and salt samples showed considerable amounts of sediment when tests were conducted on 25 and 50 g samples. All water samples tested showed some sediment. Although the amounts of extraneous matter contributed to butter from these sources would be small, these must be recognized as variable contributing sources of contamination.

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EVALUATING THE USE OF NITRIC ACID AS A DETERGENT IN DAIRY CLEANED-IN-PLACE SYSTEMS[1]

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ABSTRACT

A model cleaned-in-place system was constructed and an atomic absorption method used to evaluate the detergency of low concentrations of nitric acid. Results indicate that critical temperature-concentration combinations exist which will remove the calcium from stainless steel test plates soiled with a milk film to a level comparable to a standardized-control wash (0.0-0.2 ppm calcium in 30 ml solvent eluted from a 324 cm^2 area). Stainless steel test plates soiled with milk became resistant to wetting with water after being washed with nitric acid. Bacteriological studies were made to determine if the surface of the plates washed with nitric acid retained enough nutrients to support bacterial growth. Longer washing times than those required for complete calcium removal were necessary to reduce effectively the number of bacteria which would multiply on the surface of the test plates. Tests on corrosion showed that low concentrations of nitric acid were noncorrosive to stainless steel type 304. Low concentrations of nitric could be used routinely and for prolonged periods with little deleterious effect to the stainless steel. Plant trials indicated that nitric acid, under the conditions studied, did not remove the soil resulting from milk in contact with heated surfaces. More extensive plant trials are needed to fully evaluate the effectiveness of nitric acid in cleaning non-heated milk contact surfaces.

BASIS FOR STUDY

Interest in using nitric acid as a detergent in dairy cleaned-in-place (CIP) systems was stimulated by reports of its use in certain European countries to clean stainless steel dairy processing equipment. Research at the National Institute for Research in Dairying (NIRD) at Reading, England, has demonstrated that farm pipelines and milking equipment can be cleaned effectively using hot water acidified with nitric acid *(1)*.

With CIP cleaning it is possible to utilize temperatures, detergent types and concentrations, and fluid dynamic factors that are severely restricted in manual cleaning. Studies by Maxcy *(3)* and others show that only small amounts of milk solids are retained on non-heated milk contact surfaces after CIP rinsing. The cleaning process in CIP systems then becomes one of removing a small amount of tenaciously bound residual soil by combining the factors of a CIP system in the most efficient way possible. It was hypothesized that nitric acid, a strong but relatively noncorrosive mineral acid, may provide the needed detergency when used in conjunction with the other factors available to clean dairy CIP systems adequately and efficiently.

Using nitric acid rather than the alkaline detergents now in general use may have several advantages: cost could be lowered significantly and nitric acid cleaning would enable use of a simple, inexpensive CIP system. In addition, nitric acid would be a very low cost detergent compared to commercial alkaline cleaners. Reduced cleaning time is also a potential advantage, and a factor which is becoming of greater importance as dairy plants increasingly operate at a higher level of capacity.

A mineral acid detergent may be more effective in controlling milkstone buildup or hard water incrustations resulting from the precipitation of calcium or magnesium by alkaline cleaning. Nitric acid presents few disposal problems; the wash solution would quickly be diluted to a completely biodegradable waste product.

DETERGENCY OF NITRIC ACID

The detergency of nitric acid was evaluated by washing stainless steel test plates soiled with a dry milk film with nitric acid solutions under controlled conditions. The residual calcium remaining after the wash was eluted and quantitatively determined by atomic absorption spectroscopy using a modification of the procedure developed by Heinz, et al. *(2)*. The assumption was made that the amount of calcium remaining is indicative of total soil remaining.

Washing device

A model CIP system was constructed which allowed the controlled washing of stainless steel test plates. The test plates were of 24 gauge, number 4 finish, 304 stainless steel, and measured 25.0 x 20.5 cm. The washing device sprayed the wash solution onto the test plate approximately 6.0 cm from the top, allowing the solution to cascade down the plate. The detergent flow rate was maintained at 2.5 $\pm$ 0.1 liters per min by pumping with a peristaltic action pump. The system was designed to evaluate the detergency properties of nitric

[1]This work was supported in part by a Dairy and Food Industries Supply Association Fellowship.

[2]Present address: Food Products Division, Proctor and Gamble Co., 6000 Center Hill Rd., Cincinnati, Ohio 45224.

acid, and therefore the mechanical factor in cleaning was minimized.

Soiling

The stainless steel test plates were soiled with fresh, homogenized, whole milk. A cardboard template was made to facilitate soiling an area 18.0 x 18.0 cm on the test plate. The milk was applied by using a hand spray gun, allowing the fine droplets to accumulate until the film started to flow. The film was allowed to air dry at room temperature. After receiving the prescribed washing treatment, test plates were gently flushed with room temperature, distilled, deionized water to remove any traces of tap water containing calcium. The additional cleaning attributable to rinsing would be insignificant in relation to the prescribed washing treatment.

Calcium elution

A rubber template with inside dimensions the same as the soiled area was attached to the test plate using metal clamps. The template formed a leakproof enclosure approximately 0.6 cm in depth which facilitated the calcium recovery.

