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## Editorials

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### THE THIRTY-SEVENTH ANNUAL MEETING AT ATLANTIC CITY

THE Thirty-seventh Annual Meeting, held at Atlantic City, N. J., October 13 to 16, 1950, was one of the most interesting and successful in the history of the Association. The papers were varied, authoritative, and informing. In addition, the presentation of motion pictures introduced an element which entertained as well as informed. A feature of the meeting was the breakfasts of the various committees, boards, and groups; these facilitated exchange of ideas, fostered acquaintanceships, and enhanced friendships. In place of the usual banquet, Dr. Robert S. Harris, Professor of Nutritional Biochemistry at the Massachusetts Institute of Technology, delivered an informative address on "Recent Developments in Nutrition with Emphasis on Dairy Products." The experiment in running the meeting for four days including Sunday seemed to work out very well, facilitating the possibility of the members visiting the great dairy exposition.

The big business of the meeting was the consideration of the proposed amendments to the constitution. The main points at issue were the questions of dues and the proposals as to whether or not all members should be on the same basis. The members present voted unanimously in favor of raising dues to five dollars for every member, whether Active or Associate, with rebate of one dollar back to the respective affiliate. A large majority voted in favor of providing for only one class of members.

The proposed amendments are now being edited for sending to every Active member for mail vote as to acceptance or rejection of each item.

The next meeting will be held near Denver, Colorado.

J. H. SHRADER

## C. SIDNEY LEETE

WE knew Sid Leete for twenty years or more. We first met him when he was employed in the Department of Agriculture in Washington. He impressed us as one who was devoted to his work, who was anxious to be of help, who was well-informed in his field. When he moved to Albany, we noted his development in professional ability and personal quality. In fact we became a party to trying to pry him away from public service—in which we failed.

When the INTERNATIONAL ASSOCIATION OF MILK AND FOOD SANITARIANS, INC. faced the crisis in its affairs that followed the death of Ivan C. Weld, several members served as its Secretary-Treasurer for brief terms to tide it over until the right man could be found. Ira Hiscock, Ralph Irwin, and Paul Brooks held things together; then Sid Leete was "discovered." For ten or twelve years or so he held office. During that time the Association grew from 250 members to over two thousand. Sid helped us in getting the JOURNAL started, and steadied the rocking boat many a time. Difficulties arose from time to time of one kind or another but he always "understood." We did not realize how great was his contribution to the cause of milk sanitation in general and the Association in particular until he resigned the secretaryship. As we took over his office, we discovered the great amount of work that he had been doing—all in addition to his regular employment duties in the New York State Department of Health. We now wonder how much this shortened his life. It must have been appreciable. Why, oh why, don't we see these things before it is too late to remedy them?

He is gone. We can scarcely realize it. Gone, hastened by his service to us. Gone, how we miss him. We all liked Sid—liked him a lot. His loss is keenly felt. Dear comrade, your passing brings us up with a jolt. We stand with uncovered head and hope to see you in the morning.

J. H. SHRADER

In the passing of C. Sidney Leete, workers in the field of milk sanitation throughout the country have lost one of their best friends and staunchest supporters. Close association with him daily during the past 20 years has impressed me with his sterling qualities. His wise counsel always was freely given. He anticipated every need and did his utmost to carry his share of the load. Many people in official control and in industry will feel as I do the loss of Sidney Leete not only as a friend and associate but as a leader in his chosen field.

WALTER D. TIEDEMAN

## JOURNAL OF NATIONAL ASSOCIATION OF BAKERY SANITARIANS

FOR those of us who have spent most of our lives in the field of food sanitation, it is gratifying to see the spread of this gospel of cleanliness. We remember when we hoped that the milk industry would become as sanitary-minded as the brewing industry was. Since then, the milk industry has gone ahead. Other divisions of the food industry are recognizing the importance of such considerations. One of the latest is the great baking industry. The National Association of Baking Sanitarians has begun the publication of a journal. We welcome this addition to the periodical literature and extend our best wishes for its growth and success.

J. H. SHRADER

## IMPROVING STAINING PROCEDURES IN THE DIRECT MICROSCOPIC EXAMINATION OF MILK \*

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### INTRODUCTION

A field survey of laboratories engaged in the bacteriological examination of milk clearly indicated that non-adherence to procedures recommended in *Standard Methods for the Examination of Dairy Products*<sup>1</sup> was more general than might have been expected. One of the cardinal causes leading to non-adherence to recommended procedures for the direct microscopic method was found in the difficulties experienced and the unsatisfactory results obtained with the two optional stains prescribed. An analysis of these and several non-official stains broadly used in the laboratories visited, in turn, indicated that they were compounded by a "rule of thumb" or "hit or miss" method.<sup>2</sup> None appeared to be based on rational scientific principles applicable to the processes of staining or dyeing. Studies were, therefore, undertaken for the purpose of determining some of the basic principles which underlie the staining of milk films with the methylene blue dye. Although the greater part of the discussion presented may appear theoretical, its purpose is a practical one, namely, to establish rational and scientific channels which future researches might follow in endeavors to further improve solutions and procedures for the staining of milk films for examination by the direct microscopic count method.

\* Presented in abstract form before Section G at the meeting of the Society of American Bacteriologists, May 18, 1950, in Baltimore, Md.

### THE CONCEPT OF ADSORPTION

A survey of the literature on biological staining and on various phases of commercial dyeing was made.<sup>3-5</sup> \* On the basis of the results of extensive experimental work and of the literature survey, it appeared that the staining of milk films and of the organized cells by methylene blue is basically an adsorption phenomenon.<sup>6</sup> In order that the application of adsorption to the staining of milk films with methylene blue may be better understood, a brief discussion of the underlying principles is here presented.

Adsorption as a general phenomenon can be defined as the change in concentration of dissolved or gaseous substances at interfacial boundaries relative to the concentration in the adjacent homogeneous phase. Such surfaces may be the boundaries between any two phases, at the interface between two immiscible liquids, or between a liquid and submerged solid, as for instance at the interfaces of the various constituents of a dried milk film fixed to a glass slide and submerged into the methylene blue dye solution. The early investigators of adsorption believed generally that the increased concentration of the adsorbed substance at the interface or near the surface was the result of the operation of simple forces. In present-day pictures explaining adsorption the

\* Further references to literature on these subjects can be found in Chemical Abstracts and in *Bibliography of Solid Adsorbents* by Victor R. Deitz of the National Bureau of Standards, Washington, D. C.

analogy to the retention of the earth's atmosphere by gravitational attraction of the earth was frequently resorted to. Such early views on adsorption are usually designated as "physical theories." According to the newer theories the forces involved are those acting directly between the absorbed atoms or molecules and the atoms or molecules of the surface of the adsorbent.<sup>7</sup>

Many authoritative students of the subject of adsorption still maintain that the distinction between physical adsorption, in which one deals with the so-called *van der Waals* forces, and chemisorption, where forces are of a more intimate interatomic or intermolecular order, is fundamental. Even the simplest case of adsorption is by its very nature a complex process in which forces of both interaction and reaction come into play in some definite or intermittently changing succession. In addition, the process of adsorption is so rapid as to be almost instantaneous. In many cases it is difficult, if not impossible, to observe the order in which the two types of forces act, and more difficult to measure them. Until technics are developed to enable the observation and measurement of the two types of forces and make records of them as they occur in all cases of adsorption, the dualistic viewpoint of adsorption will continue to find adherents even among authoritative students of the subject of adsorption.

#### MONO- AND POLYMOLECULAR LAYERS OF ADSORPTION IN RELATION TO DIFFERENTIATION STAINING

If the chemical theory of adsorption, narrowed down to some conventional stoichiometric concept, is adopted, then the assumption might follow that after a single layer of molecules had been adsorbed by a surface, the process would stop, unless it is assumed that new types of forces come into action. Such appears to be the viewpoint of Langmuir. He believes that there is a strong tendency for the adsorbed film

to be one atom or molecule in thickness, but admits the possibility of additional layers. Langmuir states, for instance, that where the forces holding atoms or molecules in the second layer are greater than those in the first layer and if the pressure is sufficient, "these atoms in the first layer begin to form clusters which readily become covered by a second layer . . . ; a third, fourth, and subsequent layers thus begin to form on these nuclei even before the first layer is completed. . . ." <sup>7</sup> On the other hand, Brunauer states that it is equally possible to assume that the same forces which produce condensation also hold together all other layers. He is of the opinion that "if we deal with adsorption on a free surface, then at the saturation pressure of the gas" ( $P_0$ , or the adsorbate, which in our instance are the molecules of the methylene blue) "an infinite number of layers can build up on the adsorbent."<sup>8</sup>

If one distinguishes the relative heats of adsorption in various layers, as it is now possible to do, then it is found that the views of Langmuir and Brunauer are in accord. Bikerman<sup>9</sup> summarizes the question of mono- and polymolecular adsorption in the following simple statement: "The adsorption isotherm in chemisorption always indicates unimolecular adsorption, in *van der Waals* adsorption it is either unimolecular or multimolecular."

