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NO. 3

MARCH, 1957

*Journal of*

# MILK and FOOD TECHNOLOGY

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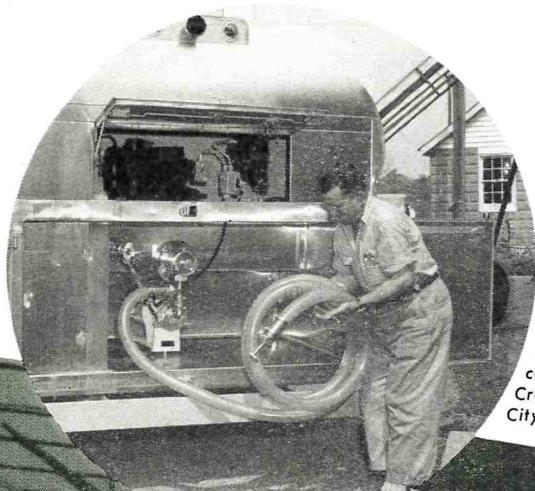
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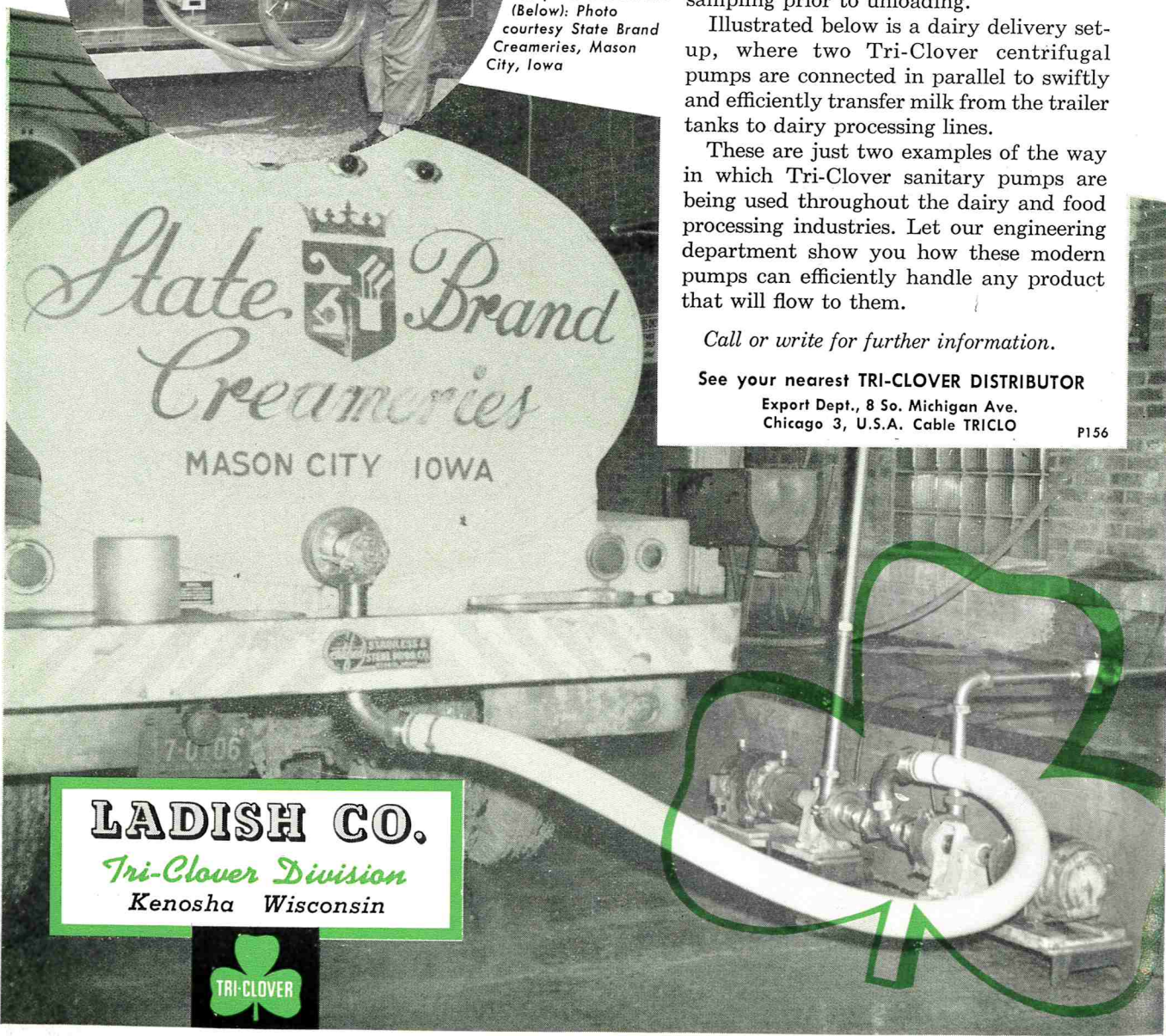
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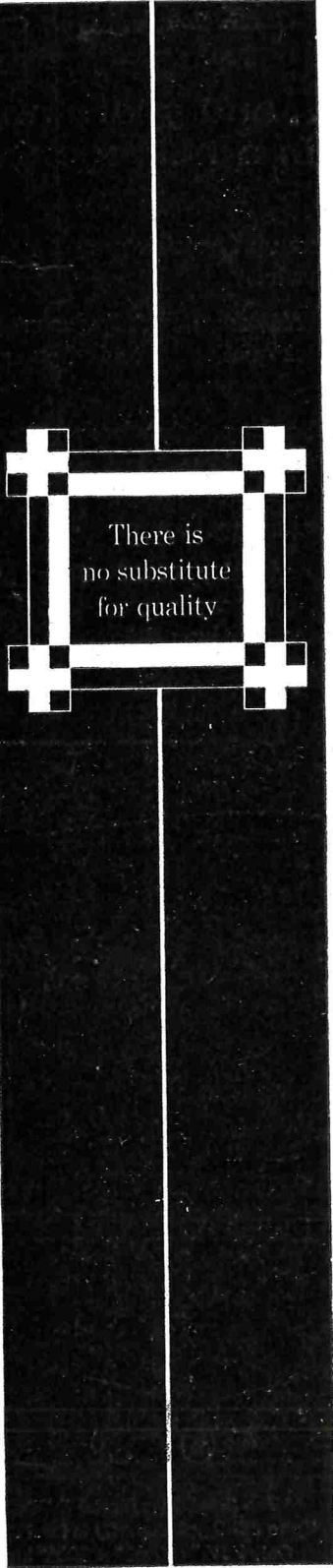


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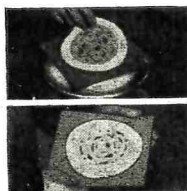




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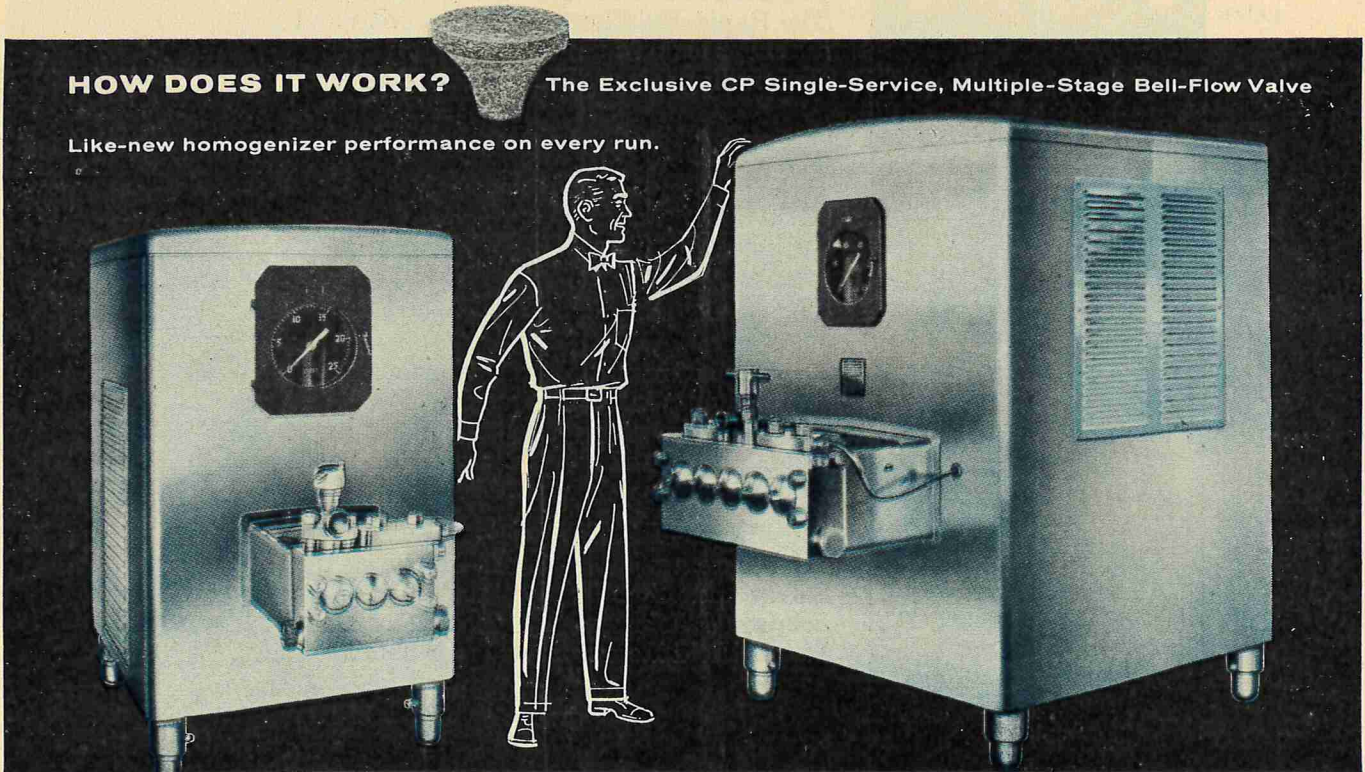
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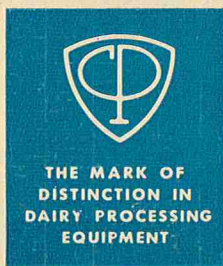
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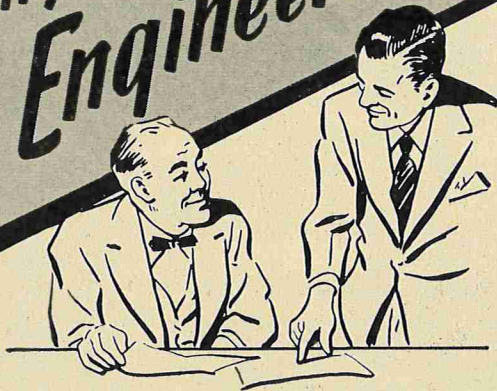


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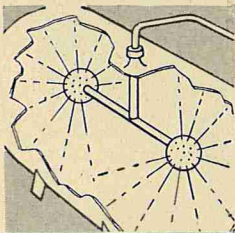
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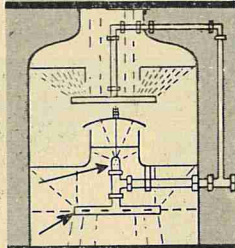


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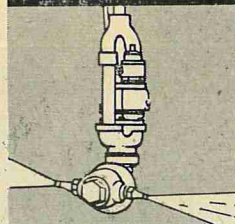
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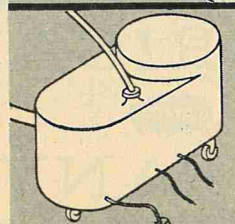
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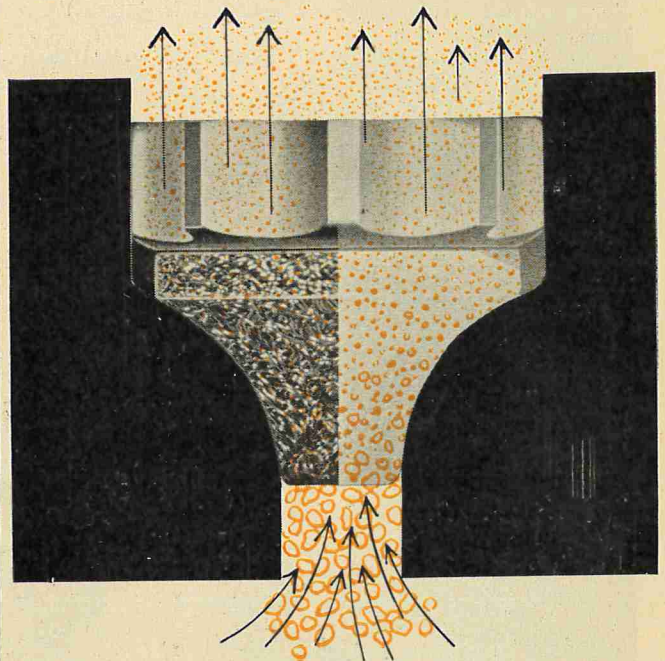
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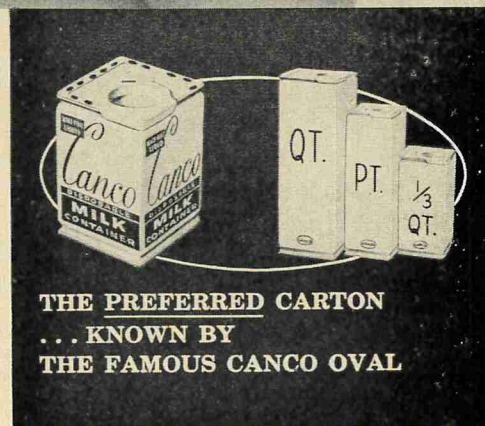
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INCLUDING MILK AND FOOD SANITATION

*Official Publication*

International Association of Milk and Food Sanitarians, Inc.