A 1.0% lanthanum solution in 1.4 M HCl containing 0.1% wetting agent (Trademark, Triton X-100, produced by Rohm and Haas Co., Independence Mall West, Philadelphia, Pa.) was used to elute the residual calcium from test plates. The stainless steel test plate with the rubber attached was positioned so that when the HCl solvent was applied it would collect in a lower corner. Six successive 5 ml aliquots of the above solvent were applied using a 10 ml glass syringe. After the application of each 5 ml aliquot, the solvent was allowed to collect in the lower corner and was removed by drawing it back into the syringe with which it was applied. The elutions were combined and after the sixth elution any solvent necessary to bring the volume up to 30 ml was added.

The eluted samples were quantitatively analyzed for calcium on Perkin-Elmer 303 Atomic Absorption Spectrometers. The instrument on which the majority of the analyses were performed was equipped with a scale expansion attachment and a strip chart recorder. Detection was distinct at concentrations as low as 0.1 ppm. Reference standards for plotting the standard curve were prepared using $CaCO_3$ added to the elution solvent. Preliminary experiments indicated that the elution method provided quantitative calcium removal.

Results

Analyses were made of test plates receiving a standardized control wash: thorough scrubbing with fine abrasive cleaner and sponge, flushing with tap water and with distilled deionized water, washing in commercial organic acid cleaner, rinsing with distilled-deionized water, and drying in air. Washed plates yielded 0.0 to 0.2 ppm calcium with the standardized elution process (30 ml solvent used to elute the calcium from a 324 cm^2 soiled area). This level of calcium provided a logical standard for cleanliness.

Repeated trials were made to determine the time-temperature-concentration relationships which would produce a state of cleanliness equal to that obtained with the standardized wash (0.2 ppm or less). The results can be summarized: *(a)* A concentration of 0.01 N nitric acid at a minimum of 130 F produced near complete (0.2 ppm or less in 30 ml solvent) calcium removal. Increasing the temperature beyond this level did not result in better cleaning as measured by the amount of residual calcium. *(b)* The length of washing time had very little effect. In experimental trials, a minimum wash time of 30 sec was evaluated. It was found that a 30 sec wash was as effective as washing times up to 2 min, the time range evaluated. *(c)* When the concentration of nitric acid was reduced below 0.01 N, the temperature required increased greatly. At 0.005 N nitric acid, a temperature of 175-180 F was required for near complete calcium removal. *(d)* Below 0.003 N, cleaning was incomplete at all temperatures. *(e)* Increasing the concentration of the nitric acid above 0.01 N did not significantly reduce the temperature required for near complete calcium removal.

Figure 1 illustrates the minimum temperature-contration combinations which were found to result in near complete calcium removal.

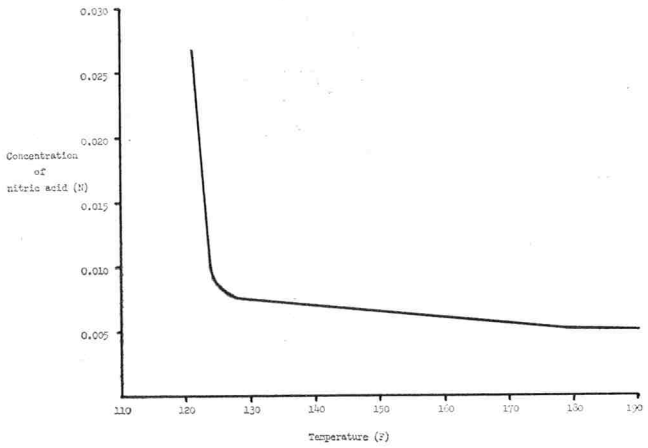

Figure 1. Minimum temperature-concentration which resulted in near complete calcium removal.

NON-WETTABILITY STUDIES

It was observed that after the test plates were washed with nitric acid solutions, the surface area which had been soiled resisted wetting although they appeared visually clean. According to the calcium residue analyses, plates retained no soil. Also, as discussed in greater detail below, test plates which received a wash treatment equal to or more rigorous than 0.01 N HNO_3 at 175 F for 2 min did not retain enough soil to provide the nutrients needed for bacterial growth.

It may be that the surfaces retained a very thin film of fat. It was reasoned that if the non-wetting condition was the result of free fatty acids or triglyceride fat adhering to the surface of the test plate, it may be possible to elute the fat with a fat solvent and detect it by gas-liquid chromatography analysis.

Plates were eluted with a high purity ethyl ether and *n*-hexane solvent. Using a conventional condens-

ation and sulfuric acid-methanol esterification technique, an attempt was made to detect the methyl esters of the fatty acids by GLC analysis using a glass column packed with 12% diethylene glycol succinate coated on 80/90 mesh diatomaceous earth.

Results

It was not possible to detect the presence of any lipid material using the method described. The concentrating procedure used and the high degree of sensivity employed resulted in the detection of much unidentified material in the control samples as well as in the samples presumed to contain fat. No consistent difference was noticed between the control samples and those presumed to contain fat.

TABLE 1. EFFECT OF WASH TIME ON BACTERIAL GROWTH ON TEST PLATES WITH HOMOGENIZED MILK AND CLEANED WITH 0.01 N NITRIC ACID AT 175 F.

Wash time	Total colony count per plate[1]	
	SPC after wash	SPC after 24 hr incubation
30 sec	40	8,400,000
1 min	19	56,000
2 min	24	36

[1]Population of soil, 320/ml (SPC)

TABLE 2. CORROSION RESISTANCE OF STAINLESS STEEL TYPE 304 TO 0.1 N NITRIC ACID AT 175-180 F

Sample	Original wt.	Wt. loss	Corrosion rate[1]	99/100 life[2]
A	39.4505 g	0.0022 g	5.6	3760
B	39.0814	0.0027	6.8	3024
C	39.2473	0.0028	7.0	2940
D	39.6620	0.0036	11.0	2310
E	39.5050	0.0026	6.5	3190

[1]Milligrams per m^2 per 24 hr.
[2]Immersion time required (days) to reduce the weight of stainless steel type 304 to 99% of its original weight.