In a previous publication<sup>6</sup> it was shown that staining milk films with methylene blue is fundamentally an adsorption phenomenon of the *van der Waals* type. The course which such a process of adsorption follows in relation to the number of layers adsorbed has a practical bearing upon the degree and clarity of differential staining of the background of the milk films and the bacteria of different species, ages, viability, and virulence.

#### SURFACE TENSION

The lowering in surface energy is intimately related to adsorption. There-

fore, the factor next in importance in our problem is surface tension. Other factors being equal, if a solution of a substance has a lower surface tension than the pure solvent, the solute is adsorbed or concentrated at the surface with a speed and to a degree proportional to the difference in the surface tension between the pure solute and the final solution.<sup>10</sup>

It is this physico-chemical property of adsorption which led to the development of the Acid-and-Water-Free stain<sup>6</sup> as a possible substitute for the stains recommended in Standard Methods. Water has a surface tension of 72.75 dynes/cm under standard conditions. The surface tension of the final aqueous carbolated stain of standard Methods is 46.0 dynes/cm. This represents a lowering in the surface tension of 26.75 dynes. Ethanol (95 percent) has a surface tension of 25 dynes/cm. The Acid-and-Water-Free stain has a surface tension which is only a few dynes lower than the surface tension of alcohol. It can readily be seen, therefore, why the aqueous carbolated Standard Methods stain with a lowering in the surface tension of 26.75 dynes should overstay within 10 to 15 seconds. It is equally understandable why the Acid-and-Water-Free stain with a lowering in surface tension of only two to five dynes should stain the milk films in one to two minutes in an even and highly differential manner.

#### THE FREUNDLICH ISOTHERM

The amount of dissolved substance (adsorbate) adsorbed per unit surface area or per unit weight of adsorbent at equilibrium is a function of temperature, pressure, and the nature of the adsorbate and the adsorbent. The functional equilibrium in adsorption, therefore, follows a path defined by certain physico-chemical laws and principles. However, such a path can be expressed in terms of a conventional stoichiometric generalization in only

some special cases. As a rule adsorption data are expressed by generalizations known as "adsorption isotherms". Many such generalizations have been proposed. This indicates that no one isotherm can cover all types of adsorption. An empirical equation known as the Freundlich Isotherm is,  $M = C P^n$ .  $M$  is the amount of material adsorbed,  $C$  and  $n$  are constants,  $n$  being less than unity;  $P$  is the pressure exerted by the solute or adsorbate. In the usual laboratory adsorption-demonstration  $P$  can be expressed numerically as the concentration or weight of the solute present in solution at the start and at different steps of the experiment. It has been frequently demonstrated that if the logarithmic values of  $M$  and  $P$  are plotted, a straight line of positive slope is obtained. The slope of the line determines the value of  $n$  and the intercept the value of  $C$ .

For a long time Freundlich's generalization was regarded as the adsorption isotherm *par excellence*. It has been very largely replaced at present by the isotherms of Langmuir,<sup>11</sup> Brunauer,<sup>8</sup> Harkins,<sup>12</sup> and their modifications. These authors dealt with adsorption of gases and vapors whereas Freundlich was concerned with adsorption from solution. It cannot be stated that the Freundlich isotherm has entirely lost its value either from a theoretical or practical point of view. Zeldowitch,<sup>13</sup> for instance, showed that the Freundlich equation can be derived from the Langmuir equation by assuming a certain type of distribution of the heats of adsorption over the surface. Vinogradov and Borodoulina have devised a nomograph for calculations by Freundlich's equation in some of their practical work.<sup>14</sup> In chromatography, which is based on differential adsorption of a type, it has been found recently that the proportion of solute in the liquid phase rises as the concentration is increased and that depending upon the particular case under study either the Langmuir type or the Freundlich

type of isotherm may apply.<sup>15, 16</sup> If the adsorption of the solute on the surface tends to a definite limit, the Langmuir isotherm would appear more applicable. In cases where adsorption increases indefinitely with increasing pressure, the Freundlich equation appears to be of greater practical value.<sup>17</sup> We found the Freundlich equation useful within the practical limits of our requirements for M and P in the study of adsorption of methylene blue by milk casein and by the different types and species of milk cultured bacteria. The Freundlich isotherm is suggested here as another scientific guide in researches intended for improvement of milk stains used in the direct microscopic examination of milk. It can be employed in determining the most useful concentrations of methylene blue dye appropriate to certain solvents, or for the attainment of special effects.

The principles underlying adsorption thus far discussed indicate the following practical points: (1) By proper selection of the pure solvent, based upon surface tension determinations as previously indicated, a staining solution can be prepared, the adsorption speed-reaction of which would be such as to result in a specific degree of stain intensity or differentiation. (2) By making a study of the isothermic curve in relation to a series of pure solvents and the particular types of material to be stained, most appropriate zones of adsorbate concentration can be established. (3) By a proper selection of a suitable combination of the above-mentioned two factors, a staining solution can be prepared which may have a wide range of application and yet possess the properties of a high degree of differentiation, without overstaining.

This can be demonstrated by an analysis of the data presented in a previous publication.<sup>18</sup> These data show that the lowering of the surface tension from that of the pure solvent to the surface tension of the final stain in the case of the aqueous carbolated

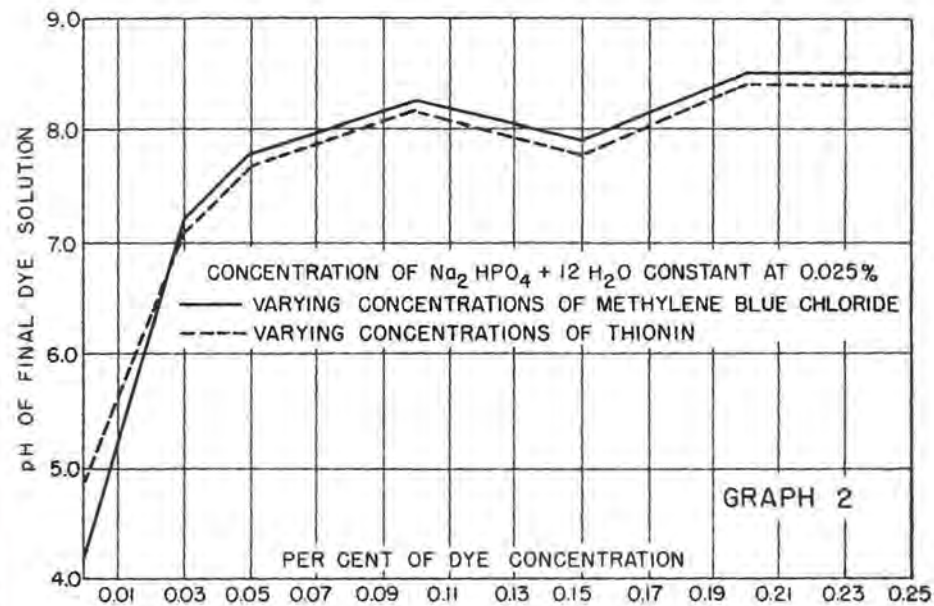
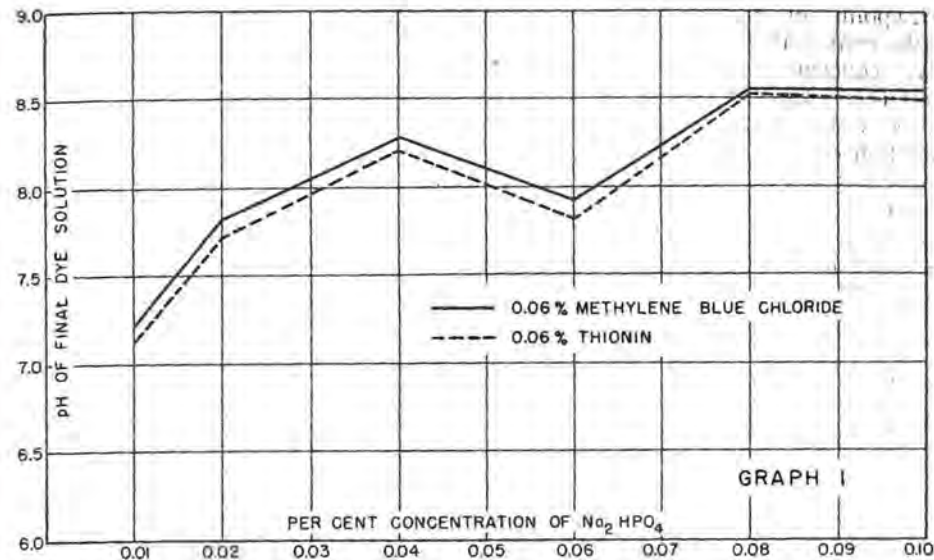
stain is equal to 26.75 dynes/cm. In the case of the optional alcohol-containing stain the corresponding lowering of the surface tension is 31.75 dynes/cm. According to one of the principles of adsorption previously discussed, the alcohol-containing methylene blue solution should stain more intensely and less differentially. This, however, is definitely not the case. The explanation can be found in the lower surface tension of the alcohol-containing solution, incident to the lower concentration of the adsorbate (the dye). It is 0.355 per cent in the aqueous carbolated stain and only 0.230 per cent in the alcohol-containing staining solution.