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Vol. 20

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No. 3

*Contents*

	<i>Page</i>
Public Health Aspects of Food Poisoning <i>Elizabeth Wilson, Milton J. Foter</i> and <i>Keith H. Lewis</i> .....	65
Bacteriology of Milk Held at Farm Bulk Cooling Tank Temperatures I. Effect of Time and Temperature During Storage, Farm Source of Milk and Season of Year. <i>E. H. Marth and W. C. Frazier</i> .....	72
Bacteriology of Milk Held at Farm Bulk Cooling Tank Temperatures II. Effect of Numbers in the Original Milk. <i>E. H. Marth and W. C. Frazier</i> .....	79
Virucidal Activity of Hypochlorites, Quaternary Ammonium Compounds, and Iodophors against Bacteriophage of <i>Streptococcus Cremoris</i> . <i>Sprague H. Watkins, Helen Hays</i> and <i>P. R. Elliker</i> .....	84
Affiliates of IAMFS, Inc. ....	88
News and Events .....	89
Index to Advertisers .....	X

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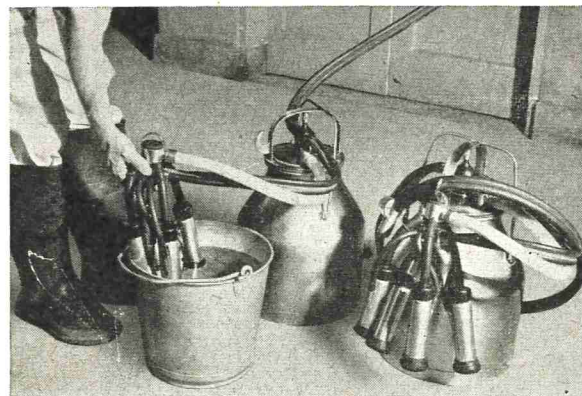
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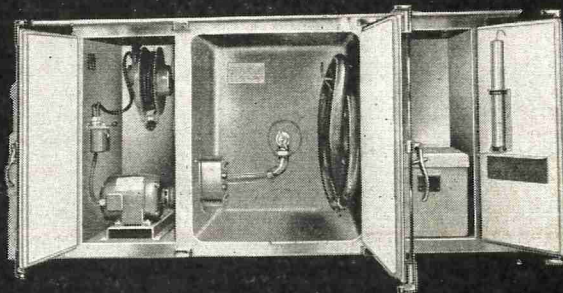
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## PUBLIC HEALTH ASPECTS OF FOOD POISONING

ELIZABETH WILSON, MILTON J. FOTER, AND KEITH H. LEWIS

Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio

(Received for publication September 19, 1956)

### INTRODUCTION

Widespread practice of food sanitation undoubtedly has contributed greatly to the remarkably low morbidity and mortality rates experienced in the United States during the recent years. There is probably less danger today of contracting disease from food in this country than in almost any other part of the world. However, a summary of disease outbreaks (11) compiled by the Public Health Service from data supplied by State Health Departments show that foodborne disease still occurs much too frequently. In 1954 there were reported 250 food or waterborne outbreaks with more than 12,000 cases. Of these, only 7 outbreaks with 452 cases were waterborne, 9 outbreaks with 200 cases were milkborne, and the remainder, 234 outbreaks with 11,700 cases were caused by ingestion of other foods.

It should be emphasized that this probably represents only a small portion of such cases occurring in the United States. There are, doubtless, many individual cases and family-size outbreaks of non-fatal illness which are not reported and never come to the attention of a physician. In many states, food poisoning is not a reportable disease and cases attended by a physician often do not reach the vital statistics records of the State Health Departments (37). Hence, any estimate of the incidence of food poisoning outbreaks based on official reports is certain to be very low in comparison to the actual number of cases occurring.

Among the total cases of established etiology, including salmonellosis, botulism, staphylococcal intoxication, and toxic chemical agents, 77 per cent were due to staphylococcal food poisoning. Next in incidence was salmonellosis which accounted for 18 per cent of the cases reported. Eight outbreaks of botulism were reported in 1954, and only one of these was attributed to a commercially prepared product (11). The improperly home-canned foods are, at the present time, the most common sources of botulism in the United States. Modern commercial canning procedures, the outgrowth of intensive research during the past 50 years, have reduced to rare instances the occurrence of botulism due to eating commercially canned food (37).

Unfortunately, staphylococcal and salmonella con-

taminations in perishable foods are less effectively controlled at the present time. Salmonella organisms of animal origin frequently contaminate meat through exposure to fecal material in slaughter houses. The investigations of Galton and her co-workers have indicated that *Salmonella* species are present in iced poultry ready for market (18) and in packaged sausage obtained from retail dealers (17). Salmonella have been demonstrated also in many spray-dried egg powders (42). The danger of infecting man increases when the reconstituted food mixture is allowed to stand at temperatures which permit multiplication of the bacteria. Another source of danger is the food-handler who is a salmonella carrier (22).

In the case of staphylococcal food contamination, the food-handler is very often the source of the microorganisms. Staphylococci occur commonly in skin lesions on the hands and face, in the nasal passages, and in the dust of clothing and floors (9); thus foods may readily be contaminated by this group of organisms. When these foods are not properly refrigerated, the staphylococci multiply and may produce the heat-stable enterotoxin responsible for inducing the food poisoning symptoms. The types of food most often incriminated are custards, cream-filled baked goods, ham, meat pies, poultry and milk products (8, 9). It is of prime importance that these foods be cooled rapidly and that they not be held for any appreciable time at temperatures much above 5°C or 41°F. The significance of adequate refrigeration for retarding bacterial growth in food is obvious, but is too often regarded with indifference.

Outbreaks of food poisoning caused by *Clostridium perfringens (welchii)* have rarely been reported in the United States. However, in the past decade they have been reported with increasing frequency in Great Britain (10, 23, 24). In the years 1951 and 1952, five per cent of the total cases of foodborne disease in England and Wales were attributed to food contaminated with this anaerobic spore-former. In most of these incidents the incriminated food was a meat preparation which had been stored many hours without adequate refrigeration after cooking. The anaerobes isolated from these foods were characterized by spores which were viable after exposure to boiling temperature for periods of one to five hours (24).

Other bacteria reported to be responsible for food

poisoning include the fecal streptococci, some shigella, and aerobic spore-formers, as well as paracolon, coliform, and proteus strains. The incidence of outbreaks caused by these organisms is relatively low in the United States, and in some instances their causative role has not been well established.

In a high percentage of foodborne disease outbreaks, the etiological agent is never satisfactorily established, and even when staphylococci are present their toxigenicity is often not demonstrated. Thus, from the public health point of view, one of the needs in dealing with the food poisoning problem is the development of practical laboratory procedures to supply more rapid and accurate identification of bacterial contaminants in foods.

In addition to foodborne disease of microbial origin, poisoning may also result from the ingestion of food containing toxic chemicals. Although chemical poisoning is rare as compared to that induced by microorganisms and their metabolic products, symptoms usually appear sooner and the illness is more prolonged. However, some of the symptoms produced by these toxic chemicals may be confused with those of microbial food poisoning (10, 21).

Detailed discussion of all phases of food poisoning is, of course, beyond the scope of this paper. The remarks that follow are intended to focus attention on a few of the important problems with which the Public Health Service is especially concerned at this time. A number of equally interesting and worthwhile topics have been omitted in the interest of brevity.

#### STAPHYLOCOCCUS FOOD POISONING

The culture media most commonly used for selective growth and characterization of staphylococci have the drawback of requiring a long incubation period (5, 52). After these media have been inoculated with the suspected food sample, incubation for 24 to 48 hours is required before sufficient growth is obtained to determine the presence of staphylococci. The result does not provide evidence of toxigenicity. The methods available for the demonstration of staphylococcal enterotoxin are even more cumbersome, time-consuming, and of questionable specificity. The use of human volunteers for this purpose is dangerous, often impractical, and not completely specific, since it has been observed repeatedly that the susceptibility of human volunteers to staphylococcal enterotoxin is quite variable (8, 37).

The laboratory animals used most extensively to demonstrate the presence of enterotoxin are the rhesus monkey and the cat (8, 10, 36). These are difficult to procure on short notice and are expensive to main-

tain. Since newly caged cats are very susceptible to infection, it is necessary to maintain them in quarantine during a course of antibiotic therapy and distemper immunization. Both the cats and monkeys are often difficult to handle, and again the specificity of their reactions is not entirely reliable. A more satisfactory test animal is urgently needed.

The use of other laboratory animals to demonstrate the presence of enterotoxin has met with little success. Among those tried are frogs, mice, white rats, guinea pigs, rabbits, dogs, and canaries. Many of these species do not have a vomiting reflex which is the criterion of reaction in man, monkeys, and cats; therefore, diarrhea has been used as the criterion (8). However, since diarrhea may be induced by a number of causes, its occurrence as an indicator of the presence of enterotoxin results in a test with unsatisfactory specificity. The use of animal reactors such as minnows, brine shrimp, and larvae is being considered since they have the advantages of availability in large numbers and ease of procurement and maintenance. As yet a demonstration that they respond in a specific, typical killing curve to known concentrations of enterotoxic filtrate is lacking.

An assay which can be performed quickly, with simple equipment, and with a high degree of reliability is urgently needed. Attempts to circumvent the deficiencies of bioassay procedures have been made in a variety of research organizations concerned with food technology, microbiology, and sanitation. The status of some of these investigations will be considered briefly.

At the Robert A. Taft Sanitary Engineering Center investigations are being directed toward the rapid presumptive identification of bacterial contaminants in foods. The aim of these studies is to develop methods which will permit screening of suspected foods in a period of 6 to 8 hours, thus making possible a presumptive identification of the bacterial contaminant during the workday in which a food sample is received. Such a method for detecting staphylococci is being tested for its effectiveness with different types of foods and with staphylococci of various cultural and metabolic characteristics. By this method it is possible to determine, after a few hours of incubation, the coagulase-producing capacity of the staphylococcus concerned. There is a high degree of correlation between the production of coagulase and enterotoxin. This test by no means replaces the need for a demonstration of the presence of enterotoxin, but it does serve as a rapid screening procedure.

It has long been believed that the presence of enterotoxin in an extract of suspected food or in a staphylococcal growth filtrate might be demonstrated

by serological means. There is evidence that experimental animals develop a tolerance to the administered enterotoxin. Some investigators have believed that antibodies are formed which could be used in a serological test, but investigations in this area have been only partially successful. Surgalla, Bergdoll, and Dack (46) used the antigen-antibody reaction in agar to follow the progress of purification of the enterotoxin. However, efforts to protect experimental animals with an enterotoxin-induced antibody have met with limited success (8).

The greatest deterrent to the development of assay procedures lies in the fact that a purified and chemically defined enterotoxin is not available. Although the material has been studied for some time, only preliminary reports of its purification and characterization have been published (1, 2, 12, 48). Conversely, studies on purification and antigenicity are made difficult by the lack of suitable assay procedures. It would be most desirable to develop a method of assay not dependent upon biological reactions, as are either an experimental animal reactor or a serological test. An ideal assay would be one in which a specific chemical reaction occurred between the enterotoxin molecule, or some portion of it, and a readily available and easily handled reagent. Such a test, naturally, awaits additional information as to the chemical nature of the enterotoxin.

In addition to these studies designed to establish the identity and toxigenicity of staphylococci found in food, other investigations have been directed toward a method of classifying staphylococci (4, 49, 50, 51) which is useful in the epidemiological investigation of food poisoning. This method of classification is based upon the susceptibility of staphylococci to bacteriophage.

Staphylococci which have been isolated from the food eaten by victims of an outbreak and those isolated from fecal material and/or vomitus of patients are typed by this means. If the staphylococci from these two sources are in the same phage grouping, these microorganisms are presumed to have a causative relationship to the food poisoning case. Persons who handled the food are also examined for the presence of exposed suppurative lesions and nasal swab examinations are made. The staphylococci thus isolated are subjected to the phage-typing procedure. The presence of staphylococci of the same phage type in a patient, in the food, and in a handler of food that has been incriminated in an outbreak is considered strong connective evidence. This procedure is being used extensively in epidemiological studies of food poisoning by the Department of Health of the City of New York. The Public Health Service has recently

added phage-typing to the laboratory services offered by the Communicable Disease Center at Atlanta, Georgia.

#### SALMONELLA FOOD INFECTION

The investigations devoted to the study of salmonella food infections have been less varied than those for the staphylococci. The illness developing from salmonella poisoning is a true infection induced by the presence of a large microbial population, rather than by the activity of a preformed toxin like that produced by the staphylococci. Hence the studies dealing with the Salmonella problem have centered about the improvement of growth media for their isolation and techniques for their identification (13, 25, 30, 40, 45).

The serological identification of *Salmonella* species is an intricate procedure requiring training, much practice, and special materials. In order to accomplish a thorough epidemiological study, detailed typing of the Salmonella found in food is indicated. It is impractical to consider complete typing in small city or county laboratories, but such service is available in the laboratories at the Communicable Disease Center in Atlanta, and at many State Health Department laboratories.

A particularly promising development related to the identification of Salmonella as a genus, and not as individual species, has been reported by Cherry *et al.* (6). This procedure relies upon the susceptibility of the members of the Salmonella group to a bacteriophage. The use of this technique would serve as a rapid method to screen isolates from large numbers of food samples.

In a discussion of "Salmonella as a Food Industry Problem", Hinshaw and McNeil (22) have indicated as the principal preventive measures the following: (a) location and elimination of animal reservoirs, (b) prevention of transmission by human carriers, and (c) storage and refrigeration.

Recent publications by Galton and her co-workers (17, 18, 20) have presented studies of the incidence of Salmonella in poultry, swine, cattle, and in certain meat products, as related to their transmission by food. Their studies on Salmonella in dogs (19) suggest the possibility of transmission by animal carriers that are household pets. They have demonstrated in these animal reservoirs many *Salmonella* species that are pathogenic for man. The actual inducement of experimental human disease with known oral doses of *Salmonella* species isolated from food has been reported by McCullough and Eisele (31, 32, 33). They have established the oral doses of *Salmonella newport*, *S. derby*, *S. bareilly*, *S. meleagridis*, *S. anatum*,

and *S. pullorum* that cause illness in man. These organisms were originally isolated from spray-dried whole eggs.

#### SHELLFISH POISONING

Two types of research relating to the transmission of disease by shellfish (clams and oysters) currently are being performed by the Robert A. Taft Sanitary Engineering Center. The first is being conducted at a branch marine laboratory in Pensacola, Florida. The investigations concern the relation of pollution in the water of growing areas to the sanitary quality of oysters, the survival of enteric bacteria in these shellfish, and the effect of commercial processing practices on their bacterial content (27).