BACTERIOLOGICAL EVALUATION

It was considered possible that the non-wetting property resulted from a soil film. As a result, it was of interest to determine if the surface of the test plates washed with nitric acid retained enough soil to support bacterial growth when in an environment in which all other growth factors were favorable.

Methodology

Duplicate test plates were soiled with homogenized milk of varying bacterial population and washed in the CIP model under identical conditions and procedures. After the washing treatment, plates were rinsed with sterile, distilled, deionized water to remove any traces of the nitric acid wash solution which may have bactericidal properties. One of the test plates was swabbed and plated immediately. The duplicate plate was placed in a glass vessel lined with porous filter paper and the bottom covered with water. A platform was positioned to hold the test plates above the water.

The covered vessel was placed in an incubator held at 89.6 F (32 C). Under these conditions relative humidity should be very near 100%. It was hypothesized that the interior of the vessel would provide optimal environmental conditions for bacterial growth except for available nutrients. If the test plates contained the nutrients necessary, bacterial growth would flourish; if nutrients were absent or severely limited, growth should be restricted. Therefore, by swabbing and plating one of two immediately after washing and swabbing and plating the second plate after a 24 hr incubation period, one could measure the extent of bacterial growth resulting from nutrients available, and indirectly the state of cleanliness of the test plates.

Results

Test plates were washed at a temperature of 175 F with a 0.01 N HNO_3 solution. Standard plate counts indicated that increased washing time resulted in a lower bacterial count after incubation. Increased washing time did not significantly affect the counts of the plates swabbed immediately after washing.

A washing time of 2 min reduced the "after incubation" counts to a level indicative of a sanitary surface. The results of tests for residual calcium also indicated no difference in the cleanliness of the test plates when the time ranged from 30 sec to 2 min. An example of the data obtained is presented in Table 1.

CORROSION STUDY

Stainless steel corrosion, resulting from nitric acid use, was given considerable attention. Mineral acids, in general, are considered to be very corrosive, and are not recommended for routine use. Nitric acid, however, is known to be far less corrosive to stainless steel than are most mineral acids. To assess the corrosion rate, the following experiment was conducted.

Plates of 24 gauge, number 4 finish, 304 stainless steel measuring approximately 5.0 cm x 16.25 cm were fabricated. The plates were washed with detergent and held in 0.1 N HNO_3 for 12 hr at 160 F to remove any foreign material. After the cleaning treatment plates were air dried, placed in a 110 F dry air oven to remove traces of moisture, cooled, and weighed with an analytical balance. Plates were immersed in 0.1 N HNO_3 and held at 175-180 F for 21 days. Following the treatment plates were cleaned in a manner similar to the procedure used before immersion and were weighed.

Results

The data in Table 2 show that no corrosion of any significance occurred on stainless steel type 304 un-

der the conditions studied. It was recognized that agitation and incorporation of air may affect the corrosion rate. No visual change in the surface of the stainless steel was observed. Prolonged and routine cleaning with nitric acid should have no deleterious effect on this type of stainless steel.

CLEANABILITY OF HEATED MILK CONTACT SURFACES

Results of atomic absorption analyses of residual calcium and of bacteriological studies indicated that low concentrations of nitric acid provided the detergency necessary to clean non-heated milk contact surfaces satisfactorily. These observations supported the results obtained at NIRD where hot water acidified with HNO_3 was used to clean farm pipelines and milking equipment *(1)*.

The nature of soil adhering to the different surfaces varies widely. For example, non-heated pipelines after rinsing contain very little milk solids, while a steam injection chamber of a deodorizer regularly forms a soil layer several millimeters thick and almost totally resistant to removal by rinsing. Soils of this nature are difficult to reproduce in the laboratory. It was therefore decided to evaluate HNO_3 cleaning of this type soil under practical plant conditions.

Experimental trial 1

The Cornell University dairy plant utilizes a system in which the HTST pasteurization unit, deodorizer, and the homogenizer are cleaned by a common CIP circuit. Rinse and wash solutions enter the system via the balance tank. After passing through the system, the solution can be returned to the balance tank for recirculation or drained to waste.

The washing cycle used was a rinse until clean water appeared followed by a 20 min wash at 192 F followed by a brief cold water rinse. Nitric acid solutions (0.02 N to 0.04 N) were circulated. At 0.035 N, the pH ranged from an initial 1.7 to a final 2.0, apparently the result of the buffering action of the milk proteins carried into the solution. At 0.04 N, a final pH of 1.7 was attained.

Results

There was considerable particulate matter in the wash solution, presumed to be coagulated protein. The first tank of the deodorizer unit (steam injection chamber) retained a thick film of milk soil. In all trials the wash treatment failed to remove the build-up from the steam injection chamber of the deodorizer unit.

Experimental trial 2

A simple mechanical adjustment makes it possible to bypass the deodorizing unit which forms a soil layer several millimeters thick and is difficult to remove by means other than a strong alkaline solution. The HTST and homogenizing unit were washed on the CIP system described above following a similar procedure.

A 0.03 N nitric acid solution (pH 1.7) was circulated for 20 min at 192 F. The system was washed in this manner for 4 days following normal plant operations. The bacteriological condition of the system was monitored by swab tests and visual inspection.