#### THE PH OF THE STAINING SOLUTION

The pH at which the adsorption system is set up further greatly influences the end-results of the process. In this connection Deitz states: "It seems to me that there are two pH effects. The staining process is a function of pH and the determination of the concentration of the dye in solution is also dependent on pH. The latter is a problem in organic chemistry and can be viewed as an indicator effect only."\* The discussion here is chiefly concerned with the effect of pH changes on the staining process. It was found that this phase of the adsorption phenomenon, as applied to the problem under consideration, can be studied by a simple method. The effect of (1) varying concentrations of buffers on the final pH of constant concentrations of the methylene blue dye, in aqueous solution, and (2) varying concentrations of the dye on the final pH of constant concentration of the buffers were determined. These results are plotted in graphs 1 and 2.

They show that aqueous solutions of 0.025 percent methylene blue chloride

\* Personal communication of June 14, 1950, from Dr. Victor R. Deitz, in Charge, Bone Char Research, National Bureau of Standards, Washington, D. C.



buffered with  $\text{Na}_2\text{HPO}_4$  to pH 7.0-7.2 can produce excellently stained milk films, so far as the differential level attained is concerned. However, because of the low pressure (P) incident to such an unusually low dye concentration, the staining time has to be considerably prolonged. In cases where

specific results are desired, such a procedure may prove useful. As guides in such special work several sets of curves similar to the examples presented may be prepared for a series of constant concentrations of the dye or of the buffer and the most appropriate combination of the two selected. From the

viewpoint of routine bacteriological milk examination stains of such low dye concentration have to be discounted. The long exposure of the dried milk films to the aqueous dye solution causes them to become waterlogged and wrinkled upon drying. In addition, it was found that the adjusted pH of the final stain changes as the solution is being used, and with time when not in use. It becomes necessary to prepare new solutions of the dye each time a set of milk slides had to be stained.

#### INFLUENCE ON ABSORPTION OF THE NATURE OF ADSORBATE

It was indicated in a preceding paragraph that the final value establishing equilibrium among the forces inherent in adsorption is dependent upon the nature of the solute or adsorbate.<sup>10</sup> Applied to the subject under consideration, this means the methylene blue dye. To determine in a limited measure the influence exerted on the outcome of adsorption of dyes of somewhat different chemical structures, a comparative study was made of thionin and methylene blue chloride. Both dyes are members of the thiazin subgroup of the quinone-imine group. Methylene blue is tetra-methyl thionin chloride. Both are basic dyes. Their molecular weights and solubilities in water and alcohol differ considerably but their maximum absorption is very nearly the same. When pH curves were plotted and as is indicated in graphs 1 and 2, the curves ran so closely together as to be considered identical for all practical purposes. Yet, when adsorption and staining tests were made it was found that the adsorption properties of thionin were considerably inferior to those of methylene blue chloride. Close observation disclosed qualitative and quantitative differences in the dye films deposited upon the bacteria, thus indicating that despite the close similarity in the chemical structure of the two dyes,

their adsorption-reaction paths in relation to the adsorbent may not be congruent.

Hansen, Fu, and Bartell found this to be true of other adsorption systems. They state that "adsorptions of aniline and phenol by sugar charcoal and the carbon blacks were . . . congruent, but adsorptions of aniline and phenol by the graphites were not congruent, and the isotherms differed markedly in form."<sup>20</sup>

In relation to improving milk staining procedures for the direct microscopic count, it must be concluded that no matter how closely related the chemical structure of two dyes may be, their adsorptive properties, and, therefore, their behavior as stains may be totally different. Each of such dyes should, therefore, be studied individually in accordance with the procedures discussed before a final or standard stain is selected.

#### INTERFACIAL STAINING INTENSIFICATION

Suitable auxiliary substances added directly to the staining solutions affect the reaction-speed and the end-results of the process of dye adsorption. Results which were considered superior to those obtainable with the stains recommended in Standard Methods were secured in this manner. This was particularly true of the addition of some of the surface active agents such as quaternary ammonium compounds. Phosphorated capryl alcohol can be cited as the most notable example among such compounds, probably due to the fact that a 1.0 percent concentration of this agent has a surface tension of 22.5 dynes/cm. However, here, as in the case of the pH adjusted stains of low concentration, the constancy of the effect of the auxiliary compounds in relation to time could not be depended upon. In addition, such procedures would be contrary to the guiding principle of simplicity that a staining solution should preferably consist

of two compounds only, one being the pure solvent and the other the appropriate solute.

The principle of introducing active agents directly at the interfaces created by submerging the milk film in the staining solution, without in any way affecting the simplicity of the staining solution as such appeared as a promising method for the improvement of milk film staining procedures. By this method suitable chemical residuals are introduced directly into the milk films prior to their immersion in the staining solution. Such chemical substances become adsorbed differentially to the surfaces of the different constituents of the milk film remaining there as chemically unchanged residuals, or they may bring about mild chemical interfacial changes. In either case the end results are such as to intensify selectively the original adsorptive properties of the organized cells in the dried milk film. As a secondary effect there may occur an intensification of the tinctorial power of the dye molecules by interfacial oxidation, reduction, or limited pH adjustment. By virtue of the particular chemical nature of the milk proteins, the adsorptive power of the milk film background for the molecules of methylene blue chloride is lessened and equalized.

Such a method for improving the utility of biological stains is not new or revolutionary. It has been tried for many years. It is presented here in its direct relation to the staining of milk films with methylene blue. As the chemicals used are not introduced into the staining solution directly but are applied at the interfaces of the staining system, it appears appropriate to refer to this staining procedure as the Interfacial Staining Intensification method.

The chemical compounds found suitable for the Interfacial Staining Intensification can be classed roughly into three groups: First, the oxidizing compounds; second, the reducing compounds; and third, the buffering

compounds. Compounds were also tested which possessed both oxidizing and buffering or reducing and buffering properties. Hydrogen peroxide, ammonium thioglycolate, and the ethers of ethylene glycol serve as examples of reagents possessing such combined influences. It is worth-while to note that thioglycolic acid and its derivatives constitute a comparatively recent family of synthetic organic compounds which are characterized by an extreme chemical versatility. Due primarily to the presence of the thio group these compounds can display a variety of reactions. Of the buffering agents  $\text{Na}_2\text{HPO}_4$  was found the best. It requires a considerable number of experimental tests before a suitable concentration can be chosen. It is of interest that in the application of the principle of Interfacial Staining Intensification to the staining of dried milk films with methylene blue, both the oxidizing and the reducing compounds tried produced similarly improved results. At first encounter this appears paradoxical. The mechanism of action of such compounds applied to the staining of milk films as here discussed is now under study.

#### EXAMPLES OF STAINING PROCEDURES BY THE INTERFACIAL STAINING INTENSIFICATION METHOD

The dye used in connection with the example-formulas given below was exclusively certified methylene blue chloride. The staining solution in all the formulas was the Acid-and-Water-Free stain described elsewhere.<sup>6</sup> However, other appropriate staining solutions can be used. The Acid-and-Water-Free stain is a simple solution of 0.6 percent certified methylene blue chloride in 95 percent ethanol.