The second study is concerned with the paralytic shellfish poison formed in certain ocean mussels and clams that are found on the Pacific Coast of the United States, Canada, and Alaska, as well as in the Bay of Fundy and Gulf of St. Lawrence. The poison is not a metabolic product of the shellfish, but is formed by the dinoflagellate, *Gonyaulax catenella* on which the mollusks feed (37, 43). This toxic material has caused death in man when only a few of the toxic shellfish have been ingested. The minimum lethal dose for man, as determined by epidemiological investigations, is probably between 20,000 and 40,000 mouse units, i.e., approximately four to eight milligrams. The research studies referred to are devoted to the improvement of assay procedures. Work is being conducted on the preparation, distribution, and use of an international reference standard for the mouse bioassay. A chemical assay procedure is also being developed for use in areas where mice are not available or are difficult to maintain in suitable condition for a routine testing program (34).

#### CHEMICAL POISONING

The symptoms of nausea and vomiting which develop in a few hours or less after ingestion of foods contaminated with antimony, arsenic, barium carbonate, and lead may be confused with the similar symptoms of staphylococcus food poisoning. The abnormal function of muscles of the eye observed in poisoning by methyl chloride, methyl alcohol, and sodium fluoride have sometimes led to its confusion with botulism. When poisoning results from the inhalation of methyl chloride as it escapes from leaking mechanical refrigerators, the symptoms may be confused with food poisoning since many persons in a household become ill within a short time (10).

Poisoning from antimony, cadmium and zinc as a rule has resulted from eating food prepared in cooking

utensils constructed of material containing these metals. Occasionally chemical poisons have been added to food accidentally because they were stored near food constituents which they resembled in appearance. Incidents of chemical poisoning resulting from such accidents have occurred when sodium fluoride, an insecticide, was used in place of baking soda and when barium carbonate, a rodenticide, was used instead of flour to prepare pastry. The belief that nickel, aluminum, copper, and tin from utensils or containers have been responsible for food illness has not been upheld by scientific investigation (10). However, a recent report implicates copper used in coloring for cake frosting in 4 cases of poisoning (29).

Aside from the hazards associated with toxic chemicals which enter foods accidentally, poisoning from materials used in processing and preserving foods and sanitizing of equipment is a possibility.

Protection of the public health from such food contamination is principally an activity of the Food and Drug Administration, based on provisions of the Food and Drug Act of 1906 (14) and the Food, Drug, and Cosmetic Act of 1938 (15). The use of chemical preservatives and coloring matters in foods is controlled, and permissible fixed limits for certain ingredients have been specified. For example, tolerances have been established for lead and arsenic contents of sprayed fruit and vegetables (47), and for antibiotic (chlortetracycline) residue in uncooked poultry (16). Constant evaluation of newly developed compounds used in producing and processing foods is required. In this connection, the Public Health Service has cooperated in research activities designed to evaluate the effects of ingesting such materials.

#### CONTROL AND PREVENTION OF FOOD POISONING

##### *Recognition and reporting*

Many of the research activities which have been discussed here are concerned with diagnostic laboratory procedures. However, public health aspects of food poisoning are not, by any means, limited to laboratory diagnosis.

One of the problems which should be studied is the development of a procedure for more adequate coverage and reporting of foodborne outbreaks. Also essential is educating the public to recognize the symptoms of food poisoning and the value of reporting them to a family physician or public health authorities. It is desirable that suspected outbreaks, even though they are of individual or family size, be reported to public health authorities immediately so that an effective epidemiological investigation can be made (7). Reliable laboratory examinations can be made only on

food specimens that are relatively fresh and have not been exposed to contamination in garbage disposal containers. Food suspected of being associated with poisoning outbreaks should be held at refrigeration temperature until it can be examined in the laboratory.

#### *Control of food handlers*

Essential to the control of food poisoning is preventing transmission of the causative organisms to food by human carriers. Care should be taken to avoid employing as food handlers persons known to have a history of an unresolved carrier state or those with active infectious disease. Although periodic physical examination of food handlers was at one time recommended and practiced, currently its value as a sanitation measure is questioned. Because of the fluctuating pattern of shedding microorganisms in subacute disease, the value of periodic examination of fecal specimens of food handlers is doubtful except when recent cases of gastro-intestinal illness are involved or the presence of a carrier is suspected. Training programs for food handlers stressing personal cleanliness and its relation to foodborne disease have proved effective means of improving food handling practices.

#### *Food sanitation practices*

The spread of disease by foods can be related directly to the sanitary quality of foods and the conditions under which they are processed, stored, distributed, and served. Improvement in the sanitary quality of foods by application of good sanitation practices will minimize the health hazards. Failure to cook food thoroughly, refrigerate it adequately, or sanitize equipment properly are among the most commonly violated sanitation practices.

The Public Health Service assists the health departments of municipalities, counties, and states in food sanitation control by developing model ordinances, codes, and operating manuals. Their provisions have the force of law only when adopted by the state or local governments having jurisdiction in a particular area. Further, the Public Health Service is legally responsible for sanitation aboard interstate carriers such as railroads, airlines, and ships. Permissible practices are defined in the Interstate Quarantine Regulations (26) and compliance is obtained through consultation, training, and cooperative planning with the carriers, as well as by direct enforcement involving inspection and rating.

The model ordinance which is best known and most widely used is that for milk (38). The topics with which it deals include the prohibition of the sale

of adulterated, misbranded, or ungraded milk and milk products; the inspection of dairy farms and milk plants; the grading of milk, its pasteurization and various aspects related to handling. Provisions for sanitary practices are proposed which aim at reducing contamination of milk at the source of supply, in transit, and in preparation for distribution to the consumer. The provisions made for determining the extent of bacterial contamination serve to evaluate the sanitary quality of the product as it reaches the consumer. Appendix F of this ordinance which deals with bactericidal treatment of utensils and equipment has been recently modified to incorporate new technical developments in chemical germicides (28).

The Public Health Service also assists with the Interstate Milk Shipment program. In states participating in the interstate shipment of milk, the central laboratory of the State Health Department is visited periodically by an official of the Robert A. Taft Sanitary Engineering Center. Each state is visited about once in three years. The Public Health Service representative evaluates the performance of each laboratory in terms of its conformity with Standard Methods for the Examination of Dairy Products (44). In turn then, the State central laboratory evaluates the performance of its own branch laboratories. Some states request that the Sanitary Engineering Center participate in this program by examining split-samples of milk which are being sent to the branch laboratories (3).

The ordinance and code regulating eating and drinking establishments recommends practices designed to encourage uniformity and effective levels of sanitary control in these establishments (39). One form provides for the grading of restaurants, while an alternative form provides only a single set of minimum standards. The recommendations provide that the establishment failing to meet the minimum standard requirement lose its permit to operate. The adoption of all or any portion of this code is optional on the part of the municipality or state.

The Public Health Service also provides a Manual of Recommended Practice for Sanitary Control of the Shellfish Industry (35) which is used by all shellfish-producing states that cooperate with the Public Health Service in the certification of shellfish producers.

The most recent model ordinance developed by the Service is the Poultry Ordinance published in 1955 (41). It provides for the inspection of poultry and the prohibition of the sale of adulterated or misbranded poultry and poultry products. Attention is directed to packaging and processing with emphasis upon sanitary practices in processing plants. An ordinance directed toward this type of food is particularly pertinent because of the marked increase in poultry consumption over the past 15 years (41). Ordinances relating to

dried milk and food and beverage vending machines are in the process of being drafted.

#### SUMMARY

Despite widespread practice of food sanitation in the United States in recent years, foodborne disease is still prevalent. Better control of the problem is visualized when (a) improved recognition and reporting make possible more effective epidemiological investigations, (b) improved laboratory techniques facilitate detection of contaminants in foods, and (c) good sanitation practices in processing, storing, distributing, and serving food are more widely adopted.

The Public Health Service is cooperating with other public and private agencies to achieve this goal through its programs of education, research, and technical services, and by developing model ordinances, codes, and operating manuals as guides for sanitation control.

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# BACTERIOLOGY OF MILK HELD AT FARM BULK COOLING TANK TEMPERATURES

## I. EFFECT OF TIME AND TEMPERATURE DURING STORAGE, FARM SOURCE OF MILK AND SEASON OF YEAR<sup>1 2</sup>

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Raw milk samples from different farms were held at 36° F., 38° F., 45° F., and at 38° F. with periodic warming to 45° F. or 50° F., and standard, thermoduric and psychrophilic plate counts were made daily for four days. Little increase in bacterial numbers took place in three days at 36° F. At higher temperatures significant increases occurred in part of the samples within one or two days. A 45° F. storage temperature was too high. No correlation was found between farm source of milk and growth of bacteria. Psychrophiles grew better in summer than in winter milk.

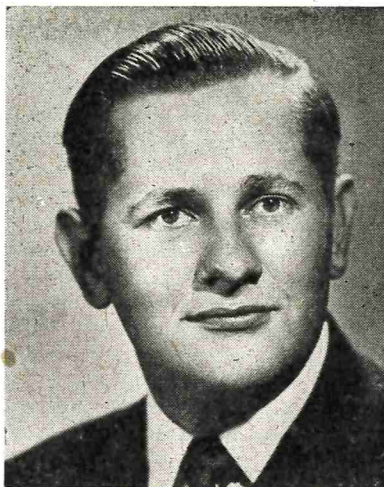
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The advent of the handling of milk in bulk on the farm has drawn attention to the growth of bacteria in milk held at low temperatures in farm coolers. The presence and subsequent growth of these bacteria may seriously affect the quality of the milk as it is collected at the farm and even may cause its rejection.

Bulk milk handling has already become an important operation on many farms. Although milk produced by the bulk method usually is picked up daily at the farm, economic pressures have brought about a movement toward every other day or even less frequent pick-up of milk.

Results of Hunter (5) and Marth, Hunter and Frazier (8) showed that milk of high quality (initial average bacteria count of 4,700 per ml.) could be held successfully in farm bulk cooling tanks for every-other-day pick-up. There was no assurance that good results could always be obtained with milks produced on different farms and that all milks with a low initial bacteria count would remain in good bacteriological condition during the storage period in the bulk cooling tank.

Ayers, Cook and Clemmer (2) found that when three different grades of raw milk were stored at 40° F. the following occurred: (a) the increase in numbers of bacteria varied in different milk samples with about



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the same initial bacteria count; (b) raw milks with an initial average of 4,295 bacteria per ml. showed an average two-fold increase in numbers of bacteria after three days and an average four-fold increase after four days; (c) milks with an average of 39,082 bacteria per ml. showed a six-fold increase after three days and a 27-fold increase after four days; and (d) milks with an average of 136,533 bacteria per ml. showed an average two-fold increase after two days, a five-fold increase after three days, and a six-fold increase after four days of holding.

Prouty (9) collected raw milk samples from three farm tanks after the fourth milking, incubated them for one and two days at 37° to 39° F., and made counts of "facultative psychrophilic bacteria" as well as standard plate counts. Psychrophilic counts increased more rapidly than did standard plate counts, but were not excessive after 24 hours of storage. After 48 hours

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a fourth of the samples had psychrophilic counts in excess of 100,000 per ml.

Claydon (3) reviewed previous reports on differences in growth of bacteria in summer and winter milk resulting from actual differences in the milk rather than higher temperatures and greater contamination in the summer. Claydon found poorer growth at 50° F. in sterilized and inoculated winter milk than in summer milk, agreeing with other workers who had found the summer milk to be the better culture medium.

Greene and Jezeski (4) have defined a psychrophilic bacterium as one that can proliferate in a relatively short time under refrigerator conditions. This definition will be accepted for the following discussion.

The experiments reported in this paper were on the changes in numbers of bacteria in raw milk held at farm bulk cooling tank temperatures as affected by: (a) time and temperature of storage; (b) the farm source of the milk; and (c) the season of the year. Other papers will discuss the effect of the initial numbers of bacteria in the milk (6), and the kinds of bacteria present and able to grow in raw milk held at 38° F. (7).

#### METHODS

##### *Sources of milk*

Milk for most of this study came from three private farms in the Madison area and a University farm. Additional samples from three other farms and another University farm were tested when holding was at 38° F. Farms where milking was done in a parlor and a pipeline milker was employed, were selected to make up six of the eight farms and all four of the farms from which samples were obtained for most of the tests, because it was thought that the pipelines often employed with a bulk cooling tank might be sources of additional contamination. The other two farms used bucket-type milking machines and cans; the cows were milked in the stanchion.

On all farms the udders and teats of the cows were wiped with a wet cloth; five farms used chlorine solution, and three used detergent solution, quaternary ammonium compound and water, respectively. The procedures for cleansing and sanitizing pipelines, milking machines and other utensils differed considerably from farm to farm, and varied from excellent to poor. Some used only alkaline detergent; others substituted an acid detergent periodically. Some sanitized with 200 p.p.m. chlorine solution, others with "hot" water at various temperatures, and others combined the two treatments. At five of the six farms with pipe-

lines they were dismantled and brushed at varying intervals. These differences in procedures at the various farms should result in the production of milks that were different bacteriologically.

Care was taken in all experiments to avoid the use of milk which might have contained antibiotics.

##### *Incubation of milk at temperatures of farm bulk cooling tanks*

A home freezer with a capacity of ten cubic feet was used to cool water in two glass baths, with continuous agitation by a power stirrer. Two magnetic stirring motors under each bath agitated milk samples, and a 600 watt heater and a thermostat in each bath served to control the temperature within plus or minus 0.1° F.

*Incubation at 36° F., 38° F. and 45° F.* About 300 ml. of raw milk were collected in a 500 ml. Erlenmeyer flask at the farm prior to cooling, packed in ice and brought to the laboratory within 45 minutes. The milk had cooled to 65° - 70° F. by the time it arrived at the laboratory. Standard, thermoduric, and psychrophilic plate counts (1) were made on the fresh milk. Petri plates for standard and thermoduric plate counts were incubated at 32° C. (89.6° F.) for two days and those for psychrophilic plate counts at 10° C. (50° F.) for 14 days.