Results

The bacteriological condition remained very good throughout the period. On the fifth day, however, a definite film of soft protein-like material was forming on parts of the piping and on the HTST plates in the heating section. The composition of the film was not determined.

GENERAL APPLICATIONS

The results of this study support those of the research at NIRD showing that low concentrations of nitric acid can be used to effectively clean non-heated milk contact surfaces; low concentrations of nitric acid do not, however, provide adequate detergency for cleaning heated milk contact surfaces. Therefore, this method of cleaning may be most applicable at the farm level or in a commercial CIP system which cleans only non-heated milk contact surfaces. To evaluate its effectiveness in commercial plant systems of this type, more extensive trials under practical plant conditions are necessary.

The non-corrosive properties of low concentrations of nitric acid solutions (e.g., 0.01-0.1 N) make it excellent for milkstone/hard water incrustation control or removal. Its use in this function could become an integral part of many cleaning programs.

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FOOD REGULATORY MICROBIOLOGY-THE INTERNATIONAL SITUATION[1]

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Microbiology lacks the exactitude of the physical sciences, and thus our difficulties of methodology, interpretation, etc., are manifold. But within one country, such as ours, these difficulties begin to look inconsequential when we look instead at the international situation. Each country of the world has its own standards of microbiological control and these standards differ markedly. There are differences in general concepts of food hygiene, differences in economic and technological advancement as applied to production, processing, distributing, and serving foods. There are differences in educational opportunities for professionals. There are major differences in the availability of professionals and laboratories. It is estimated that 60% of all of the microbiologists in the whole world are located here in the United States. Despite that fact, microbiologists are in short supply here in this country. They are in much shorter supply in the world as a whole.

Basic laboratory techniques differ in other parts of the world. One important factor is that the culture media, reagents, and antisera are quite different. In this country, we have a few major manufacturers of media. They are not standardized. Nevertheless, a standardized method here in this country can specify a particular brand. But these brands are generally unavailable outside of the United States and Canada. In England, Oxoid is popular. In Japan, the brands Eiken and Nissui are popular. In the Soviet, no prepared media are available and microbiologists must prepare their own media using meat and vegetable infusions.

Standardizing of Microbiology

There are some definite advantages to standardizing international microbiology. First, standardization of methods will facilitate international shipment of foods. At the present time, an exporter must determine what microbiological method the importing country will use. If he uses a different method, he may end up with a shipload of food unacceptable to the importing country. Second, international bacteriological standards could be useful if they are applicable to the particular foods in question. Third, microbiological control on an international basis will enhance food protection, particularly when applied to those foods from areas of poor sanitation.

Present International Activities

Now let's look at some of the international activities as they are today.

Canada and the United States have had a reciprocal agreement on the bacteriological quality of shellfish and shellfish growing waters since 1948. A similar agreement applies to one specific area in Japan near Hiroshima, and has been in effect since 1962.

Under the auspices of the U. S.-Japan Program for Development of Natural Resources, a panel on toxic microorganisms was established in 1963, primarily to exchange information on botulinal and fungal toxins. Representatives of government agencies in the two countries meet annually to plan cooperative activities, and to review recent development on occurrence and elimination of toxins from foods. In 1968, the panel sponsored a conference at the University of Hawaii. The 1,600 manuscript pages of proceedings will be published later this year. This panel has exchanged translations and microbiological methods for examination of foods. The Chairman is Dr. C. W. Hesseltine, Head, ARS Culture Collection Investigations, Northern Utilization Research and Development Division, ARS, USDA, Peoria, Illinois 61604.

Most other attempts at standardization and general international control have been related to methods of sampling and analysis. There are several agencies at work.

Agencies

The International Organization for Standardization (ISO) is a primarily European group. Subcommittee 6 of ISO has conducted a series of collaborative studies on methods for microbiological examination of meat and meat products, which was recently published in part in *Fleischwirtschaft (1)*. The specific de-

[1]Presented at Round Table on Food Regulatory Microbiology, Annual Meeting of the American Society for Microbiology, Miami Beach, Florida, May 6, 1969.

tails of the ISO methods are somewhat different from those we use in the United States. Further information regarding this Subcommittee may be obtained from Dr. I. B. Krol, Central Institute for Nutrition and Food Research, Zeist, The Netherlands.

The World Health Organization through its Expert Committee on Food Hygiene has sought to obtain and evaluate the views of expert food microbiologists from throughout the world. It also supports or participates in the activities of other organizations working on food sanitation. The proceedings of their meeting of October, 1967 was recently published *(4)*. This booklet gives short interpretive paragraphs about a wide variety of problems in food microbiology.

The joint FAO International Atomic Energy Agency (IAEA), Division for Atomic Energy in Agriculture is developing microbiological criteria and methods for evaluating irradiated foods; at least two panel meetings have been held, but a report has not yet been published. Further information may be obtained from Dr. H. E. Goresline of the IAEA at Kaertnerring, Vienna I, Austria.

The Codex Alimentarius Commission, through its several committees, is developing standards for several categories of foods such as cocoa and chocolate, sugars, processed fruits and vegetables, and so on. These committees have only an incidental interest in microbiology; however, the Codex Committee on Food Hygiene under the chairmanship of Mr. Robert Shelton of the Division of Microbiology, FDA, is heavily involved with microbiology. This committee is drafting basic provisions on food hygiene applicable to all foods. According to the committee, "Food hygiene comprises conditions and measures, necessary for the production, processing, storage and distribution of food, designed to insure a safe and sound wholesome product fit for human consumption."