##### Example No. 1

1. Prepare and dry milk film as prescribed in *Standard Methods for the Examination of Dairy Products*.
2. Defat as prescribed in *Standard Methods*.

3. Fix in 95 percent ethanol containing 5 percent of a 50 percent solution of ammonium thioglycolate (or a suitable derivative of same) for two minutes.
4. Stain with Acid-and-Water-Free Stain for two minutes.
5. Lightly rinse in tap water (not running) having a pH not lower than 7.0.
6. Thoroughly dry and examine microscopically.

#### Example No. 2

1. Place 0.1 ml of ammonium thioglycolate, (or a suitable derivative of same), of 50 percent strength into a 15 ml vial.
2. Place 10 ml of milk previously properly stirred into the vial containing the thioglycolate. Shake.
3. Allow vial to rest 10 to 15 minutes.
4. Prepare milk film from content as per Standard Methods.
5. Defat. Fixing in this case is not necessary.
6. Stain with the Acid-and-Water-Free Stain for two minutes.
7. Rinse and dry as in the case of Example No. 1.

#### Example No. 3

1. Prepare and dry milk film and defat as per Standard Methods.
2. Fix for two minutes in 95 percent ethanol containing 5 percent of hydrogen peroxide of 12 percent strength. (12 percent hydrogen peroxide can be prepared by adding 5 ml of 30 percent hydrogen peroxide to 7.5 ml of distilled water).
3. Stain with the Acid-and-Water-Free stain for two minutes.
4. Rinse and dry as previously described.

#### Example No. 4

1. Place 0.1 ml hydrogen peroxide into a 15 ml vial.
2. Place 10 ml of previously stirred milk into vial and shake. Allow vial to rest 10 to 15 minutes.
3. Prepare, dry, and defat milk film as per Standard Methods.
4. Stain with Acid-and-Water-Free Stain as above described.
5. Lightly rinse, dry, and examine microscopically.

#### SUMMARY

A discussion is presented of the physico-chemical phenomenon of adsorption as it applies to the staining of milk films with a solution of methylene blue chloride. Several phases of adsorption were analyzed in relation to the attainment of specifically desired

degrees of differential intensity in the staining of the proteins of the milk film and the bacteria of different species, ages, viability, and virulence. Effects produced upon the final results by the use of a staining method designated as the Interfacial Staining Intensification are described, and examples of staining procedures are presented.

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(Continued on page 331)

## INACTIVATION OF BACTERIOPHAGE OF THE LACTIC ACID STREPTOCOCCI AT HIGH AND LOW pH LEVELS \*

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BACTERIOPHAGE infection of the lactic acid streptococci of starters is a problem frequently encountered in cheese making. Among the various measures contributing to the prevention and elimination of this condition from a cheese factory, the cleaning and sanitizing operations play an important role.

Since both acid and alkali cleansing agents are used extensively in plants processing dairy products, it appeared desirable to study the resistance of bacteriophage in an environment of both high and low pH values.

Hunter and Whitehead<sup>1</sup> observed a rapid destruction of bacteriophage at room temperature at and beyond the limiting pH values of 11.8 and 2.5. They presented no detailed data concerning this observation.

#### METHODS

Three bacteriophage strains contained in whey filtrates were used in this study. The filtrate was prepared by inoculating the lactic acid streptococcus and its homologous bacteriophage into sterile milk and incubating the culture at 30° C. After 24 to 48 hours sufficient acid usually was produced to cause coagulation. If such was not the case, either acid or rennet was added to induce coagulation. The coagulated milk was first filtered through a cloth-cotton pad to remove the coarser curd particles. The result-

ing filtrate was then passed through a sterile Seitz filter. The final filtrate, free from bacteria, contained the bacteriophage particles. Care was exercised in all trials to develop and maintain a high bacteriophage titer in all test filtrates.

In making the determinations the pH value of the whey filtrate was adjusted to the desired level by using approximately 2 N lactic acid or 2 N sodium hydroxide as the particular determination required. A Beckman pH meter, Industrial model with glass electrode, was used for measuring the pH value. The amount of acid or alkali necessary to adjust the pH of the test whey to the required level was predetermined by titrating an identical test portion. This made it possible to make the pH adjustment with a minimum lapse of time, usually a period of a very few seconds.

The experiments were carried out at room temperature (21-23° C). A 10-ml portion of whey filtrate containing the bacteriophage was placed in a 50-ml beaker, and the pH adjusted to the desired level. At intervals of 1 minute and continuing for a period of 10 minutes, a 0.5-ml portion of the adjusted whey filtrate was transferred to a sterile test tube containing bromthymol-blue indicator. The pH of this aliquot was restored rapidly to the neutral point by the addition of dilute acid or alkali, as required. A 10-ml portion of freshly inoculated milk containing the homologous lactic streptococcus was then added, mixed

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by inverting the tube several times, and then incubated at 30° C.

Following an incubation period of 14 to 16 hours, the milk culture tubes were observed for evidence of acid coagulation. Cultures that presented no evidence of coagulation or that required an incubation period in excess of the control culture for acid coagulation were assumed to contain active bacteriophage as the cause of the retarded coagulation. To obtain further evidences of the presence or absence of active bacteriophage, sub-cultures were made to additional milk tubes inoculated with the homologous host cultures.

Previous to making the sub-culture the whey and curd was separated. A drop of the clear whey served as the inoculum for the bacteriophage in the sub-culture. This technique was followed in order to reduce the possibility of transferring, along with the bacteriophage particles, large numbers of host cells that may have developed considerable resistance to the bacteriophage strain and which, by rapid growth in the sub-culture, might obscure the presence of bacteriophage active against the original host culture. Previous studies<sup>2</sup> conducted in this laboratory have shown that some cultures of lactic acid streptococci will rapidly develop such a resistance to the homologous bacteriophage. The cultures were incubated at 30° C and observed after 14-16 hours for acid coagulation. The data presented in this study represent the findings as observed in the sub-cultures.

#### EXPERIMENTAL RESULTS

##### High pH Levels

*HP strain of bacteriophage:* No inactivation occurred at pH levels of less than 11.3 at exposure periods up to 10 minutes. Inactivation was complete after an exposure period of 1 minute in all trials conducted at pH levels above 11.6. At pH levels of 11.3 to

11.4, 11.4 to 11.5, and 11.5 to 11.6, the longest periods of time required for inactivation were 8, 7, and 4 minutes respectively. In each of the ranges 11.4 to 11.5 and 11.5 to 11.6, inactivation was complete in several of the trials even after 1 minute of exposure.

*No. 6 strain of bacteriophage:* No inactivation occurred at pH levels below 11.1 at exposure periods up to 10 minutes. Inactivation was complete in all trials at pH values of 11.3 and above, even after 1 minute exposure periods.

*W. strain of bacteriophage:* No inactivation occurred at pH levels below 11.1 at exposure periods up to 10 minutes. Complete inactivation in all trials, and at all exposure periods did not occur until a pH level of 11.8 was reached. The results of the different trials with this strain were somewhat more variable than those secured with the other two strains.

##### Low pH Levels

*HP strain of bacteriophage:* The first evidence of inactivation occurred at a pH level of 2.75 and at exposure periods of 10 minutes. At pH levels of 2.59 and lower, inactivation was complete in all trials and at all exposure periods.

*No. 6 strain of bacteriophage:* The earliest evidence of inactivation occurred at a pH level of 2.72, this evidence being present at the longer exposure periods. Inactivation was complete in all trials and in all exposure periods at pH levels below 2.54.

*W. strain of bacteriophage:* No evidence of inactivation during the 10 minute exposure period was observed until a pH level of 2.72 was reached. Inactivation was complete in all trials and at all exposure periods at pH levels of 2.54 and lower.

#### DISCUSSION OF RESULTS

The three strains of bacteriophage used in this study showed some degree of variation in their resistance to high

pH levels. The no. 6 strain was the first to be inactivated, the HP strain next, and the W strain last. The critical pH levels for 1 minute exposure periods were 11.3, 11.6, and 11.8 respectively for the three strains.

At low pH levels the differences in resistance occurring among the three strains appeared to be slightly less than those noted for the high pH levels. The first evidence of inactivation of the HP strain occurred at a pH level of 2.75, and inactivation was complete after an exposure period of 1 minute at a pH of 2.59. With the other two strains, inactivation first appeared at a pH level of 2.72 and it was complete at a pH level of 2.54.