The milk was incubated for four days at 36° F., 38° F. or 45° F. in the previously described equipment and a daily standard plate count (SPC), thermoduric plate count and psychrophilic plate count (PPC) were made. When milk was first placed in the bath it was stirred two hours both to insure rapid cooling to the desired temperature and to stimulate the two hour agitation of milk when it first enters the farm bulk cooling tank. Fifteen to 20 minutes of cooling brought samples of milk to the desired temperature. Milk samples were agitated for two minutes prior to sampling for plating, for experiments had shown that a representative sample could be obtained after one minute of agitation. Marth, Hunter and Frazier (8) had similar results on sampling milk in two farm bulk cooling tanks.

Studies were made on the bacteriology of samples of raw milk held at various storage temperatures for four days, rather than to try to simulate the addition of seven succeeding milkings, so as to avoid the complications and uncertainties involved in the introduction of seven new lots of milk that probably would differ from the original milk in both numbers and kinds of bacteria. It was realized, of course, that continued incubation of one sample was likely to result in greater numbers at any time after 12 hours than

when successive samples of fresh milk were added.

*Incubation at 38° F. with periodic increases to 45°*

*F.* The procedure for incubation of milk at 38° F. was followed except that at ten hour intervals stirring and warming of the milk was begun. The temporary warming of the milk was accomplished automatically by means of a time clock to start and stop stirrers and heaters at the desired times. The milk samples were warmed to 45° F. in ten minutes and held there for 110 minutes while being stirred constantly. The milk samples were cooled back to 38° F. in 20 minutes and held there for nine hours and 40 minutes when the cycle was repeated. This experiment was designed to simulate changes in the temperature of milk in farm bulk cooling tanks that occur when fresh warm milk is added at successive milkings as reported by Marth, Hunter and Frazier (8).

*Incubation at 38° F. with periodic increases to 50°*

*F.* The procedure was like that just described except that the milk samples were warmed to 50° F. in 12 minutes, held there for 108 minutes with constant stirring, then cooled back to 38° F. in 28 minutes, and held there for nine hours and 32 minutes. This procedure simulated temperature changes in milk during successive milkings when a bulk cooling tank was employed that cooled more slowly.

## RESULTS

### *Effect of time and temperature of storage*

Logarithms of average numbers of bacteria in raw milks held at various cooling temperatures are shown in Figure 1 which is based on the number of samples

indicated in Table 1. Logarithms of average numbers are used because the influence of individual results is not minimized as much as by averages of logarithms. This is important, for in work of this nature the sample with unusual results may be of greatest significance. Figure 1 shows that there was on the average a decrease in numbers of bacteria during the first two days of storage at 36° F. By the third day psychrophiles had increased, but both SPC and PPC gave increased counts after four days. The milks held at 38° F., which were barely Grade A for pasteurization, exhibited rapid and continuous increases in numbers of bacteria by both SPC and PPC methods and exceeded the Grade A standard after one day. Storage of the milk at 38° F. with periodic warming to 45° F. or 50° F. apparently did not increase rates of growth to any extent. However, holding milk at 45° F. resulted in rapid growth from the start as judged by SPC or PPC methods. Most of the milk samples tested would be classed as Grade A for pasteurization as received from the farm, but most samples exceeded the 200,000 bacteria per ml. within three days at storage temperatures above 36° F.

Table 1 indicates the percentage of samples that showed increases in numbers of bacteria as indicated by the SPC method and the ranges of those increases, and Table 2 does the same for the PPC method. SPC counts in Table 1 indicate no increase in numbers after three days at 36° F. and increases in four days in only one-eighth of the samples. At 38° F., however, increases began during the first day and increased in rate through the succeeding days. Milks held at 38° F., but warmed periodically to 45° F. or 50° F., had no increase after one day, slow increases for the most part during the second day and more rapid increases thereafter. At 45° F. increases were early and rapid.

TABLE 1 — EFFECT OF STORAGE OF RAW MILKS AT VARIOUS COOLING TEMPERATURES ON PERCENTAGES OF SAMPLES SHOWING INCREASES IN STANDARD PLATE COUNTS AND RANGES OF THOSE INCREASES

Storage temperature	No. of samples	Days of storage																
		1			2			3			4							
		3-6X <sup>a</sup>	7-10X	>10X	3-6X <sup>a</sup>	7-10X	>10X	3-6X <sup>a</sup>	7-10X	>10X	3-6X <sup>a</sup>	7-10X	>10X					
°F.																		
36	16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.25	0.00	6.25					
38	57	5.26	1.75	0.00	10.65	0.00	5.26	8.77	1.75	10.65	10.65	3.50	19.30					
38/45 <sup>b</sup>	20	0.00	0.00	0.00	5.00	0.00	5.00	10.00	0.00	10.00	15.00	5.00	25.00					
38/50 <sup>c</sup>	12	0.00	0.00	0.00	0.00	0.00	0.00	8.33	0.00	8.33	8.33	25.00	33.33					
45	23	13.04	4.35	13.04	13.04	4.35	30.43	0.00	4.35	69.56	8.70	4.35	82.61					

<sup>a</sup>3-6X = Three to six-fold increase over original number of bacteria.

<sup>b</sup>Milk stored at 38° F. except when warmed periodically to 45° F.

<sup>c</sup>Milk stored at 38° F. except when warmed periodically to 50° F.

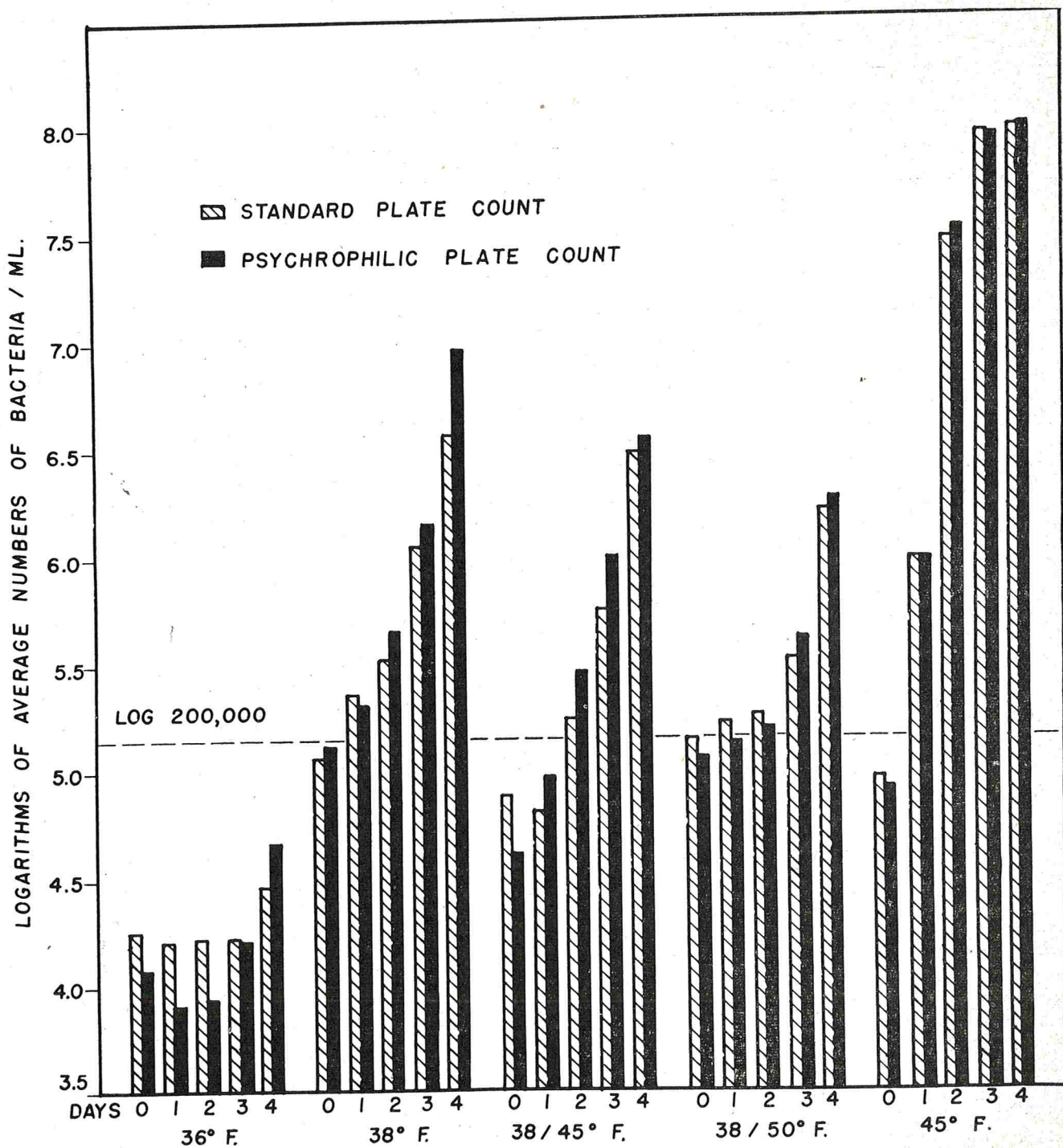


FIGURE 1. Logarithms of average numbers of bacteria in raw milks held at various cooling temperatures for one, two, three and four days.

Increases in psychrophiles, shown in Table 2, in general paralleled those by the SPC method, except to be higher by the third and fourth days.

Because there were no significant changes in numbers of thermodurics during any of the periods of storage at any of the storage temperatures, the Ther-

moduric Plate Counts have not been tabulated.

*Effect of farm source of milk*

Attempts were made to correlate the results of storage tests on milk samples from the various farms

TABLE 2 — EFFECT OF STORAGE OF RAW MILKS AT VARIOUS COOLING TEMPERATURES ON PERCENTAGES OF SAMPLES SHOWING INCREASES IN NUMBERS OF PSYCHROPHILES AND RANGES OF THOSE INCREASES

Storage temperature	No. of samples	Days of storage																					
		1			2			3			4												
		3-6X <sup>a</sup>	7-10X	>10X	3-6X <sup>a</sup>	7-10X	>10X	3-6X <sup>a</sup>	7-10X	>10X	3-6X <sup>a</sup>	7-10X	>10X										
°F.																							
36	16	0.00	0.00	0.00	0.00	0.00	0.00	6.25	0.00	0.00	25.00	0.00	12.50										
38	57	4.26	0.00	0.00	10.65	0.00	6.39	12.78	4.26	12.26	17.04	6.39	21.30										
38/45 <sup>b</sup>	20	15.00	0.00	0.00	5.00	5.00	5.00	25.00	0.00	20.00	25.00	10.00	45.00										
38/50 <sup>c</sup>	12	0.00	0.00	0.00	0.00	0.00	0.00	25.00	8.33	0.00	16.67	8.33	66.64										
45	23	30.25	8.70	13.05	26.10	8.70	39.15	4.35	8.70	73.95	4.35	4.35	91.30										

<sup>a</sup>3-6X = Three to six-fold increase over original number of bacteria.

<sup>b</sup>Milk stored at 38° F. except when warmed periodically to 45° F.

<sup>c</sup>Milk stored at 38° F. except when warmed periodically to 50° F.

with methods of milk production on these farms. It was true that, in general, good procedures of cleansing and sanitizing resulted in milk with lower numbers of total bacteria and of psychrophiles than milk produced on farms with poorer procedures. The effect of numbers of bacteria in the original milk on the growth of bacteria during storage will be discussed in a following paper (6). It is obvious, however, that the lower the original count of a sample of milk is, the longer it will take to reach the limiting numbers for a grade. No definite relationship was evident, however, between original numbers of psychrophiles in the milk and their rate of growth during low temperature storage. High and low count milks showed slow growth of bacteria at some times and rapid growth at other times. Successive samples of milk from one farm often showed greater differences than samples from different farms. Even milk from the farm with the poorest production method did not consistently contain rapidly growing psychrophiles; yet milk from a good farm often showed rapid bacterial growth.

Because of the lack of correlation between procedures of cleansing and sanitizing on the farms and differences in rates and amounts of growth of bacteria in stored milk samples from these farms, no data are presented here.

#### *Effect of season of the year*

For the purpose of comparison the year has been divided into two seasons, the summer from May through September and the winter from October through April. The growth of psychrophilic bacteria in raw milks held at farm bulk cooling tank temperatures

of 38° F. and 45° F. during these two seasons is summarized in Table 3. It will be observed that not only did a greater percentage of the milk samples show three-fold or greater increase in numbers each day during the storage period at 38° F. and 45° F. in the summer than in the winter, but also that the average increase over the original number was greater. The effect was more striking when storage was at 45° F. than at 38° F.

#### DISCUSSION

It should be kept in mind that the experiments just described involved incubation of the original samples of milk at various temperatures for a total of four days. In practice, however, a second milking would dilute the original milk by half about twelve hours later. A third milking would dilute the first two milkings, and so on. Each fresh addition presumably reduces numbers of bacteria of the mixture in the cooling tank if previous growth has taken place, but the dilution effect decreases with each additional milking. Therefore, the longer the time between pick-ups of milk, the more important will be the growth of bacteria in the first milkings that enter the tank. The results of the experiments reported here show that growth can be rapid after the first day at 38° F. or higher. Any set of storage conditions found satisfactory under the conditions of the experiments reported here, should be all the better under practical conditions, which, for the reasons cited, would permit still poorer growth of bacteria.

It was thought that the raw milk used in these experiments might exhibit a so-called "germicidal" or

bacteriostatic effect. Actually, however, this bacteriostatic effect probably is of little or no significance in the milk in the cooling tanks, because the effect is greatly reduced by low temperatures, although more prolonged, and because the psychrophiles that grow in the cooled milk do not seem to be affected appreciably by the bacteriostatic substances, as will be shown in a later paper (7).

Experiments should be conducted, of course, on a number of farms to study the effect of addition of fresh milk at each milking on the numbers of bacteria in the milk in the farm bulk cooling tanks. It would be interesting, too, to obtain samples of milk from a large number of farms to see whether any of them chance to carry persistently some of the strains of psychrophiles able to grow rapidly at the temperatures of farm bulk cooling tanks.