This Codex Committee feels that the hygiene rules it sets should not be mandatory because enforcement would be difficult. The Committee lays out general principles in codes of hygienic practice, and requests individual countries to develop legislation designed to encompass these objectives.

A document, still in its early stages, on eggs and egg products contains the general statement that *Salmonella* tests should be conducted. A similar requirement is stated in the code for dessicated cocoanut. The Committee has agreed to ask the advice and counsel of other international bodies qualified to evaluate methods of analysis and sampling. FAO member nations have been urged to place additional specialists in food microbiology on this important committee.

The last but perhaps the most important example of international microbiology is the work of the International Committee on Microbiological Specifications for Foods (ICMSF), a standing committee of the International Association of Microbiological Societies (IAMS). The American Society for Microbiology is part of IAMS, and therefore has a stake in the ICMSF. This group of approximately 20 food microbiologists from twelve nations, has worked under the chairmanship of Dr. Fred S. Thatcher, Food and Drug Directorate, Tunney's Pasture, Ottawa, Canada. The committee's work started in 1962 and it has met almost yearly since that time. The first report was published in 1963 *(2)*. The second consists of a book entitled, *Microorganisms in Foods. Their Significance and Methods of Enumeration. (3)*. A full review of this book is to be found in the April 1969 issue of the ASM News of the American Society for Microbiology. It should be made available to every food microbiologist. The book is primarily a collection of methods of analysis recommended for use on foods in international commerce. It also includes considerable information on the interpretation of bacteriological data. The methods are, at this time, simply a collection, since committee members found it difficult to agree on which were best. Collaborative and comparative studies on these methods are now under way. Also methods and statistics of sampling of products in international trade are under study.

The first appendix of the book is a list of the program objectives of the committee. The overall purpose is to appraise public health aspects of microbiological content of foods in international commerce, and to recommend analytical methods and guides to interpretation. The Committee has agreed to offer recommended microbiological limits for some foods, to recommend and define methods of analysis and sampling, to conduct collaborative testing of methods, to establish channels of communication among scientists, to consider the feasibility of establishing international centers for reporting foodborne illness. The Committee has also agreed to formulate group statements on the significance of specific microorganisms in foods, to serve as a consultative body of food microbiologists for international agencies such as WHO, FAO, UNICEF, etc., to maintain liaison with other allied committees such as those of the Codex Alimentarius, to recommend areas of research, and to establish means by which international exchange of funds can be made for research and other studies under the scope of the Committee's functions.

This Committee is largely self-supporting, having received grants from government agencies in the United States, and in Canada. Also, the World Health Organization, the Pan American Health Or-

ganization, The Pasteur Institute, and the Carlo Erba Institute for Therapeutic Research have contributed, as have several large private firms in the United States, Sweden, Canada, Japan, and the United Kingdom. A meeting in May 1969, in Dubrovnik, Yugoslavia, was supported by Public Law 480 funds. The PL 480 program uses soft currency credits which the United States has available from the foreign sale of surplus agricultural products. The largest contributor to date is the U. S. Department of Agriculture, Agricultural Research Service, which has given the Committee $50,000 to be used for collaborative studies of methods. The Committee would welcome additional contributions from private or public sources. International cooperation is an expensive business.

The membership of this Committee is limited to 20 persons. At this time they are: Dr. Fred S. Thatcher, Chairman and Dr. David S. Clark, Secretary-Treasurer, both of Canada; Dr. Howard E. Bauman of the Pillsbury Company, USA; Dr. R. Buttiaux from France; Dr. C. Cominazzini from Italy; Dr. Claude E. Dolman from Canada; R. Paul Elliott, USA; Dr. Eugene Gangarosa, USA; Dr. Harry Goresline from the International Atomic Energy Agency in Austria (He is a U. S. citizen.); Dr. Betty Hobbs, UK; Dr. Hiroo Iida of Japan; Dr. Maurice Ingram, UK; Dr. Keith H. Lewis, USA; Dr. Helgor Lundbeck from Sweden; Dr. G. Mocquot from France; Dr. David Mossel from the Netherlands; Dr. N. P. Nefedjeva from the Soviet Union; Dr. Joseph C. Olson, USA; Dr. Fernando Quevedo from Peru; and Bent Simonsen from Denmark. The Committee also has an observer from the World Health Organization. This has been Dr. Z. Matyas, from Czechoslavakia.

The Committee has formed subcommittees in Latin America and Eastern Europe, and is considering forming other subcommittees in other areas of the world.

The leading role of the ICMSF Committee was recently recognized by FAO, when the Codex Committee on Methods of Analysis and Sampling, at their meeting in November 1968, agreed to support the use of the ICMSF methods.

The international picture in microbiology of foods is still somewhat confusing; however, through common cross membership, in some instances, and through publication and other communications, the various international groups are showing remarkable progress and cooperation. At a time when the politicians and the military are having international problems, the microbiologists are developing ever closer relationships.

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REPORT OF THE BAKING INDUSTRY EQUIPMENT COMMITTEE, 1968-1969

This committee has had two meetings with the Baking Industry Sanitation Standard Committee since our 1968 report. The fall meeting was held in Houston, Texas October 17-19, 1968. At this meeting Standard No. 29, Electric Motors, was carefully reviewed and approved for publication. The effective date for this standard will be January 1, 1970. Standard No. 30, Delivery Cabinets, Distribution Racks, Dollies, Baskets, and Trays, and Standard No. 31, Pie Making Equipment, were reviewed but not approved.