With the HP strain the first evidence of inactivation occurred at a slightly higher pH value than with the other strains but with all strains inactivation was complete at the pH level of 2.54.

The results secured in this study

confirm the findings reported by Hunter and Whitehead.<sup>1</sup>

Because of the tolerance of the bacteriophage to both high and low pH levels, it is questionable that the ordinary alkaline and acid detergents used in the cleansing of dairy equipment would be of great value in controlling an outbreak of bacteriophage infection, particularly if used at low temperatures.

Since the effectiveness of alkalis and acids as germicides is associated with the temperatures at which these are used, data in this direction need to be secured before definite conclusions can be drawn.

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#### IMPROVING STAINING PROCEDURES

(Continued from page 328)

## A METHOD FOR MEASURING THE CLEANLINESS OF MILK CANS<sup>1, 2</sup>

J. M. JENSEN AND J. WATERSON

Michigan Agricultural Experiment Station, East Lansing

PRACTICAL measures are needed badly for measuring the cleanliness of milk cans. Dairy plant operators often find themselves ignorant of the cleaning effectiveness provided by their can-washing procedures because it is difficult to determine the extent of cleanliness that is being secured. Perhaps it is for this reason that milk cans are often considered weak links in quality milk production. They have been shown to contribute both bacterial and extraneous-matter contamination to milk. Roadhouse (1948) demonstrated by swabbing with clean cotton swabs that dirt remained on the interior of cans, thus suggesting that dust particles were deposited from dust-laden air that was blown into the cans during the drying stage of can washing. Claydon (1948) likewise demonstrated the presence of extraneous matter in cream cans, which had accumulated as a result of long storage. Tuckey *et al.* (1946) made examinations of milkstone scraped from the interior of cans to determine its bacterial content. The scrapings from a can with a heavy deposit was weighed and contained approximately 8 grams. However, no concerted effort was made to measure the soil content of cans in this study. As in other studies, the measure of can sanitation was largely made by determining bacterial population.

Ultraviolet light has also been used to aid the inspection of cans for cleanliness. While this light shows the presence of milkstone, density is not revealed.

<sup>1</sup> Journal Article No. 1149, Michigan Agricultural Experiment Station.

<sup>2</sup> This study was made possible through a research fund provided by Swift and Company, Chicago, Illinois to study quality problems in milk and cream.

### EXPERIMENTAL

In order to determine the effectiveness of can washing under various conditions of control, investigation was undertaken at the Michigan Experiment Station in 1947 to develop some practical means of measuring the degree of cleanliness in cans. A scrubbing procedure was first used, somewhat similar to that by Claydon (1948). A quart of sediment-free water was used with a detergent containing wetting agent (25%) and condensed phosphate (75%). The detergent was chosen for its milk-stone loosening properties that had been demonstrated in earlier studies (1946). On the completion of scrubbing a standard Langsenkamp-Wheeler milk sediment tester was used to draw a sediment test off the bottom of the can with the can in a tilted position. When a fibre dairy scrub-brush was used in this test of can cleanliness it was apparent that the brush contributed a great amount of fibre-brisle parts that were deposited on the sediment discs. Various scrubbing devices were therefore tried for washing entirely clean cans. These included (1) a stainless steel sponge, (2) a new bronze sponge, (3) a fibre brush, (4) a nylon bottle brush, and (5) a wad of clean cheese cloth. The results of the washings, (shown in figure 1), revealed that fibre brushes and metal sponges shed fibre and filings that would give a distorted reflecting of the cleanliness of the cans. The bottle brush, although used vigorously, shed only very little, thus indicating that a brush of this kind is not hazardous in contributing to extraneous matter retained in utensils on which it is used. The bottle brush was awkward to use inside the

can where vigorous rubbing was needed to loosen the milkstone-like soil. For such washing, the cheese cloth was sufficiently effective and left no residue that could be mistaken for soil. Cheese cloth was, therefore, selected as the rubbing "agent" in preference to the others that were tried.

### Method of Grading Can Washing Results

Various methods of grading the sediment pads were explored. The usual standard milk sediment chart for matching purposes was out of the question because of the character of the dirt on the pads. Weighing the pads met with little success. The variation in weight of unused pads often exceeded the variation that existed between pads before and after being used. Dissolving dirt from the pads by boiling them in strong acid and alkali, then measuring the amount of light that would pass through the solution by means of a photometer, was moderately successful. A numerical value was secured that was somewhat proportional to the amount of deposit. However, varying amounts of undissolved residue remained after boiling and this could not be accounted for with the photometric measurements. This method was, therefore, considered inaccurate and cumbersome, and was abandoned.

Finally, on the basis of many tests, it was decided to grade the pads on their outward physical appearance. The pads were divided into four groups. Those in Grade I were fairly clean and white. Those pads which were only slightly soiled were placed in Grade 2. Grade 3 consisted of dirty pads, and Grade 4 was made up of excessively dirty pads. Four representative pads were mounted as shown in figure 2, and used for matching during the segregation of the pads into their various grades.

### RESULTS

#### The Effect of the Condition of Cans on Can Washing Results

Can cleanliness was evaluated on 180 producer cans from the College Creamery. Sixty-one of these cans were hand-scrubbed and tagged for identification before being put into regular use along with 119 cans, also tested, that had not been hand scrubbed. After every third washing in a rotary can washer of the

### Can Washing Method Used in the Recommended Tests

Cans that had been machine washed and were ready to be returned to the patrons were selected at random for the hand-washing, sediment-testing trials. Each can was washed using a quart of sediment-free water, and approximately a half-tablespoonful of condensed-phosphate-wetting-agent detergent. Vigorous washing with a clean wad of cheese cloth was applied over the entire inner surface with at least two rounds of washing or until the cans had a feeling of cleanliness. Sediment tests were made on the wash water to determine the difference in amounts and in types of sediment that existed in the cans.



Figure 1

Showing appearance of sediment discs resulting from scouring a new clean milk can with (1) cheese cloth, (2) a nylon bottle brush, (3) a worn fiber brush, (4) a new fiber brush, (5) a new bronze sponge and (6) a new stainless steel sponge.





Figure 2

A sediment grading chart used for grading milk can residues that are washed free after the milk cans have received their regular washing treatment.

College Creamery, one pre-scrubbed and one or more nonpre-scrubbed cans from each of five producers were tested for cleanliness. The sediment pads were labeled to record the date of the test and other conditions. After testing, the identifying tags were removed and wires were fixed to can handles to distinguish them from untested cans, thus preventing reusing these cans for testing during the balance of the study. In this manner, records were accumulated from the testing of pre-scrubbed cans from 3 to 39 days\* following scrubbing.

The results shown in Table 1, indicate a definite improvement in cans as a result of pre-scrubbing, since 70.4 percent of these cans were Grade 1 and 2, in contrast to 56.3 percent in these grades from the nonpre-scrubbed group. Also, there were only 6.6 percent of Grade 4 discs in the pre-scrubbed

\* A few sediment discs were lost prior to grading

group compared with 16.8 percent in the nonpre-scrubbed group.

DISCUSSION OF RESULTS

The deposit secured on sediment discs by the washing procedure used is not comparable with that secured by Claydon (1948) or Roadhouse (1948), both of whom showed the presence of loose dust-like residue. The washing applied in the tests herein reported was designed to set free as much as possible of the residual films that adhered to cans after repeated washing in mechanical can washer.

In the can washing trials higher results in favor of the pre-scrubbed group of cans were expected. This was supported by the testing procedure. While this difference was not as great as may have been anticipated, it is likely that too much lasting result has been expected from occasional hand scrubbing.

In the operating of the test, the use of clean wads of cheese cloth for each can is important. Otherwise soil from cans that gave Grade 3 and 4 pads is carried over to succeeding cans.

While the test provided a practical means for determining can cleanliness, it was not assumed that all the milkstone soil was set free when Grade 4 discs were secured. Nor was all the loosened material from the wash water transferred to the sediment pad. It was deemed most expedient and highly sufficient to use a method that employed the sediment tester rather than filtering the entire amount of wash water.

The tests served to demonstrate convincingly the differences that existed in

cleanliness of cans. While there was no follow-up made to determine farm-handling practices, it appeared that the cleanest cans found received some washing attention on the farm. It also appeared that those with the densest films were the result of improper application of chlorine treatment. Further study making note of farm treatment of cans would be valuable.