#### SUMMARY

Raw milk samples from farms employing different methods and conditions of production were held at 36° F., 38° F. and 45° F. for four days and tested daily by the standard, thermoduric and psychrophilic plate count methods. Similar tests also were made on raw milk samples held at 38° F. with periodic increases to 45° F. or 50° F. in imitation of the warming effect of the addition of subsequent milkings.

At 36° F. there was, on the average, a decrease in numbers of bacteria during the first two days of hold-

ing. Psychrophiles had increased a little by the end of three days, and both psychrophiles and bacteria estimated by the standard plate count method had increased after four days. This storage temperature kept bacterial numbers in all milk samples tested low enough to meet the standard for Grade A raw milk for pasteurization as delivered from the farm, even through the fourth day. The percentage of 36° F. samples showing significant increases in standard plate counts was zero until after four days when only 6.25 per cent showed appreciable rates of growth. By this time 12.5 per cent of the samples showed over ten-fold increase in psychrophiles. A storage temperature of 36° F., then, seems to be satisfactory for holding these milks for three days.

Raw milk samples held at 38° F. showed, on the average, rapid and continuous increases in numbers of bacteria as estimated by either plate count method. Because the original samples had for the most part fairly high bacterial counts, the average counts exceeded the Grade A standard after one day. Milk samples at 38° F. in which increases in numbers of bacteria took place showed increasing rates of growth on succeeding days of storage and increasing percentages of the samples showed appreciable growth. So percentages of samples giving seven-fold or greater increases by the standard plate count method rose from 1.75 per cent after one day to 5.26 per cent after two days, 12.3 per cent after three days and 22.8 per cent after four days. Psychrophilic plate

TABLE 3 — EFFECT OF SEASON ON GROWTH OF PSYCHROPHILIC BACTERIA IN RAW MILKS HELD AT FARM BULK COOLING TANK TEMPERATURES

Storage temperature °F.	Storage time Days	Season			
		Summer <sup>a</sup>		Winter <sup>b</sup>	
		Samples with 3X or greater increase per cent	Average increase X initial no.	Samples with 3X or greater increase per cent	Average increase X initial no.
38 <sup>c d</sup>	1	8.9	4	3.2	4
	2	16.0	33	6.5	5
	3	35.6	327	22.6	9
	4	53.4	382	41.9	25
45 <sup>e</sup>	1	64.3	94	33.3	4
	2	85.7	4,463	55.5	18
	3	92.9	10,680	66.7	321
	4	100.0	15,508	100.0	647

<sup>a</sup>May through September.

<sup>b</sup>October through April.

<sup>c</sup>Includes milks stored at 38° F. except when warmed to 45° F. or 50° F. for two hours at ten hour intervals.

<sup>d</sup>56 samples during summer and 31 during winter.

<sup>e</sup>14 samples during summer and 9 during winter.

counts gave higher results than these after three and four days.

Apparently the first three days of storage of milk at 38° F. with periodic warming to 45° F. or 50° F. did not increase rates of bacterial growth over those in milk at 38° F. continuously, but by the fourth day a larger percentage of the samples showed rapid growth in milk samples periodically warmed.

Holding milk at 45° F. resulted in rapid bacterial growth from the start as judged by either method of counting. Also the percentage of samples showing seven-fold or greater increases was considerably larger at all stages of storage. Obviously 45° F. is too warm a temperature for milk in a farm bulk cooling tank.

No significant changes in numbers of thermophilic bacteria were observed in the raw milk samples during any of the periods of storage at any of the storage temperatures.

No correlation could be observed between methods of milk production on eight farms and rates or amounts of bacterial growth in milk samples stored at the various temperatures.

Psychrophilic bacteria grew in more of the raw milk samples held at 38° F. and 45° F. and at a greater rate in these samples when the samples were obtained from May through September than when they were procured from October through April.

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# BACTERIOLOGY OF MILK HELD AT FARM BULK COOLING TANK TEMPERATURES

## II. EFFECT OF NUMBERS OF BACTERIA IN THE ORIGINAL MILK<sup>1 2</sup>

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Raw milk samples containing less than 50,000 bacteria per ml. were compared with samples over 50,000 per ml. by means of bacterial counts made during four days of storage at 36° F., 38° F., 38° F., with periodic raises to 45° F. or 50° F., and 45° F. More samples of the high count milks showed appreciable growth within one, two, three or four days than of the low count milks, but psychrophiles often grew faster, especially after two days, in the low count milk. Raw milks are more likely to remain Grade A for a desired storage period if they contain low numbers of bacteria and if their storage temperature is 38° F. or lower.

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In general it is to be expected that raw milk with a high bacterial content will be likely to contain more psychrophilic bacteria than milk with low numbers of bacteria and the greater the number of psychrophiles there are in a milk sample, the shorter should be its keeping time in a farm bulk cooling tank. The work reported here to investigate the possibility involved a study of; (a) the effect of initial numbers of bacteria in milks on the rate of growth of the bacteria when the milks were held at farm bulk cooling tank temperatures, and (b) the number of samples initially acceptable as "Grade A for pasteurization" that became unacceptable during storage.

Ayers, Cook and Clemmer (2) stored raw milks of three different grades at 40° F., 50° F., and 60° F. From this work the following conclusions were drawn: (a) in two samples of approximately the same initial bacterial count, the increase after holding was not always similar; (b) the milk held at 40° F. showed a relatively small increase in numbers after four days of holding compared to the milk at 50° F. or 60° F.; (c) the milk with a low initial bacteria count (average of 4,295 per ml.) showed an average two-fold increase after three days of storage and an average

four-fold increase after four days of holding at 40° F.; (d) the milk with an average initial bacteria count of 39,082 per ml., when stored at 40° F., showed average two-, four-, six- and 27-fold increases after one, two, three and four days, respectively; (e) the milk with an average initial bacteria count of 136,533 per ml., when stored at 40° F., showed average two-, five- and six-fold increases after two, three and four days, respectively; (f) seventy-two hours of storage at 50° F. and 48 hours of storage at 60° F. were required to produce counts in high quality milk comparable to those in low quality milk after 24 hours of incubation at the same temperatures, and (g) the milk showed a high bacteria count when held at a high (60° F.) temperature for a prolonged period of time even if the original count was low.

Marth, Hunter and Frazier (6) reported that when milk with a low initial bacterial count (average of 4,700 per ml.) entered a farm bulk cooling tank for storage until picked up every other day, its standard and psychrophilic plate counts sometimes showed increases between the entry of milks from the second and fourth milking. Hunter (3) showed that these increases in the standard plate count of individual samples were two-fold and in the psychrophilic plate count were two-, three- and nine-fold.

### METHODS

#### *Sources of milk*

Milk for these experiments came from one University and three private farms in the Madison area except milks held at 38° F. which came from two University and six private farms and milks held at 45° F. which came from three private and two University farms. All of the farms chosen, except two of those used in the 38° F. storage experiments and one of those used in the 45° F. storage experiments, utilized a pipeline milker in the production of milk. Six of these farms and one private farm used bucket-type milkers in their production procedure. A brief des-

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<sup>2</sup>The research reported herein was conducted as a part of the North Central Regional Research Project NC-3, One Story Dairy Barns and Related Structures.

TABLE 1 — THE EFFECT OF ORIGINAL NUMBERS OF BACTERIA IN RAW MILKS STORED AT 36°F. ON NUMBER OF SAMPLES SHOWING INCREASES IN BACTERIAL COUNTS AND ON AVERAGE INCREASES

SPC/ml. of original milk	No. of samples	Counting method	3 Days <sup>a</sup>		4 Days	
			No. of samples showing increases	Average increases	No. of samples showing increases	Average increases
1,600 to 50,000	14	SPC <sup>b</sup>	0	—	2	21,500X
		PPC <sup>c</sup>	1	3X <sup>d</sup>	5	17,000X
51,000 to 62,000	2	SPC	0	—	0	—
		PPC	0	—	1	6X

<sup>a</sup>No samples showed a 3X or greater increase in numbers of bacteria after one and two days of holding.

<sup>b</sup>SPC = standard plate count.

<sup>c</sup>PPC = psychrophilic plate count.

<sup>d</sup>3X = Three-fold increase over original number.

cription of the milk production methods on the farms will be found in another paper (4).

#### Storage of milks

Milks in these experiments were stored for four days at 36°F., 38°F., 38°F. with periodic increases to 45°F., 38°F. with periodic increases to 50°F., and 45°F. as previously described by Marth and Frazier (4). Numbers of bacteria in the milk were determined before storage and after each of four days of storage by means of the standard plate count (SPC) and psychrophilic plate count (PPC) (1).

### RESULTS

#### Storage at 36° F.

The effect of original numbers of bacteria in raw milks stored at 36°F. on numbers of samples showing increases in bacterial counts and on average increases is summarized in Table 1. A three-fold increase in numbers of bacteria over those present originally in the milk was considered as evidence of growth and hence significant in all of the results reported.

Unfortunately most of the milk samples obtained for storage at 36°F. had fewer than 50,000 bacteria per ml. The results showed that all samples, regardless of original numbers of bacteria, showed no significant increase in numbers in the first two days and only one sample out of 16 showed a three-fold increase after three days. The large increases in numbers of bacteria during the fourth day indicate that even storage of low count milks at as low a temperature as 36°F. for four days might be unsuccessful.

#### Storage at 38° F.

The effect of original numbers of bacteria in raw

milks stored at 38°F., a temperature commonly used in farm bulk cooling tanks, on numbers of samples showing increases in bacterial counts and on average increases is summarized in Table 2. Results with milks held at 38°F., but warmed periodically to 45°F. or 50°F., are included here because they did not differ appreciably from those obtained when the milk was held at 38°F.

The results show that average increases in numbers of bacteria in milk samples showing growth during the first two days of storage were similar for both lower count and higher count milks, but that by the end of the third day increases were considerably more rapid in the low count milks. By the fourth day psychrophiles were multiplying at an increased rate in the poor milks. It should be noted, however, that only a tenth of the low count milks showed any significant growth by the SPC method after two days, but about one-sixth of the high count milks showed growth. After three days only 16 per cent of the low count milks exhibited increases by the SPC method, but about 38 per cent of the high count samples showed appreciable growth. After four days, too, a lower percentage (36 per cent) of the low count samples showed growth than of the high count samples (69 per cent). These results indicate, then, that there is a greater likelihood of appreciable growth of bacteria taking place in milk with over 50,000 bacteria per ml. initially than in milk with lower counts. However, occasional samples of either low or high count milk may undergo storage for two, three or even four days at 38°F. without appreciable increase in numbers of bacteria.

*Storage at 45°F.*

The effect of original numbers of bacteria in raw milks stored at 45°F. on numbers of samples showing increases in bacterial counts and on average increases is summarized in Table 3.

Although fewer samples of low count milk than of high count milk showed growth during the first day, those that gave increases had a rapid rate of growth, faster than in the high count samples. Growth was still more rapid by the end of two days. Three and four days are obviously too long a holding period at 45°F. The results suggest that even a two day holding period at 45°F. for good milk is risky.

Results obtained when raw milks were held at 36°F., 38°F., or 45°F. indicated that: (a) appreciable growth is more likely to occur in milk samples originally containing over 50,000 bacteria per ml. than in milks with less than that number; (b) some samples, both high and low count, show little increase in numbers in the first two days or even after longer periods at the two lower temperatures; (c) in those low count samples in which growth took place the psychrophiles usually grew faster than in milks with higher counts; and (d) 36°F. is to be recommended as a temperature for milk in farm bulk cooling tanks, 38°F. should be fairly successful but permits more rapid bacterial growth, and 45°F. is definitely too high a temperature.

*Acceptability of stored milks*

The number and percentage of initially acceptable milks that became unacceptable as "Grade A for pasteurization as delivered from the farm" after one, two, three and four days of storage at cooling temperatures are summarized in Table 4.

At 36°F. none of 16 samples became unacceptable, even through four days of storage. It should be noted that 14 of the 16 samples had original counts of less than 50,000.

Most of the samples held at 38°F. were all right after two days and 42 of the 52 were acceptable after four days. This was despite the fact that original counts on many of these samples were not far below the 200,000 per ml. level. Milks stored at 38°F. but periodically raised to 45°F. or 50°F. kept as well as those held at 38°F. The slightly better results with the milks undergoing rises in temperature probably are because of generally lower original numbers of bacteria in those samples.

As was to be expected, poorest results were obtained by storage at 45°F. where 25 percent of the samples were unacceptable within a day and 65 per cent within three days.

## DISCUSSION

It is evident from the results that the acceptability of milk as Grade A for pasteurization after storage in a farm bulk cooler will depend upon; (a) the original number of bacteria in the milk as it affects the number of bacteria that must be added to exceed the 200,000 per ml. limit, and (b) the rate of growth of bacteria in the milk at the storage temperature. The results have indicated that high count milk samples are more likely to support growth of low temperature bacteria than low count samples, yet at 38°F. or above certain samples of either high or low count milk may show appreciable bacterial growth within

TABLE 2 — THE EFFECT OF ORIGINAL NUMBERS OF BACTERIA IN RAW MILKS STORED AT 38°F.<sup>a</sup> ON NUMBER OF SAMPLES SHOWING INCREASES IN BACTERIAL COUNTS AND ON AVERAGE INCREASES

SPC/ml. of original milk	No. of samples	Counting method	1 Day		2 Days		3 Days		4 Days	
			No. of samples showing increases	Average increases	No. of samples showing increases	Average increases	No. of samples showing increases	Average increases	No. of samples showing increases	Average increases
940 to 50,000	58	SPC <sup>b</sup>	3	5X <sup>d</sup>	5	19X	8	390X	19	641X
		PPC <sup>c</sup>	1	4X	4	24X	11	472X	27	262X
50,000 to 2,100,000	31	SPC	2	5X	6	19X	13	60X	22	338X
		PPC	5	4X	9	26X	18	92X	24	325X

<sup>a</sup>Includes samples held at 38° F. and raised periodically to 45° F. or 50° F.

<sup>b</sup>SPC = standard plate count.

<sup>c</sup>PPC = psychrophilic plate count.

<sup>d</sup>5X = Five-fold increase over original number.