The spring meeting was held in Chicago, Illinois February 28 and March 1, 1969. At this meeting four new standard task committees were appointed as follows: *(a)* Baking pans, *(b)* Coolers, bread cake, pie and doughnuts, *(c)* Donut icing machines, *(d)* Small batch and ingredient containers.

The baking industry is actively supporting the Office of Certification which issues authorizations and certifications for the use of the BISSC seals on equipment conforming to the concerned standard. Authorizations have been issued to approximately fifty manufacturers of bakery equipment including several foreign manufacturers. Many of the largest bakery chains are specifying, when ordering bakery equipment that the equipment shall conform to BISSC standards. To aquaint sanitarians with BISSC standards the *Journal of Milk and Food Technology* is publishing the complete list of standards. Also, sanitarians may obtain, without charge, complete sets of BISSC Standards by writing: Ray Walter, *Executive Secretary,* c/o BISSC, 521 Fifth Avenue, New York, New York 10017.

It is hoped that through increased publicity and educational efforts by BISSC, that the bakery standards will become as well known and accepted as the 3-A Standards.

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LOUIS A. KING, JR., American Institute of Baking, 400 East Ontario, Chicago, Illinois 60611.

FRED VITALE, Continental Baking Company, Inc., P. O. Box 731, Rye, New York 10580.

HAROLD WAINESS, Wainess & Associates, 510 North Dearborn Street, Chicago, Illinois 60610.

ASSOCIATION AFFAIRS

ANNOUNCEMENT CONCERNING THE SANITARIANS AWARD FOR 1970

Announcement is made that nominations will be accepted for the annual Sanitarians Award until June 1, 1970, and the members of the International Association of Milk, Food and Environmental Sanitarians, Inc. are requested to give consideration to the nomination of individuals whose professional work in the field of milk, food, or environmental sanitation has been outstanding.

The Award consists of a Certificate of Citation and $1,000 in cash, and is sponsored jointly by the Diversey Chemical Corporation, Klenzade Products, Inc., and Pennwalt Corporation. It is administered by the International Association of Milk, Food and Environmental Sanitarians, Inc., and is presented annually. The next presentation of the Sanitarians Award will be made at the 57th annual meeting of the Association which is to be held at Cedar Rapids, Iowa, in August 1970.

The Executive Board of the Association has established the following rules and procedures governing the Sanitarians Award.

Eligibility:

1. *General Criteria*

To be eligible for nomination the Sanitarians Award offered annually by the International Association of Milk, Food and Environmental Sanitarians, candidates must:

a. Have been a member of IAMFES in good standing for a period of five years prior to the date when the Award is to be presented;

b. Be a living citizen of the United States or Canada who, at the time of nomination, is employed as a professional sanitarian in the field of milk, food, and/or environmental sanitation by a county, municipality, state or federal government provided that in the odd years beginning with 1969 the Sanitarians Award will be limited to state and federal employees and the even years to county and municipal employees.

Members of the Executive Board, members of the Committee on Recognition and Awards of the International Association of Milk, Food, and Environmental Sanitarians, and industry members shall not be eligible for the Award. Race, sex or age shall not enter into the selection of the Award recipient.

c. Have made a meritorious contribution in the field of milk, food or environmental sanitation, to the public health and welfare of a county, counties, district, state or federal government with the United States or Canada.

d. Have completed the achievements and contributions on which the nomination is based during the seven-year period immediately preceding January 1, of the year in which the Award is to be made.

2. *Additional Criteria*

a. Co-workers are eligible for nominations if both have contributed equally to the work on which the nomination is based and each independently meets the other qualifications for nomination.

b. Where co-workers are selected to receive the Award, each shall receive a certificate and share equally in the cash accompanying the Award.

c. No person who has received, or shared in receipt of the Award, shall be eligible for renomination for this Award.

Nominations

Nominations of candidates for the Sanitarians Award may be submitted by the Affiliate Associations of the IAMFES, or by any member of the Association in good standing except members of the Executive Board, members of the Committee on Recognition and Awards, and employees of the sponsoring companies. Nominations from persons who are not members of the Association cannot be accepted. No member or Affiliate may nominate more than one candidate in any given year.

Each nomination must be accompanied by factual information concerning the candidate, a resume of his work and achievements, evidence supporting his achievements and if available, reprints of publications. A form for the submission of nominations may be obtained upon request from H. L. Thomasson, Executive Secretary, International Association of Milk, Food and Environmental Sanitarians, Inc., P. O. Box 437, Shelbyville, Indiana 46176.

Submission of Nominations

The deadline for submission of nominations is set annually, and all nominations and supporting evidence must be postmarked priod to midnight of that date. The deadline this year is June 1, 1970. Nominations should be submitted to Dr. A. N. Myhr, Chairman, Committee on Recognition and Awards.

Selection of the Recipient

The Committee on Recognition and Awards of the International Association of Milk, Food and Environmental Sanitarians, Inc., has full responsibility for selecting from among the candidates nominated the recipient of the Sanitarians Award. In judging the contributions of each candidate, the Committee will give special consideration to (*a*) originality of thought, mode of planning, and techniques employed, (*b*) the comprehensive nature of the candidate's achievements, and (*c*) their relative value as they affect the health and welfare of the area served by the candidate. The Committee will give consideration also to the efforts of the candidate to establish professional recognition in the area in which he serves, as well as to his research, administrative development, program operation and educational achievements. Additional information or vertification of submitted information will be requested when considered necessary by the Committee. Testimonial letters in behalf of a candidate are not desired.