SUMMARY

A method for determining the extent of cleanliness of machine washed cans was developed. The method consisted of hand washing individual cans with one quart of water to which a detergent was used containing a mixture of wetting agent-condensed phosphate. Abra-

sion during washing was applied with clean wads of cheese cloth. A sediment test made of the wash water with a Langsenkamp-Wheeler tester provided pads that were graded into four classes according to density of sediment.

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TRICHINOSIS PREVENTIVE MEASURE \*

Dear Sir:

In the past several years, hundreds of cases of trichinosis have been reported to the New York City Health Department. Undoubtedly this is only a small proportion of the number that actually occur. The victims may suffer severe pain, extreme weakness, high fever and the infection may even result in death.

Since the majority of these cases occur as a result of eating improperly cooked pork, the steps that you take in handling pork and meat products are important in preventing you, your employees and your customers from being infected with trichinosis.

Health Department inspectors will visit your restaurant in the near future to make certain that you are taking the following precautions:

1. All pork must be thoroughly cooked, baked, fried or processed to kill the trichinae parasite. Every portion of the pork must receive this heat treatment. As pork is a poor conductor of heat, the center portion must be frequently tested with a thermometer until a temperature of at least 137° F. is reached, at which point the trichinae are killed. This is the only safe way to know whether the meat has been sufficiently heat treated.
2. All ground beef sold as hamburgers which may be ordered rare must be free from pork. The restaurant owner must know whether the butcher or the restaurant supply house delivers hamburger meat that contains pork. If any of the ground meat contains pork, then it must be thoroughly cooked, fried or grilled to kill the parasite.
3. All orders requesting the serving of rare pork products must be courteously refused.
4. Under no circumstances, should pork chops be submitted for veal chops, or shredded pork for chicken in salads or chow mein. If your meat grinder has been used for grinding pork, it should be thoroughly cleaned and washed before using for grinding beef.
5. Chefs should be instructed not to taste the raw ground pork products. This is a dangerous practice.
6. Smoked or tenderized pork is not free of trichinae. These products usually receive insufficient heat treatment to kill trichinae. These products must also be heated to an internal temperature of 137° F. in order to destroy trichinae.

Your cooperation in carrying out the above principles is earnestly requested. Please feel free to call the Health Department for any additional information which you may desire on this subject.

Very truly yours,  
 EDWIN LUDEWIG  
 Director, Bureau of Food & Drugs

\* Copy of letter sent to restaurateurs by New York City Department of Health.

TABLE 1  
 SHOWING CLASSIFICATION OF CAN SEDIMENT PADS  
 FROM PRE-SCRUBBED AND NONPRE-SCRUBBED CANS

Sediment Grade	Number		Percent	
	Pre-scrubbed	Nonpre-scrubbed	Pre-scrubbed	Nonpre-scrubbed
1	24	34	39.3	28.6
2	19	33	31.1	27.7
3	14	32	23.0	26.9
4	4	20	6.6	16.8
Total	61	119	100.0	100.0

## REPORT OF COMMITTEE ON FOOD HANDLING EQUIPMENT \*

The last annual report of this committee was far from optimistic. This year we feel that we report some worthwhile accomplishments and are looking forward to greater opportunities for future expansion.

Although the committee did not meet during the year, except at this annual meeting, considerable work was done and progress made by correspondence and by activities of individual members. The committee's policy was formulated on the premise that the goal of high sanitary standards for food equipment acceptable to the greatest number could best be attained through collaboration with similar committees representing other national associations and food industry organizations. The initial efforts of this committee and other members of the Association has resulted in the appointment of similar committees by four other national health or sanitation organizations to work in close cooperation with your committee. The U. S. Public Health Service welcomed this coordinated teamwork and offered its full collaboration.

The National Sanitation Foundation was asked to support this program and to solicit the cooperation of industry. The Foundation responded with technical, moral and financial support. Representatives of the committees of each of the five Associations and the U. S. Public Health Service were invited to and attended a meeting at the Foundation headquarters, School of Public Health, University of Michigan, Ann Arbor, Michigan on September 6, 7 and 8, 1950. The main order of

business was to form a "Joint Committee on Food Equipment Standards" and to establish what might be called a "charter", stating the membership, officers, purpose and procedure of the Joint Committee and the relationship and responsibility of each member to his parent organization.

A tentative draft of this "charter" was prepared by a sub-committee of three incorporating the recommendations of all members of the Joint Committee. It has been reviewed by each member and some minor changes were suggested but we believe that the basic principles will be retained. This is a new and broader undertaking by this Association and for that reason, we urge every member to give it careful study before passing judgment on the recommendations offered by your committee.

### THE CHARTER

#### JOINT COMMITTEE ON FOOD EQUIPMENT STANDARDS OF THE NATIONAL SANITATION FOUNDATION

##### *Introduction:*

It is generally recognized by representatives of industry and public health that uniform, nationally accepted sanitation standards for food equipment are essential. Accepting this principle, representatives of the leading national organizations of public health sanitation officials suggested the creation of this committee and outlined certain procedures whereby representatives of industry and public health sanitation would work together toward this goal.

##### *Membership:*

The membership of the committee shall consist of:

A. Public Health Sanitation officials  
1. A representative of the United States Public Health Service.

2. The Chairman or a member of the appropriate committee of each of the following organizations:

International Association of Milk and Food Sanitarians

National Association of Sanitarians Engineering Section, American Public Health Association

Conference of State Sanitary Engineers

Conference of Municipal Public Health Engineers

A Secretary appointed by the National Sanitation Foundation

A public health sanitation delegate-at-large who is a member of the Council of Consultants of the National Sanitation Foundation

Delegates of such additional national organizations as may be recommended by the committee and approved of by the Council of Consultants of the National Sanitation Foundation.

B. Industry and Business representation

The National Sanitation Foundation shall, with the advice of its Industrial Advisory Board, encourage that branch of industry or business most directly concerned with any particular standards which are to be developed, to appoint or form a "task committee". This committee or its representative or representatives shall be invited to participate in the Committee sessions at which standards affecting its interests are being considered.

##### *Officers*

###### *Chairman and Vice Chairman*

The members of the committee shall at least every two years elect a chairman and a vice-chairman. The chairman, and in his absence, the vice-chairman shall preside at all committee meetings. He shall appoint such sub-committees as are authorized by the committee.

##### *Secretary*

The secretary shall keep records of all decisions of the committee and shall prepare and distribute the preliminary and final drafts of standards. He shall handle all correspondence and shall be the liaison official between the committee and the Foundation. With the consent of the Foundation and the advice of the chairman, he shall arrange for and announce meetings of the committee and shall prepare the agenda therefor. He shall represent the committee, whenever necessary, at meetings of Industry Task Committees to present suggestions or conclusions of the committee or to assist and encourage them in preparing preliminary standards for consideration by the committee.

He shall be the representative responsible for conferring with any test or research organization or official whose work may be considered by the committee. He shall invite to committee meetings any such official who may furnish the committee with information to assist in arriving at decisions.

He may perform any other such duties and act as the executive officer of the committee.

##### *Determination of Projects*

Projects may be recommended by industry, members of this committee, public health officials, the Council of Consultants, the staff of the National Sanitation Foundation or others.

Any project, before being formally undertaken, should first be approved by the National Sanitation Foundation in accordance with its policy of assigning priorities.

##### *Operation of Committee*

Whenever possible or practical the Secretary will attempt to encourage the appropriate Industry Task Committee to prepare preliminary standards for submission in writing to committee members as far in advance of the committee meeting as possible.

After consideration and revision of the preliminary draft of proposed

\* Presented at Thirty-seventh Annual Meeting of the International Association of Milk and Food Sanitarians, Inc., Atlantic City, N. J., Oct. 13-16, 1950.

standards by all participants in this program a revised draft shall be submitted to the appropriate task committee for final review, including, when necessary and agreed to, joint meetings for adjudicating differences. Whenever practical such standards shall also be submitted to the appropriate representative or representatives of the users, designers, or purchasers of equipment, materials or facilities affected by such standards for review and comment.

Final approval of standards by the committee may be by agreement arrived at in meetings or by mail.

#### *Responsibility of Committee members to their Parent Organizations*

It is assumed that, by their appointment, each committee member is authorized to perform all of the functions, outlined in these by-laws. It will generally not be possible for him to seek approval of the full membership of the Executive Committee of his organization before the standards are approved by the committee. It is, however, recognized that he will consult with his committee or members of his organization whenever practical so his broad recommendations may generally conform to the views or broad policies of the membership of the organization. He may also submit final, approved drafts for formal approval and support by his organization.