TABLE 3 — THE EFFECT OF ORIGINAL NUMBERS OF BACTERIA IN RAW MILKS STORED AT 45° F. ON NUMBER OF SAMPLES SHOWING INCREASES IN BACTERIAL COUNTS AND ON AVERAGE INCREASES

SPC/ml. of original milk	No. of samples	Counting method	1 Day		2 Days		3 Days		4 Days	
			No. of samples showing increases	Average increases	No. of samples showing increases	Average increases	No. of samples showing increases	Average increases	No. of samples showing increases	Average increases
1,600 to 50,000	16	SPC <sup>a</sup>	3	153X <sup>c</sup>	8	4,093X	10	10,941X	15	10,435X
		PPC <sup>b</sup>	6	136X	10	6,120X	13	10,808X	16	15,283X
50,000 to 760,000	7	SPC	4	7X	4	55X	7	104X	7	261X
		PPC	6	6X	7	70X	7	165X	7	431X

<sup>a</sup>SPC = standard plate count.

<sup>b</sup>PPC = psychrophilic plate count.

<sup>c</sup>153X = 153-fold increase over original number.

two days. The explanation must be, of course, that these certain samples contain special kinds of psychrophiles that are able to grow fairly well at the storage temperature, while the other samples are without these actively growing psychrophiles or contain them in very small numbers. The assumption must be made that occasional samples of milk, both those of high and those of low counts, will contain appreciable numbers of psychrophiles able to grow actively at 38°F. or above. Therefore there is some risk involved in storage of milks for more than two days of these temperatures. Kinds of psychrophiles from milks stored at farm tank cooler temperatures will be discussed in a later paper (5).

As has been mentioned in the preceding paper (4), it should be kept in mind that in these experiments

a sample of milk was held at its storage temperature for four days, but in practice a new milking would enter the tanks every 10 to 12 hours, reducing the bacterial count if it were low count milk. This would tend to lengthen the possible storage time. The so-called germicidal power of raw milk probably had little, if any, effect on the results because the psychrophiles are, in general, little affected.

#### SUMMARY

Raw milk samples of two bacteriological groups (under 50,000 bacteria per ml. and over 50,000 bacteria per ml.) were obtained from six farms that employed pipeline milkers and from two farms that employed bucket-type milking machines in their

TABLE 4 — THE NUMBER AND PERCENTAGE OF INITIALLY ACCEPTABLE MILKS THAT BECAME UNACCEPTABLE AS "GRADE A FOR PASTEURIZATION AS DELIVERED FROM THE FARM" AFTER ONE, TWO, THREE AND FOUR DAYS OF STORAGE AT COOLING TEMPERATURES

Storage temperature (°F.)	No. of samples with initial bacteria count of <200,000/ml. <sup>a</sup>	Samples with bacteria count of >200,000/ml. after storage <sup>a</sup>							
		1 Day		2 Days		3 Days		4 Days	
		(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
36	16	0	0.00	0	0.00	0	0.00	0	0.00
38	52	1	1.95	5	9.60	9	17.30	10	19.50
38/45 <sup>b</sup>	18	0	0.00	2	11.11	3	16.68	7	38.92
38/50 <sup>c</sup>	10	2	20.00	2	20.00	2	20.00	5	50.00
45	20	5	25.00	6	30.00	13	65.00	14	70.00

<sup>a</sup>Based on standard plate count.

<sup>b</sup>Milk stored at 38° F. except when periodically warmed to 45° F.

<sup>c</sup>Milk stored at 38° F. except when periodically warmed to 50° F.

production procedures. These milk samples were stored at 36°F., 38°F., 38°F. with periodic raises to 45°F., 38°F., with periodic raises to 50°F., and 45°F.

Milk samples, most of which contained less than 50,000 bacteria per ml., showed comparatively little growth after three days at 36°F., but rapid growth in some samples had begun by the end of the four days.

Results at 38°F. were similar to those at 38°F. with periodic raises to 45°F. or 50°F. All of the results indicated that average rates of increase in numbers of bacteria in milk samples showing growth during the first two days of storage were similar for both lower and higher count milks. However, a smaller percentage of the milk samples with low counts than of those with high counts showed appreciable growth of bacteria after two, three and four days at 38°F. Some samples of either low or high count milk may undergo storage for two, three or even four days at 38°F. without appreciable increase in numbers of bacteria.

Storage at 45°F. produced rapid growth of bacteria, faster usually in the low count milk than in the high count. Apparently a two-day holding period at 45°F. would be risky.

At all storage temperatures appreciable growth is more likely to occur and to happen earlier in milk samples originally containing over 50,000 bacteria per ml. than in milks with lower numbers.

Growth of psychrophiles usually was more rapid especially after two days in the lower count milks than in the higher count ones.

When tests were made on milks that originally were acceptable as "Grade A for pasteurization as delivered from the farm" to see how long they would remain acceptable at the various storage temperatures, it was found that: (a) all samples tested remained acceptable for four days at 36°F.; (b) most samples held at 38°F. remained acceptable after two days and

four-fifths of them after four days; (c) milks stored at 38°F. but periodically raised to 45°F. or 50°F. kept as well as those held at 38°F.; and (d) poorest results were obtained by storage at 45°F. where 25 per cent of the samples were unacceptable within a day and 65 per cent within three days.

Of the storage temperatures tried, 36°F. was best for storage for two days, 38°F. was fairly good and 45°F. was unsatisfactory. The results suggested that very good milk could be held for three or four days at 36°F. without significant increases in numbers of bacteria.

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# VIRUCIDAL ACTIVITY OF HYPOCHLORITES, QUATERNARY AMMONIUM COMPOUNDS, AND IODOPHORS AGAINST BACTERIOPHAGE OF *STREPTOCOCCUS CREMORIS*<sup>1</sup>

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Recent introduction of iodophors as sanitizing agents for the dairy industry has occasioned interest in their ability to destroy bacteriophage (virucidal activity). A number of studies have shown the iodophors to represent effective germicidal agents against a variety of nonsporeforming bacteria (4, 5, 9, 10, 11). However, there have been no reports on the activity of iodophors with respect to destruction of bacteriophage (phage).

Hypochlorites in a concentration of 50 ppm and quaternaries at 200 ppm have been shown to inactivate *Streptococcus cremoris* phage in 15 seconds (12). Similar results were obtained in a subsequent study (3) using essentially the same techniques. Since phage destruction represents an important consideration in present day sanitization of dairy plants, it was considered desirable to compare the ability of iodophors, hypochlorites, and quaternaries to destroy representative strains active against lactic streptococci used in starter cultures.

## EXPERIMENTAL

*S. cremoris* phage strain 144F was employed in the virucidal trials as a phage representative of those infecting starter cultures. This particular strain showed somewhat greater resistance to germicides than others tested in preliminary studies. The virucides selected were typical of those employed in general dairy and food plant sanitizing procedures. The quaternary was alkyl dimethyl ethyl benzyl ammonium chloride, the iodophor was a liquid preparation containing approximately 10 per cent iodine solubilized in 85 per cent nonionic wetting agent, and the hypochlorite was sodium hypochlorite.

Quaternary concentrations were determined by the Furlong and Elliker method (7). The iodophor was titrated to a colorless endpoint with standard thiosulfate. The standard thiosulfate titration (2) for deter-



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mining available chlorine was used for the sodium hypochlorite. Virucide solutions were buffered at pH 5.0 with M/100 acetate-acetic acid and at pH 8.0 and 9.0 with borate-boric acid.

The technique for evaluating bactericidal activity was similar to that used by Parker and Elliker (12) with the following modifications: Thirty-ml capacity, wide-mouth, screw cap bottles were substituted for test tubes. Thiosulfate inactivator solutions for the iodophor and hypochlorite and lecithin-tween 80 inactivator solutions for the quaternary were prepared in M/100 phosphate buffer at pH 7.1. The medium used to cultivate the sensitive host and for plaquing procedures represented a type developed especially for lactic streptococci (6).

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Whey filtrates of a phage strain of *S. cremoris*, designated as 144F were prepared in the manner described by Parker and Elliker (12) with the following variations: The artificially coagulated milk containing propagated phage was clarified by centrifugation rather than filtration through cheese cloth. The resulting clear filtrate was passed through a Seitz filter and the cell free filtrate was transferred to a sterile bottle containing an excess of CaCO<sub>3</sub> to neutralize whey acidity. A 1:100 dilution of this filtrate in distilled water was used for virucidal trials.

The following plaquing procedure was employed to determine the numbers of phage surviving virucidal treatment: A 1-ml. aliquot from an inactivator tube or appropriate dilution blank was transferred to a test tube containing 0.25 ml. of a 5-hour old broth culture of the sensitive host. After 1 minute, 3 ml. of a semi-solid (0.85%) agar, cooled to 45°C. were poured into the host-phage mixture. The contents were mixed by rotating the tube and then pouring into a petri dish containing approximately 20 ml. of hardened solid agar. After incubation of the plates at 30°C. for 12 hours the plaques were fully developed and countable.

RESULTS

Table 1 summarizes the results obtained with buffered solutions of the virucides against phage strain 144F. Sodium hypochlorite completely inactivated phage in 30 seconds at a concentration of 25 ppm and in 15 seconds at 50 ppm. The quaternary showed a lower rate of virucidal activity than the hypochlorite and failed to completely inactivate phage in 30 seconds at concentrations of 25 and 50 ppm. The iodophor was comparatively ineffective against the phage during a 60-second exposure period in concentrations of 25 and 50 ppm.

A series of trials was made to determine the concentration of each virucide required to inactivate phage during short exposure periods. Virucides were prepared in tap water and were not buffered. As indicated in Table 2 the quaternary was effective in destroying phage in 30 seconds at 100 ppm. A sharp increase in virucidal activity occurred between 25 and 50 ppm. The iodophor (Table 3) was ineffective against phage in concentrations as high as 200 ppm during a 60-second exposure period. There was less increase in activity between 10 and 200 ppm than was anticipated with the iodophor. The hypochlorite on the other hand accomplished complete destruction of phage at levels of 12.5 to 100 ppm during a 30-second exposure period (Table 4).

The pH levels obtained on dilution of the iodophor were relatively high because of the low buffering

TABLE 1 — COMPARISON OF VIRUCIDAL ACTIVITY OF A QUATERNARY AMMONIUM COMPOUND, IODOPHOR, AND SODIUM HYPOCHLORITE AGAINST *Streptococcus Cremoris* PHAGE STRAIN 144F

Virucide	Conc.	Final pH	Plaque count after following exposure times:		
			15 sec	30 sec	60 sec
Quaternary	25	8.95	17 <sup>a</sup>	10	0
	50	8.9	11	14	0
Iodophor	25	5.1	Lysed <sup>b</sup>	Lysed	TC <sup>c</sup>
	50	5.1	TC	TC	TC
Sodium hypochlorite	25	8.1	2	0	0
	50	8.1	0 <sup>d</sup>	0	0

<sup>a</sup>Initial count of phage-germicide mixture: 23 x 10<sup>6</sup> per ml. Plaque count after exposure represents number per ml of a 1:10 dilution of phage-germicide mixture.

<sup>b</sup>Lysed denotes lysis of all developing bacteria due to excessive number of surviving phage.

<sup>c</sup>TC denotes plaques too numerous to count but less than in lysed plates.

<sup>d</sup>O denotes no plaques on plates, i.e. less than 10 per ml remaining in phage-germicide mixture.

TABLE 2 — VIRUCIDAL ACTIVITY OF VARIOUS CONCENTRATIONS OF QUATERNARY AGAINST *Streptococcus Cremoris* PHAGE STRAIN 144F DURING A 30-SECOND EXPOSURE PERIOD

Conc.	Final pH	Plaque count <sup>a</sup> following treatment
(ppm)		(No/ml)
12.5	7.0	110 x 10 <sup>4</sup>
25	7.15	6 x 10 <sup>4</sup>
50	7.25	6
100	7.25	0
200	7.3	2

<sup>a</sup>Initial count of phage-germicide mixture: 126 x 10<sup>6</sup> per ml. Plaque count of survivors represents No/ml of a 1:10 dilution of phage-germicide mixture.

TABLE 3 — VIRUCIDAL ACTIVITY OF VARIOUS CONCENTRATIONS OF IODOPHOR AGAINST *Streptococcus Cremoris* PHAGE STRAIN 144F DURING A 60-SECOND EXPOSURE PERIOD

Conc.	Final pH	Plaque count <sup>a</sup> following treatment
(ppm)		(No/ml)
10	7.3	32 x 10 <sup>3</sup>
25	7.2	286 x 10 <sup>2</sup>
50	7.25	147 x 10 <sup>2</sup>
100	7.2	59 x 10 <sup>2</sup>
200	7.0	144 x 10 <sup>2</sup>

<sup>a</sup>Initial count of phage-germicide mixture: 153 x 10<sup>6</sup> per ml. Plaque count of survivors represents No/ml of a 1:10 dilution of phage-germicide mixture.

capacity of the preparation used. However, at the pH level encountered, the iodine should have been more virucidal than at pH 5.0. Studies with a highly buffered iodophor (Table 5) resulted in extremely low pH levels at higher concentrations and when the level of iodophor reached 100 ppm, rapid destruction of phage occurred. Results of subsequent studies, however, indicated that this destruction was primarily the effect of the low pH rather than virucidal activity of the iodophor.

Other studies have demonstrated aqueous solutions of iodine to be more active in destruction of bacterial spores than are the same concentrations of iodophors (7). Consequently, aqueous solutions of iodine were employed at various levels ranging from 10 to 100 ppm against phage strain 144-F. The aqueous solutions showed somewhat greater activity than iodophors against phage but did not approach the hypochlorites in degree of destruction.

Studies in this laboratory on nonsporeforming bacteria had shown that iodophors were most germicidal at low pH levels and, as the pH increased from 5.0 to 9.0 in some trials, the rate of destruction was markedly lower. Therefore effect of pH on activity of both iodophors and aqueous iodine solutions against lactic streptococcus phage was investigated at levels varying from pH 4.0 to 9.0. With both forms of iodine phage destruction was greatest in the range of pH 7.0 to 9.0, but still did not approximate rate of destruction by hypochlorites. Phage exposed to buffer solutions containing no germicide was not destroyed during 60 seconds of exposure at pH levels of 5.0 to 9.0. However, the buffer at pH 4.0 did cause appreciable phage destruction. Thus phage destruction for iodophors was greatest in the alkaline range and at pH levels at which the iodophor has been least effective against bacteria. At these pH levels only a trace of color remained in the iodophor solutions.