If after reviewing the nominations and supporting evidence, the Committee decides that the work and achievements of none of the candidates have been significantly outstanding, the Award shall not be made. In this connection, it is fundamental that if meritorious professional achievement cannot be discerned the Award shall be omitted for a year rather than to lower the standards for selections of a recipient.

Dr. A. N. Myhr
Dairy Science Dept.,
University of Guelph
Guelph, Ontario, Canada

NOTICE TO MEMBERSHIP

In accordance with our Constitution and By-laws which requires our Second Vice-President and Secretary-Treasurer to be elected by mail ballot, you are hereby notified that President Sam O. Noles, at the annual meeting in Louisville, Kentucky, August, 1970, appointed Earl O. Wright, Dairy and Food Dept., 116 Dairy Industry Bldg., Iowa State University, Ames, Iowa 50010 as Chairman of the Nominating Committee for 1970.

Nominations for the office of Second Vice-President and Secretary-Treasurer are now open and any member wishing to make a nomination should send a picture and biographical sketch of his nominee to Mr. Wright not later than March 1, 1970.

Roy Fairbanks, Secretary-Treasurer,
IAMFES, Inc.

CEDAR RAPIDS, IOWA OUR 1970 ANNUAL MEETING CITY

George L. Baldwin, Chairman, Committee on Publicity

The State of Iowa sprawls green and growing for four hundred miles between the nation's two largest rivers, and in this lush valley, the nation grows its food. For more than a hundred years, our black earth has produced in abundance and we have built towns and cities whose prosperity is as permanent as the land itself. And of these cities, none has matched the accomplishments of Cedar Rapids. Its metropolitan population of 178,000 people enjoys an annual per household income considerably higher than other Iowans and well above the national average. Cedar Rapids has been described as a community which has grown beyond the awkwardness of a town cut-up into cliques into a unified city with a community consciousness; beyond antagonism or even tolerance into cooperation and fellowship. For those who have fought alone with heavy opposition or dull unseeing indifference, Cedar Rapids is a new atmosphere in which to live. The people of Cedar Rapids have wanted every institution in their city — a college, a church, a business — to be the best in the state; and they are ready to help make it so. The only thing Cedar Rapids has ever known in its history is a continuous strong rate of growth. We have never been a boom town or suffered setbacks through depression or adversity. The Department of Commerce of the United States referred to Cedar Rapids as one of the most depression proof economies of our United States. We were also described in an article in Life Magazine as one of the four most liveable communities in our country. One of the most difficult jobs we face in Cedar Rapids is to adequately describe the community to outsiders without appearing to be bragging or boasting. We don't delude ourselves by assuming we are the center of the universe, however, we feel that any evaluation of the Cedar Rapids record will document that we are one of the finest spots on the circumference of the earth. We are constantly seeking to improve the quality dimensions in all aspects of our community responsibilities.

HOWARD BROWN ACCEPTS POSITION WITH FLORIDA DEPARTMENT OF AGRICULTURE

The Dairy Division, Florida Department of Agriculture and Consumer Services, have employed Mr. Howard Brown as a chemist in their laboratory in Jacksonville. Howard had previously been with the City of Jacksonville Division of Public Health as Director of Environmental Health since 1943. Pre-

vious to that, Howard was an instructor at Purdue University where he did research work with dairy products and instructed courses in dairy bacteriology and other laboratory courses. Howard got his Bachelor of Science in Agriculture from Iowa State University and his Masters degree in Chemistry and Bacteriology from the University of Illinois.

Howard is a member of the Association of Food and Drug Officials, American Public Health Association, International Association of Milk, Food and Environmental Sanitarians, Inc., and the Florida Association. He is a member of the Canadian Public Health Association, Florida Public Health Association, and the American Dairy Science Association.

Howard resides with his wife in Jacksonville and his son is a sanitarian with the Lake County Health Department.

THE 1970 KENTUCKY FIELDMEN'S AND SANITARIANS' CONFERENCE

The 1970 Kentucky Fieldmen's and Sanitarians' Conference will be held at Mammoth Cave, Kentucky, on February 17 and 18.

Confirmed speakers as this announcement goes to press include:

1. Mr. Ralph C. Pickard, Director of the Division of Environmental Health, Kentucky State Department of Health, Frankfort, Kentucky.
2. Mr. Dan Noorlander, Equipment Research Director of Milk Producers, Inc., Arlington, Texas.
3. Mr. Joe Johnson, Director of Government Relations of Milk Producers, Inc., Little Rock, Arkansas.
4. Mr. Floyd Fenton, Chief of Standardization Branch (Dairy Division), Consumer and Marketing Service, USDA, Washington, D. C.
5. Mr. Russ Rooks, Field Operations Director, Interstate Milk Producers Cooperative, Philadelphia, Pennsylvania.
6. Mr. Paul Freebairn, Director of Special Chemicals Department, Pennwalt Corporation, Philadelphia, Pennsylvania.
7. Mr. George Turner, Ag. Engineering Specialist, University of Kenutcky, Lexington, Kentucky.

An awards luncheon is scheduled for February 18 to recognize the 1970 outstanding Kentucky Fieldman, sanitarian, and dairy industry man.