#### *Review by Council of Consultants*

The final draft of the standards shall be submitted by the Executive Director of the National Sanitation Foundation to the Council of Consultants for review and approval.

#### *Publication*

After approval, as required by the National Sanitation Foundation procedures, the National Sanitation Foundation shall publish standards so they can be used as guides by industry and control officials and they also shall be used as guides in awarding its Seal of Approval.

### RECOMMENDATIONS

We believe that the interests of the members of this Association, the food industry and the public, can best be served by collaboration with other organizations desirous of improving the sanitary design of food handling equipment and of improving the sanitary procedures of handling food.

We recommend that these tenets be carried out in two steps.

1. Your committee will work with the Joint Committee on Food Equipment Standards of the National Sanitation Foundation and appropriate task committees of industry to establish acceptable sanitation Standards for food equipment. The chairman, or other member selected by a majority, will represent the committee and serve as a member of the Joint Committee. Approval of a Standard will be by a majority vote of the committee unless the selected member is otherwise specifically authorized to act for the committee. The approved National Sanitation Foundation Standard may carry the endorsement of the committee but not the endorsement of the International Association of Milk and Food Sanitarians.

2. After an approved Standard has had a fair trial and has been studied by members of this Association and its affiliates, it should be brought up for Association approval at a regular business meeting. When a Standard is approved by the Association, the National Sanitation Foundation should be advised that such Standard carries the endorsement of this Association and that this fact may be so indicated on the Standard.

### STANDARDS UNDER CONSIDERATION

Industry task committees and the Joint Committee have not only oiled the machinery but have already given it a trial run. The Joint Committee at the meeting on September 6-8 worked with representatives of appropriate in-

dustry groups and with technical experts on the following tentative Standards:

1. Soda Fountain and Luncheonette Equipment
2. Food Service Equipment
3. Dishwasher and Associated Equipment and Facilities.

The Soda Fountain and Luncheonette Equipment Standard has been revised, reprinted and is now in process of being studied by each member of your committee and parallel committees of other Associations. The other two above Standards are being reprinted, as revised at the Joint Committee meeting, and will soon be in the hands of the individual members for study.

The opportunity for rapid progress looks promising but approval of Standards will not be the ultimate goal. The Standards will not only have to keep pace with progress; they should always be in the lead but not so far ahead that they cannot be followed by the majority. There will be instances in which it will not always be practical to apply every feature of the Standard. For example, in the Standard for Mechan-

ical Dishwashing Machines, it is recommended that pre-flushing facilities always be provided. Lack of space or the additional cost may discourage a small operator from installing a mechanical washer to replace his hand washing facilities. At the other end of the scale, there will be those who want something different from the majority. If that difference has sanitation merit and is practical, we are anxious to know of it in order to amend the Standard so the majority may reap the benefit.

It should be understood that the endorsement of these Standards by the Association does not bind any member to abide by the specifications. They are intended to serve as a guide to good practice. They have the force and effect of law only when official agencies incorporate them in laws, ordinances or codes.

C. W. WEBER, *Chairman*  
LEWIS DODSON  
F. H. DOWNS, JR.  
JOHN FAULKNER  
W. A. MACLINN  
JEROME TRICHTER  
JAMES H. MCCOY

### Public Relations Training at University of Denver

The difficulties sanitarians face in acquainting the public with phases of their work is receiving marked attention in the curriculum of the sanitary science department of the University of Denver.

Milton Miller, director, has required students to enroll in at least one major class in journalism, radio, newscasting or public relations.

Cooperating with Miller are the departments of radio and journalism which tailor courses to help the student sanitarians.

## THE DDT CONTENT OF MILK PRODUCTS<sup>1</sup>

H. D. MANN AND R. H. CARTER

*Bureau of Entomology and Plant Quarantine*

AND

R. E. ELY

*Bureau of Dairy Industry*

*Agricultural Research Administration, U. S. Department of Agriculture  
Washington, D. C.*

IT HAS been shown by a number of investigators that DDT<sup>2</sup> is eliminated in milk from cows that have been sprayed with this material or have been fed on forage containing it as a residue from insecticide applications.

This investigation was undertaken to determine the effect that various processing operations would have on the DDT in the original milk and to determine the DDT content of the finished milk products.

Milk from a number of cows that had been receiving DDT in soybean-oil solution was combined to make a supply sufficient to carry out the processing on a pilot-plant scale. The milk products were prepared by members of the Dairy Products Research Laboratories,

Bureau of Dairy Industry, by the adaptation of the usual commercial procedures to pilot-plant operations, because insufficient milk was available to operate commercial-size equipment. Nevertheless, the products obtained were considered to be representative of those that might have been manufactured on a large scale.

Samples of raw whole milk, raw skim milk, raw cream, pasteurized whole milk, pasteurized cream, butter, buttermilk, whey, and cheddar cheese were analyzed for their DDT content by the Schechter-Haller colorimetric method<sup>1</sup> modified to include several treatments with the sulfuric acid-sodium sulfate reagent.

Determinations of the butter-fat content of the various products were made by the usual procedures.

TABLE 1

BUTTER-FAT AND DDT CONTENT OF MILK AND MILK PRODUCTS

Product	Butter fat %	DDT (p.p.m.)	
		In product	In fat only
Raw whole milk	5.0	7.5	150
Raw skim milk	—	.2	96
Raw cream	70.0	67.2	120
Pasteurized whole milk	5.0	6.0	100
Pasteurized cream	70.0	70.2	—
Buttermilk	—	1.9	167
Whey	.3	.5	125
Butter	80.0	100.0	122
Cheddar cheese	38.5	47.0	—

<sup>1</sup> Report of a study in part under the Research and Marketing Act of 1946.

<sup>2</sup> 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.

The results of the analyses for DDT content and butter fat are given in table 1.

These results indicate that the DDT content of the fat in the various products is fairly constant and that little reduction of the amount of DDT in the original milk had been effected by pas-

teurization of the milk or by its conversion into milk products.

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## HOLLANDAISE SAUCE\*

For the safe preparation, handling and serving of hollandaise sauce, the following rules should be observed:

1. Wash hands thoroughly with soap and hot water before starting the preparation of this product. Dry with individual towels. Workers with open cuts, burns, sores, or stomach upset should not prepare this product.

2. Wash all utensils such as sauce pans, crocks, wire beaters, ladles, thoroughly with soap or other detergent and hot water, rinse and place in boiling water for two minutes. Receptacles should be picked up by handle and fingers should not come in contact with inside portion of containers or utensils.

3. Only clean shell eggs should be used.

4. Break the eggs out and separate whites from the yolks with utmost care to avoid any contact between the egg contents and the fingers.

5. Butter must be fresh print butter or tub butter which has not been previously handled. Butter chips kept in ice water and butter returned from patron plates should not be used. The

butter must not be touched with the hands.

6. Hollandaise sauce prepared in a kitchen should be used only within a two hour period after preparation. Hollandaise sauce older than two hours should be discarded and not reused. Under no circumstances should left over hollandaise sauce be "cracked" to salvage the butter fat nor should it be used as a base for other sauce. This is especially hazardous. It is preferable to run short during the latter part of the lunch or dinner period to avoid left-overs. Only a short time is required to prepare a new batch.

7. Discard your cheese cloth or other materials used for straining since a properly prepared sauce is smooth and glossy and requires no straining.

8. Gravy boats when used should not be excessively filled to avoid any waste since left over sauce must be discarded and not reused.

9. Under no circumstances should hollandaise sauce be sampled with the fingers. For sampling use a clean spoon which should not be reused.

10. For additional information telephone Mr. S. Plotkin, Supervisor in Charge of Food Poisoning Investigations, 125 Worth Street, New York City, WORTH 2-6900, Extension 246.

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## FAT DETERMINATIONS IN MILK AND MILK PRODUCTS

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FOR years the Babcock method has been employed for determining the fat content in milk and certain milk products. However, this procedure has numerous disadvantages. The sulfuric acid used as a reagent frequently chars the sample to such a degree that it is difficult to read accurately the fat column. The neck of the Babcock bottle is small; and unless care is observed, burns may be inflicted on the worker when pouring the acid. In this technique two and even three centrifugations are performed for each test. This is time-consuming, and furthermore difficulty is frequently encountered in trying to clean the Babcock bottle upon completion of the test.