Results of still another study on the pH effect demonstrated rapid destruction of phage in buffer solutions without germicide at pH 2.0 and 3.0, some destruction at pH 4.0, comparatively no destruction at pH 5.0, 6.0, 7.0, 8.0 and 9.0, and some destruction at pH 10.0.

Similar studies on the pH effect on hypochlorite action indicated progressively faster phage destruction as the pH decreased from 9.0 to 4.4. The order of increased activity with lowering of pH was similar to that obtained in other studies with hypochlorites on bacterial spores and nonsporeforming bacteria. Activity of the quaternary against phage was greatest in the range of pH 7.0 to 9.0 used for comparative trials of the different germicides in these studies.

An interesting effect on plaque formation was observed in the case of phage surviving iodophor treat-

TABLE 4 — VIRUCIDAL ACTIVITY OF VARIOUS CONCENTRATIONS OF SODIUM HYPOCHLORITE AGAINST *Streptococcus Cremoris* PHAGE STRAIN 144F DURING A 30-SECOND EXPOSURE PERIOD

Conc.	Final pH	Plaque count
(ppm)		(No/ml)
12.5	8.3	0 <sup>a</sup>
25	8.7	0
50	8.9	0
100	9.2	0

<sup>a</sup>Initial count of phage-germicide mixture: 140 x 10<sup>5</sup>/ml. Plaque count of survivors represents No/ml of a 1:10 dilution of phage-germicide mixture.

TABLE 5 — VIRUCIDAL ACTIVITY OF VARIOUS CONCENTRATIONS OF AN ACIDIFIED IODOPHOR AGAINST *Streptococcus Cremoris* PHAGE STRAIN 144F

Conc.	Final pH	Plaque count <sup>a</sup> after exposure for:		
		30 sec	60 sec	120 sec
(ppm)		(No/ml)	(No/ml)	(No/ml)
10	7.4	50 x 10 <sup>3</sup>	30 x 10 <sup>3</sup>	15 x 10 <sup>3</sup>
25	6.8	31 x 10 <sup>3</sup>	12 x 10 <sup>3</sup>	68 x 10 <sup>2</sup>
50	5.6	105 x 10 <sup>3</sup>	39 x 10 <sup>3</sup>	105 x 10 <sup>2</sup>
100	2.9	0	0	0
200	2.3	0	0	0

<sup>a</sup>Initial count of phage-germicide mixture: 130 x 10<sup>5</sup>/ml. Plaque count of survivors represents No/ml of a 1:10 dilution of phage-germicide mixture.

ment. Many of the plaques formed from phage that survived exposure to iodophor were smaller in size than normal. This effect was not observed with the other germicides. A number of the small plaques were picked and the phage subcultured to determine whether or not it represented a different genetic type. However, the subcultured phage always gave rise to normal size plaques. This suggested that treatment with the iodophor either caused a slight denaturation of the phage or perhaps the iodophor adsorbed sufficiently onto the phage particle to partially inhibit its development in plaques on the agar plates.

## DISCUSSION

The marked difference in bactericidal and virucidal activity of the three types of germicides studied here suggests important differences in mode of action against microorganisms in general. The hypochlorites appear to be the least selective since they rapidly destroy nonsporeforming types and bacteriophage, and are the most active of the three germicides against resistant bacterial spores. The quaternaries are highly selective against nonsporeforming types, dependent to



a considerable degree on pH (13), and are comparatively slow against spores (7) and phage. The slower activity of quaternaries and iodophors against phage and bacterial spores suggests the possibility that these compounds encounter difficulty in penetrating bacterial spores and phage fast enough to rapidly inactivate them. Whether the spore coat and outer shell of the phage particle provide such a selective barrier for quaternaries and iodophors has not been established.

Differences in mode of action for the germicides studied here are suggested further by the high bacteriostatic activity of quaternaries against vegetative cells of sporeforming species and other gram positive types, and, in the case of iodophors, by their high rate of destruction of most nonsporeforming bacteria.

The hypochlorite used in this study demonstrated virucidal activity comparable to destruction exhibited against bacterial cells. Sodium hypochlorite was effective in very low concentrations and in short time intervals. Higher concentrations of germicide and longer exposure periods were required for phage inactivation with the quaternary than with the hypochlorite as anticipated on the basis of earlier studies (12). The virucidal activity of the iodophor, however, was surprisingly different than its bactericidal activity. Control studies on bacterial destruction made along with phage runs showed that concentrations of iodophor which completely inactivated bacterial cells were relatively ineffective against phage. The fact that the aqueous iodine solutions proved somewhat superior to the iodophor suggests the possibility that reaction between the iodine and nonionic wetting agent carrier retarded the attainment of a virucidal concentration of free iodine in solution. Allawala, Naseem and Riegelman (1) have discussed these reactions in a recent publication.

The superiority of the hypochlorite under the experimental conditions employed in this study and particularly its ability to destroy phage in very low concentrations during short time intervals recommends its use in sanitizing steps for phage control in dairy plants and possibly other food or industrial fermentations where phage represents a problem.

#### SUMMARY AND CONCLUSIONS

A comparison was made under laboratory con-

ditions of the relative effectiveness of representative hypochlorite, quaternary and iodophor germicides in destruction of a lactic streptococcus bacteriophage. Factors affecting activity of the three germicides against bacteriophage also were studied.

Sodium hypochlorite completely inactivated phage of *S. cremoris* in concentrations as low as 12.5 ppm during a 30-second time interval. A concentration of 100 ppm quaternary was required to provide the same degree of activity. The iodophor was ineffective against the phage in concentrations as high as 200 ppm during a 60-second time interval.

The results suggest that an active hypochlorite preparation should provide the most effective agent for sanitizing procedures for control of bacteriophage in dairy plants.

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## NEWS AND EVENTS

### SERIOUS LAG IN SANITATION SALARIES REPORTED

Problems of recruitment of qualified engineers and sanitarians by local health departments are reflected in the first of five reports by the Committee on Salaries of the Conference of Municipal Public Health Engineers.

Failure of health departments by substantial margins to pay the going price for competent personnel in environmental health services is resulting in a growing shortage, especially of engineers.

The Committee surveyed the salaries of engineers, sanitarians, sanitary inspectors, veterinarians, and other environmental health personnel in 371 full-time local health departments. In its report are shown the rise in salary levels from 1951 to 1956.

According to the report, salaries have been rising steadily at rates varying from 5 to 13 percent each year, but they fail to match those paid in similar

professional categories outside of health departments.

Salaries for engineers are well below those paid to other members of the profession. In 1952-54, the median salary was \$1,000 below the median for county and municipal engineers and \$2,400 below the median for all engineers. Under the circumstances, the number of vacant engineering positions in local health departments is increasing rapidly.

Starting salaries in local health agencies for engineers with no experience in 1956 ranged from \$4,250 to \$4,650. The same year engineering schools reported a median starting salary of \$5,040 and predicted that their 1957 graduates will expect around \$5,250. In addition to paying higher salaries, private employers tell the young engineer that his interview and moving expenses will be paid, that he will receive an annual bonus, overtime pay and free medical and hospital care.

While top salaries for sanitarians have been rising

more rapidly since 1954 than for any other salary group, the median salary since 1952 has been \$2,000 or more below that of engineers in local health departments and approximately \$750 below the median salary paid to male professional and technical workers in the United States.

Seven percent of the sanitarian positions studied were vacant. One-third of these were available to college graduates with no experience, offering a starting salary of \$4,479. vacant positions for sanitarians offer higher salaries than filled ones. Among 675 filled positions with the same requirements, the median starting salary was \$4,347.

Members of the reporting Committee are: Walter A. Lyon, chairman, William T. Ballard, Herbert J. Dunsmore, Reinhart W. Koch, John W. Lemon, Eric W. Mood, Louis W. Pickles, Jack C. Rogers, and Lester A. Sanger.

Copies of the report may be obtained by writing to Walter A. Lyon, Philadelphia Department of Public Health, Room 630, City Hall Annex, Philadelphia 7, Pa.

### NEW KLENZADE SAN-SPRAY FOR SANITIZING BULK TANKS

Klenzade Products, Inc., Beloit, Wisconsin, announces a new injector type spray unit for sanitizing farm bulk tanks and udder wash water in milking parlors. The unit, called "San-Spray", consists of a molded nylon feeding and proportioning jet, and container for holding Klenzade X-4, XY-12, Iodophor or Klenzade KDS-3. The spray nozzle and jet feeder assembly is attached to a regular garden hose connected with the water supply. Proportioning is done by the jet which feeds the sanitizing solution to the stream emitted by the spray nozzle. The unit also has a small valve which shows "on" and "off". When the valve is in the "off" position, only water is dispensed. When the valve is in the "on" position, the proper amount of sanitizer is added to the water stream. Klenzade San-Spray needs no adjusting and is always ready for use. The container holds one pint of concentrated sanitizing solution which will make up to the equivalent of 32 gallons of sanitizing solution. Feature of this equipment is its low cost, simplicity and versatility. It saves both time and sanitizing products because an entire bulk tank can quickly be sanitized in a minute or two. The San-Spray unit need not be disconnected from day to day but can remain permanently attached to the water supply line for immediate use at any time. Retail price of the proportioning unit only currently is quoted at \$14.50 list and \$22.95 list for the complete assembly including 15 feet of rubber hose and spray nozzle.

### NEW BOOK ON DAIRY MICROBIOLOGY PUBLISHED

Five dairy bacteriologists have teamed up to write a new book, *Dairy Microbiology*, published by Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Prepared as an interpretive source of information for students, sanitarians, dairy fieldmen, laboratory technicians, and others in the dairy industry, this book is concerned with the microbiology of milk and dairy products. Important characteristics of organisms found in dairy products are cataloged, and their pertinence to dairy processing presented.

Factors influencing growth and death of microorganisms are discussed in detail. Sources of contamination are then considered, as well as the applications of microbiology to specific dairy products.

The first six chapters emphasize the application of well-known facts in the science of microbiology to the various pertinent phases of the dairy industry. The main dairy organisms are described, first according to their classical taxonomic arrangement and then according to the physiological groupings in which the various organisms that produce the same changes in milk are placed together.

The authors deal with the factors affecting the growth of microorganisms, stressing the means of preventing growth where it is unwanted and of stimulating it where it is desired. They discuss in considerable detail the physical and chemical methods of killing microorganisms in milk and on dairy equipment. General directions for examining dairy products for microorganisms are given, with explanations of underlying principles.

The organisms, important to the dairy field, are covered in a logical manner starting with their classification and going on to their biological properties and finally their influence on technology of various products.

Sanitarians and fieldmen should find the chapters on destruction of microorganisms, sources of microorganisms in milk, and the microbiology of market milk of particular interest.

In recognition of the growing problem of waste disposal and the utilization of dairy by-products, the closing chapter discusses these subjects extensively. The utilization of dairy by-products promises to be an area of considerable economic importance and therefore deserves special emphasis.

Pictorial, graphic and tabular illustrations are given throughout the book.

The authors are: Dr. E. M. FOSTER, Professor of Bacteriology, University of Wisconsin; Dr. F. E. NELSON, Professor of Dairy Bacteriology, Iowa State College; Dr. M. L. SPECK, Professor of Dairy Bacteriology, North Carolina State College; Dr. R. N.

DOETSCH, Associate Professor of Bacteriology, University of Maryland; and Dr. J. C. OLSON, JR., Professor of Dairy Bacteriology, University of Minnesota.

### SOUTHERN REGIONAL GRADUATE SUMMER SESSIONS IN STATISTICS

The fourth Southern Regional Graduate Summer Session in Statistics will be held during the summer of 1957 from June 12 through July 20 at the Virginia Polytechnic Institute, Blacksburg, Virginia. The three previous sessions were held at the Virginia Polytechnic Institute, the University of Florida, and North Carolina State College in that order. In 1958, the summer session will be held at Oklahoma Agricultural and Mechanical College. Students who attended past sessions came from all of the forty-eight states, South America, India, Finland, Canada, Australia, Hawaii, China, the Philippines, and Africa. The enrollment at each of these sessions has exceeded one hundred graduate students.

The summer sessions are designed to carry out a recommendation of the Southern Regional Education Board's Committee on Statistics, on which the four institutions initiating the program are represented. The sessions will be of particular interest to (1) research and professional workers who want intensive instruc-

tion in basic statistical concepts and who wish to learn modern statistical methodology, (2) teachers of elementary statistical courses who want some formal training in modern statistics, (3) prospective candidates for graduate degrees in statistics, (4) graduate students in other fields who desire supporting work in statistics, and (5) professional statisticians who wish to keep informed of advanced specialized theory and methods.

Each annual summer session lasts six weeks and each course offered carries approximately five quarter hours of graduate credit. The program may be entered at any session, and consecutive courses will be offered in successive summers. The summer work in statistics may be applied towards residence requirements at any one of the cooperating institutions, as well as certain other institutions, in partial fulfillment of residence requirements for graduate degrees.

The faculty for the 1957 session at the Virginia Polytechnic Institute will include Evan J. Williams, Principal Research Officer, Commonwealth Scientific and Research Organization, Division of Mathematical Statistics, Melbourne, Australia; Daniel B. DeLury, Director of Statistics, Ontario Research Foundation, Toronto, Canada; J. L. McHugh, Director, Virginia Fisheries Laboratories; and the following staff members from the Virginia Polytechnic Institute: Willard

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Of particular interest at this summer session will be the lectures by D. B. DeLury on the Sampling of Biological Populations. This course will concern itself with the study of statistics of biological populations with specific reference to marine research. It will include some of the more recent research in this field by Dr. DeLury, and it will be accompanied by a laboratory conducted by J. L. McHugh dealing with problems, exercises, and discussions. E. J. Williams will give a course on the Analysis of Variance from the regression point of view with generalizations to multivariate analysis. Much of his lectures will deal with new material that he is now putting in book form. R. A. Bradley will be giving a course on Rank Order Statistics and will include some of the more recent research that he has published or is in the process of publishing in this field. J. E. Freund will give a course in Stochastic Processes, with particular reference to engineering applications. He will present many of the more recent advances in this field as

well as his own research. The other graduate courses will include: Probability by C. W. Clunies-Ross, Statistical Inference by W. O. Ash, Theory of Least Squares by R. L. Wine, Statistical Methods by C. Y. Kramer, Engineering Statistics by L. S. Brenna, and Sampling by R. J. Freund. Seminars which will include many of the foremost statisticians in the eastern part of the United States, will be conducted each afternoon Monday through Thursday from 3:00 to 4:30. These seminars will be on some of the more recent research now being carried on in the field of statistics.