For further information, please contact Dr. C. Bronson Lane, 104 Dairy Products Building, University of Kentucky, Lexington, Kentucky 40506.

NEWS AND EVENTS

DAIRY AND FOOD ENGINEERING CONFERENCE KELLOGG CENTER UNIVERSITY OF MICHIGAN

Dairy and Food Engineering Conference, to be held on February 24 and 25, 1970, at the Kellogg Center for Continuing Education, Michigan State University. This conference is sponsored by the Departments of Agricultural Engineering and Food Science and the program is developed through cooperation with the food industry, government and other educational agencies. The primary objective of the conference is to provide a forum for discussion of the latest engineering developments of interest to the food industry.

The following topics wil be the themes for the four half-day sessions of the conference:

1. Instrumentation and Process Control
2. New Developments in Pumping of Liquids and Suspensions
3. Batch and Continuous Blending and Mixing Systems
4. New Developments in Heat Exchangers.

Each half-day session has been planned to cover a broad range of information from basic concepts to discussion of specific developments in the area of the theme. In most cases, the speakers have been selected, based on the knowledge and experience with the subject matter being discussed.

A proceedings of the conference will be published shortly after the conference is completed. These proceedings are made available to all conference participants and to others who may request directly from the Continuing Education Service at Michigan State University.

DAIRY SOCIETY INTERNATIONAL MOVE APPROVED

At Dairy Society International's Twenty-third Annual Meeting, held in New Orleans on November 2, near-final action was taken on a plan which has been in the making for more than a year. This is to broaden the milk producer support for DSI by including the National Milk Producers Federation, as well as American Dairy Association, which currently is carrying half of the Society's budget. The Federation support will be largely "in kind," consisting of the furnishing of Washington headquarters and supplying a wide range of services. ADA will continue financial support, but at a reduced rate. In return, DSI will handle, for the two organizations, certain international activities.

The Board of Directors of DSI, after being informed that the plan had been approved by the Federation's Executive Committee (ADA had approved it a year ago), voted to confirm the arrangements, as did the membership at its meeting later that day. The timing of the physical move of the Society into the Federation's building at 30 F Street, Northwest, has been left open, since the current lease of the offices on 19th Street has until May to run. The move will mean severing the day-to-day ties with Dairy and Food Industries Supply Association — next to ADA the Society's largest supporter — which has always provided many facilities (printing, switchboard, etc.) for DSI. Representatives of DFISA companies, however, continue as active members of the DSI Board of Directors.

The Society will retain its name, its corporate identity, and its Board of Directors, but the Board, instead of remaining the Society's governing body, will serve in an advisory capacity to an ADA/NMPF nominated Executive Committee. As reported to the membership by Lyman D. McKee, chairman of the special Industry Support Coordinating Committee, the Executive Committee "will control budgets, fix policy, generally supervise DSI activities and periodically report to ADA and Federation Boards."

VENDO COMPANY EXECUTIVE RECEIVES AWARD AT NATIONAL CONVENTION IN NEW ORLEANS

George H. Hansen, vice president, manufacturing, of the Vendo Company tonight was honored here with the Arthur J. Nolan Award at the annual banquet which concluded the Convention-Exhibit of Automatic Merchandising. Established by the National Automatic Merchandising Association to recognize outstanding performance in the field of public health, the award has been presented only twice before.

In honoring Hansen, William H. Martin, president of the association, cited his contribution to vending industry sanitation programs over a number of years. He said Hansen helped to establish the U. S. Public Health Service Code on the sanitation of vending machines and the associations' vending machine evaluation program. He also was chairman of the manufacturers' division of the association's Public Health Committee for a number of years.

Nolan, after whom the award is named, helped pioneer the long-standing public health standards of the vending industry.

EMERGENCY HEALTH SERVICES DIGEST AVAILABLE

The response to the first issue of a new HEW periodical, *Emergency Health Services Digest*, by a test group of physicians and other professionals has been so favorable that the publication has been released for distribution to all interested in emergency services. Published by the Health Services and Mental Health Administration's Division of Emergency Health Services, the first issue contains summaries of 57 articles selected from current professional literature. Each article deals with some aspect of planning, programming, training or delivery of emergency medical and health services.

The *Digest* is available from the Public Information Office, Division of Emergency Health Services, 6935 Wisconsin Avenue, Chevy Chase, Maryland 20015. It may be purchased from the U. S. Government Printing Office, Washington, D. C. 20402, at 40 cents per copy.

SEMINAR NOTICE

The Departments of Food Science and Technology and Agricultural Engineering at the University of Massachusetts will sponsor a seminar "Heat Processing of Foods-Sterilization and Nutrient Retention with Computer Applications." The seminar will be held in Chenoweth Laboratory on March 23 to March 27, 1970.

Seminar registrants will be limited to fifty in number. University staff, and industry experts will comprise the seminar instruction staff. A registration fee will be charged.

Further details may be obtained from Professor Kirby M. Hayes or Dr. Charles R. Stumbo, Department of Food Science and Technology, University of Massachusetts, Amherst, Mass. 01002.

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The minerals calcium and magnesium in water cause it to be hard. The greater the concentration of these minerals the *harder* the water. They combine with soaps to form scum and with milk residue in milking machines and bulk tanks to form milkstone deposits. These deposits in the pipeline offer a breeding ground for bacteria. Also, hard water can cloud and deposit mineral film on equipment surfaces, causing stains which are difficult to remove. Hard water greatly affects the cleaning efficiency of detergents, causing increased usage and higher costs.

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