The total tuition fee will be \$38.00 for the six-week term. Doctoral courtesy will be offered to those holding doctoral degrees. Living and other expenses at the Virginia Polytechnic Institute are reasonable. The Virginia Polytechnic Institute is located at Blacksburg, Virginia on the scenic Alleghany Mountain plateau 2100 feet above sea level. The summer climate is delightful.

Inquiries should be addressed to Boyd Harshbarger, Head, Department of Statistics, Virginia Polytechnic Institute, Blacksburg, Virginia.

**LABORATORY REPORT**

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*\*Write for booklet giving highlights of single-service spoon study recently made by William T. Ingram, Consulting Engineer and Adjunct Professor of Public Health Engineering, New York University.*

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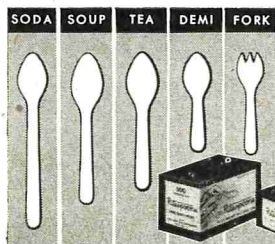
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**PIPELINE CHATTER**

Mr. H. L. Thomasson, Executive Secretary  
International Association of Milk &  
Food Sanitarians  
P. O. Box 437  
Shelbyville, Indiana

Dear "Red":

I wrote you sometime ago that I expected that I would be leaving the United States for an appointment in India. Now, I guess, it is official. At any rate, I have turned in my resignation and there has been no indication that the resignation was not accepted.

I will be leaving Fairmont Foods Company the 8th of February, and will report in Washington the 18th of February for an indoctrination course, which I am told lasts about three weeks. After that I will be given transportation tickets and a passport and sent on my way. The position, as I indicated to you in my previous letter, is that of Dairy Technology Advisor to work with the officials of India in the development of the dairy industry in that country. It sounds like a real challenging position, and it should be interesting.

I would appreciate very much if you would change

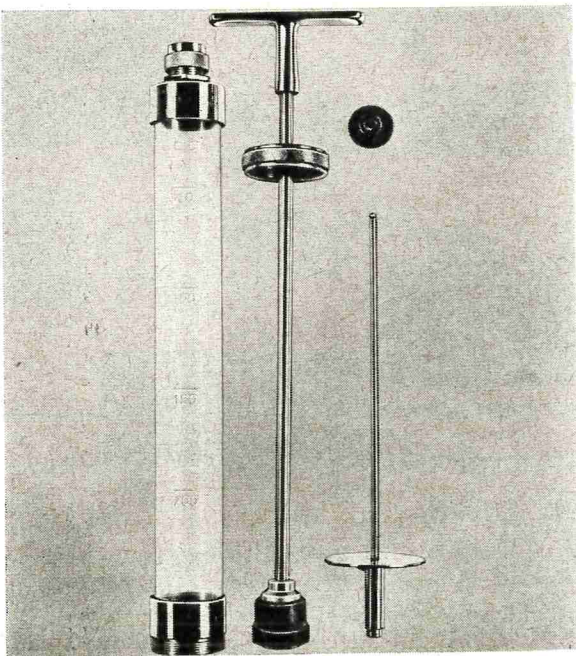
my subscription address to The American Embassy, (I. C. A.), New Delhi, India. I will enclose my check for dues.

Yours very truly,  
H. L. Templeton

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Oakite Products, Inc., manufacturers of industrial cleaning and sanitizing compounds and equipment, have announced the following change in their nationwide technical field service organization.

B. B. Herron has been transferred from Odessa to Beaumont; Horace V. Wells from San Antonio to Corpus Christi, Texas; and R. W. Krajicek from Billings, Montana, to Lake Charles, Louisiana. New representatives, all of whom recently completed Oakite's intensive eight week training program, are Robert H. Bourbonnais, assigned to Lansing, Michigan; Harry H. Thomas to Cedar Rapids, Iowa; William G. Caffee to Birmingham, Alabama; Andrew C. Johnston to Washington, D. C.; and Theo. L. Matula to San Antonio, Texas.



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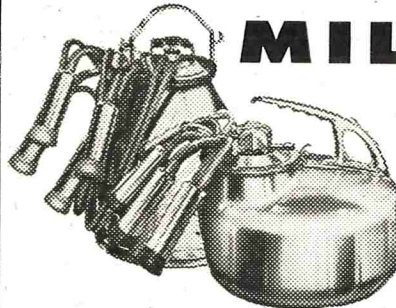
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### INDEX TO ADVERTISERS

Advanced Instrument Co. ....	VIII
American Can Co. ....	IV
American Seal Kap Corp. ....	VII
Babson Bros. Co. ....	Back Cover
Baltimore Biological Laboratory ....	VIII
Chr. Hansen's Laboratories ....	X
Creamery Package Mfg. Co. ....	II, III
Classified Ads ....	VIII
Difco Laboratories ....	Page 91
Johnson & Johnson ....	I
Klenzadé Products Co. ....	III
Langsenkamp-Wheeler Brass Wks. ....	IX
Lazarus Laboratories	
Division West Disinfecting Co. ....	VIII
Oakite Products Co. ....	IX
Oval Wood Dish Corp. ....	Page 92
Penn Salt Mfg. Co. ....	Inside Back Cover
Rohm & Haas ....	VI
The Heil Co. ....	VII
Tri-Clover Division of	
Laddish Co. ....	Inside Front Cover
Universal Milking Machine Co.	
Division National Cooperatives ....	X

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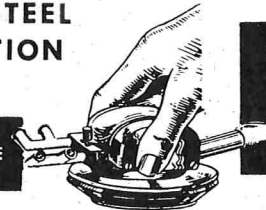
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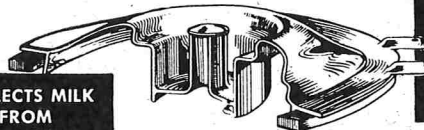
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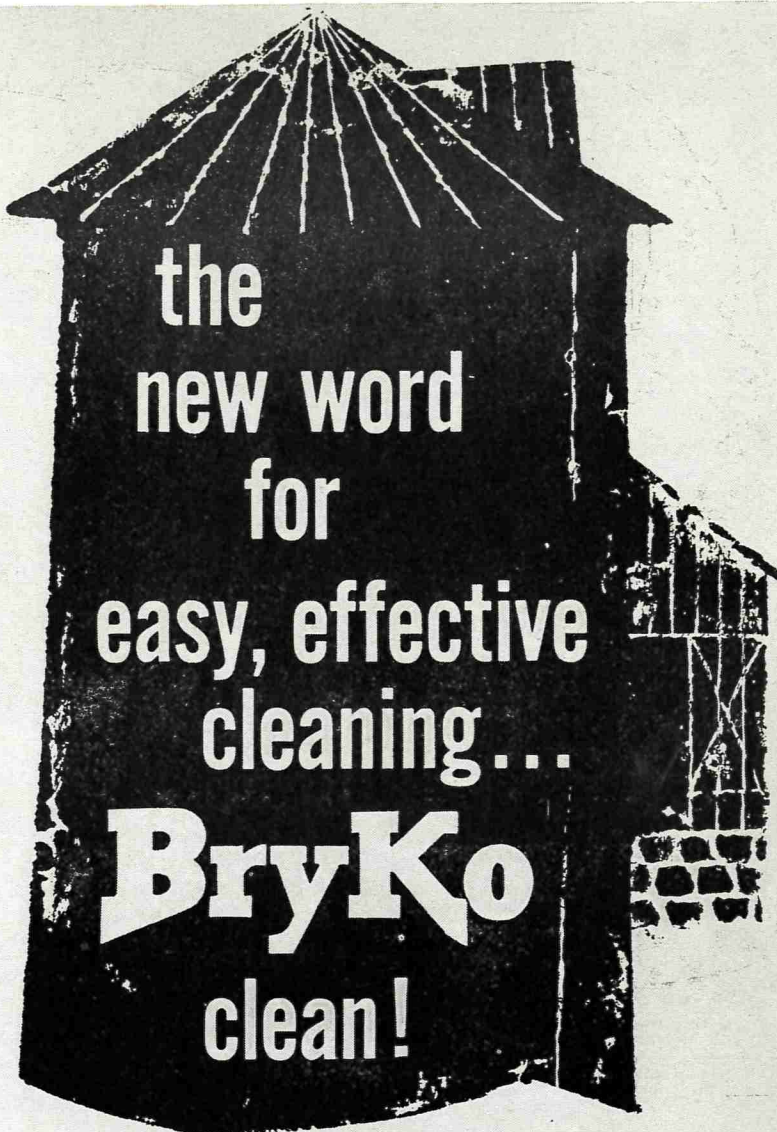
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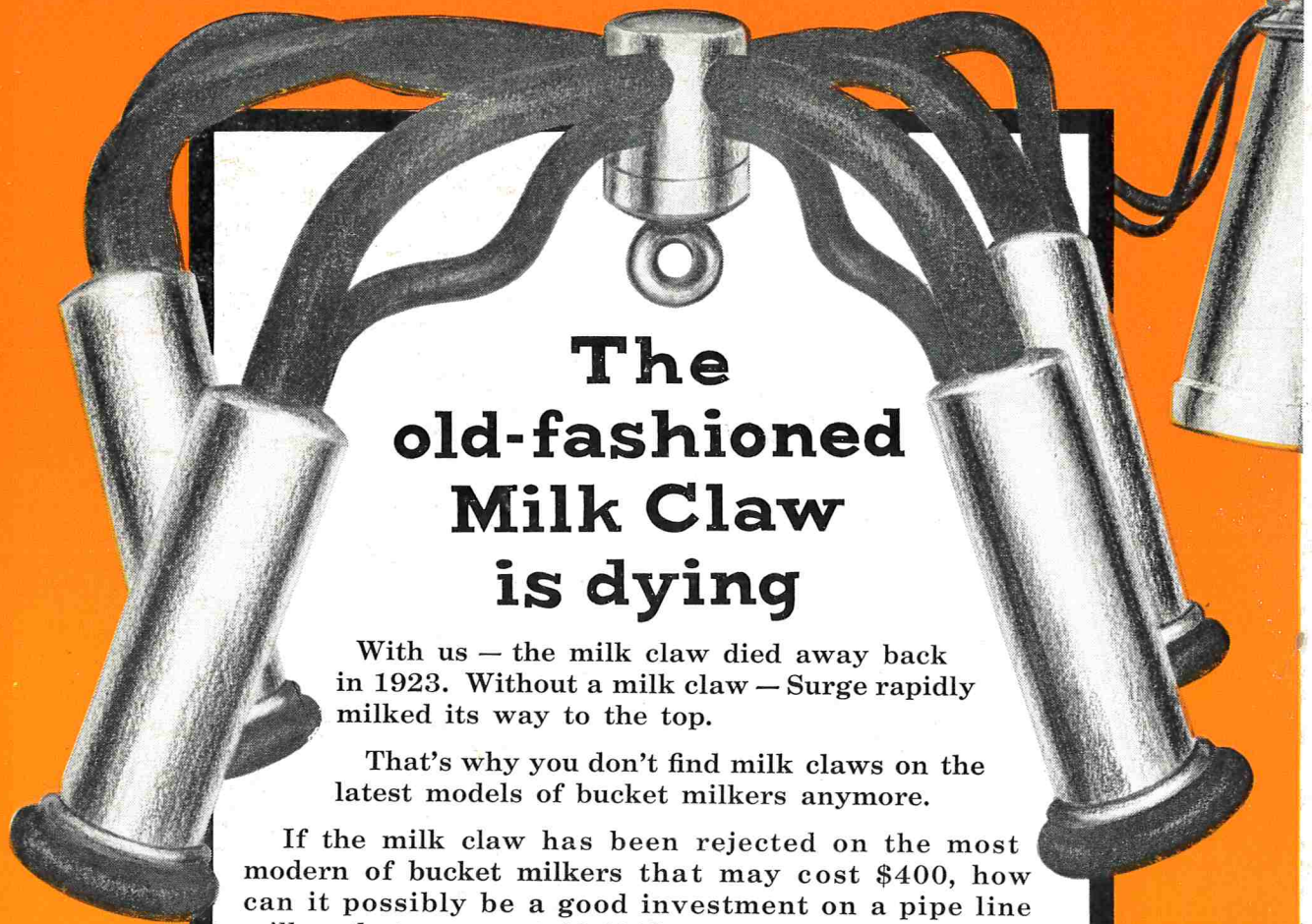


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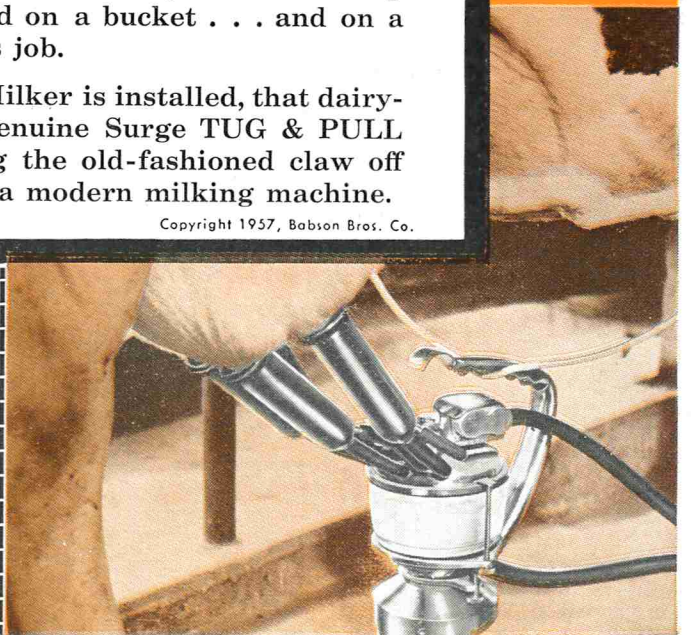
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