The technique suggested by Schain<sup>1</sup> eliminates most of the above-mentioned disadvantages. This procedure depends upon the formation of a protein-detergent complex to break up the emulsion and thereby liberate the fat present in the mixture. We have reported elsewhere on the value of this technique.<sup>2</sup>

Since then we have made numerous improvements on the Schain method and have employed such technique on milk and milk products, comparing it at the same time in all instances with the Babcock procedure.

## MATERIALS

**Solution A**—A supersaturated solution of a fat dye was prepared by mixing oil red O in isopropyl alcohol. This was then added to a standardized nonionic detergent, polyoxyethylene sorbitan monolaurate in ethyl alcohol. This mixture was shaken well.

**Solution B**—This is a standardized anionic detergent dioctyl sodium phosphate.

## PROCEDURE FOR TESTING MILK AND MILK PRODUCTS

(a) *Quantity of sample employed for testing the various products*

1. Milks (raw, homogenized, pasteurized, chocolate, skim, and butter-milk)

Weigh out 18 grams and employ an 8 percent milk test bottle. (For approximate results, 17.5 ml can be used).

2. Creams (sweet or sour)

Weigh out 9 grams, and employ a 9-gram, 50 percent cream test bottle.

3. Cheeses

Weigh out 4.5 grams and employ a 9-gram, 50 percent test bottle. The cheese must be finely divided and warmed (to liquefy where possible).

4. Ice-cream

Weigh out 9 grams of the representative sample and employ a 20 percent ice cream test bottle. The ice cream is warmed (enough to liquefy) and mixed thoroughly. Fruit and nuts, if present, are finely divided.\*

5. Butter

Weigh out 4.5 grams of a representative sample utilizing a 9-gram 50 percent cream test bottle.

6. Evaporated milk

Weigh out 4.5 or 6 grams of sample utilizing either an 8 percent milk test bottle or a 9-gram, 20 percent ice cream test bottle.

7. Sweetened condensed milk

Weigh out 9 grams of a representative sample, utilizing a 9-gram, 20 percent ice cream test bottle.

(b) *Technique for the Test*

1. A well mixed sample (amount specified above) was placed in a 50 ml beaker.

2. 3.5 ml of Solution A were then added and mixed thoroughly by stirring with a glass rod.

3. 10 ml of solution B were then added without delay and again the mixture was stirred thoroughly.

We found that this proportion 3.5 ml of Solution A and 10 ml of Solution B, can be mixed before the test so that 13.5 ml of the mixture were added directly to the sample and this was stirred well.

4. The mixture was then placed in an official Babcock milk or cream bottle (depending on the sample), and 1 ml of distilled water was used to wash the mixture out of the neck of the test bottle.

5. The bottle and its contents were then placed in a boiling water bath.

6. After exactly 5 minutes in the boiling water bath, the bottle was removed; and with boiling water from the bath the liquid mixture was brought up into the neck of the flask, so that the fat column could be read.

7. The Babcock test bottle was set aside for 15 minutes, after which time the percentage of fat was recorded, as in the Babcock technique. If any fat adheres to the side of the neck of the flask, it is cleared and the column of fat evened by adding from one to several drops of 1N NaOH.

This method was tested on many samples of raw and homogenized pasteurized milks, buttermilk, skim milk, chocolate, condensed and evaporated milks, cream, ice cream, butter and cheese. The findings in all instances were clear-cut and agreed with the official Babcock test findings. The fat column, due to the fat dye has an even column colored red with only a slight

meniscus and is easily read. The test can be carried out quickly, accurately and with a minimum of time and equipment.

## SUMMARY AND CONCLUSIONS

We have simplified the technique suggested by Schain for determining the fat content in milk. Schain originally employed the proportions of 7 ml of Solution A, the nonionic detergent and 20 ml of Solution B, the anionic detergent. We have found the proportion of 3.5 ml of Solution A and 10 ml of solution B, much more effective, and have combined these proportions and made one solution. This solution containing 3.5 parts of Solution A and 10 parts of Solution B, proved most efficient, when using 13.5 ml for each test and mixing it thoroughly with the sample. Instead of the water-bath controlled at 82° C, we used a boiling water-bath. This saves the expense of a controlled water-bath and simplifies the method of analysis. Furthermore we have applied our modification to determining the fat content in milk products as well.

We recommend this modification not only because of its simplicity, but also because the results are clear-cut enabling the worker to quickly determine the fat content in milk and most milk products.

## Note:

We have a paper which concerns itself with the use of other detergent mixtures as the reagent of choice. This will appear in the January issue.

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## MEAT INSPECTION

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### PUBLIC HEALTH REASONS

PUBLIC health administrators sometimes ignore meat inspection apparently because mortality and morbidity tables do not show impressive numbers of persons killed or made sick from consumption of meats and meat products. The number of meat-borne diseases is not large, nor are human infections frequent for most of them. Among the animal diseases which are meat-borne are anthrax, brucellosis, beef and pork measles, salmonella infections, trichinosis, and tuberculosis. Also, meat from animals with pyemia and septicemias contains thermostable toxins which may cause food poisonings and toxemias in persons.<sup>1</sup> Many cases of food poisoning originate from meats, although in most of these, the meat was probably contaminated during slaughter or processing.<sup>2</sup> Q-fever epidemics have been reported among packing house workers presumably from slaughtering infected animals.<sup>3</sup> No reports of human infection from ingestion of infected meats have been noted. Botulinus poisoning occasionally occurs from contamination of meat products after slaughter.

Of the above, salmonella infections and food poisoning occur quite often.<sup>2</sup> Both are contracted from other sources, and it is impossible to even estimate the number of cases caused by meats. More accurate figures are available for trichinosis, which man contracts only from consumption of meats. Findings on 5,313 human autopsies in the United States extending over a number of years reveal that one in six per-

sons have or have had the larvae of this parasite in their muscles.<sup>4</sup> Only a few clinical cases or deaths are reported since it takes a heavy initial dose of the larvae to produce typically clinical disease, although many of the undiagnosed infections probably produce various symptoms extending over several weeks or months.

A part of the human brucella infections occur from handling infected raw meats, especially that of hogs.<sup>4</sup> No definite reports have been noted of infection from ingestion of such meats when insufficiently cooked. There is ample epidemiological evidence that ingestion of raw milk may produce infection. It appears logical that ingestion of infected meat may also produce infection, though this is probably infrequent as pork is not commonly eaten raw or rare.

The writer believes that more meat-borne infections occur from pathogens which are introduced during slaughter, processing, handling, and preparation than from meat of diseased animals. These are usually food poisonings and salmonella infections. The chances for this are greatly reduced by effective meat inspection.

As is true for inspection of all foods, prevention of human infections is only one of the reasons for an inspection program. Preventing the sale of products otherwise unfit for food is also important. Meat animals are subject to a large number of infections, parasitic infestations and physiological disturbances which, even though they do not cause specific human infections, make some parts or even the entire carcass unfit or undesirable for food.

Meat food products may be adulterated by illegal and undeclared mixtures of meats, use of excessive cereals or water, and by the use of preservatives which are detrimental to the health of the consumer. These should be controlled.

### HISTORY OF MEAT INSPECTION IN U. S.

Meat inspection may be said to have started in this country in 1891 when the U. S. Bureau of Animal Industry began inspection of pork for export, although it is reported that a few cities had meat inspection ordinances before that time.<sup>1</sup> This inspection was later expanded to include all meats and meat food products from cattle, sheep, goats, and swine intended either for interstate trade or for export. The acts of Congress merely directed the U. S. Bureau of Animal Industry to inspect all such meats and authorized the promulgation of detailed regulations necessary for carrying out their intent.

These regulations contain (a) provisions for both antemortem and post-mortem inspection of every animal slaughtered, (b) requirements regarding construction, sanitation, and cleanliness of the buildings and equipment, (c) minimum times and temperatures of processing and storage, and (d) delimitation of ingredients such as cereals and nitrates which can be used in meat products. This inspection will be referred to hereafter as Bureau inspection.

About 65 percent of the commercial slaughtering in the United States in 1946 was done in 477 plants under Bureau inspection.<sup>5</sup> This represented over 84 million food animals during the 1947 fiscal year.<sup>6</sup> Of these, approximately 1 in 8,000 were condemned on ante-mortem inspection, 1 in 260 were condemned in their entirety post-mortem, while parts of 1 in 65 were also condemned. In addition, 1 in every 9 cow and calf livers and an unreported number of hog livers were

condemned. There were nearly 10 million pounds of meat and meat food products condemned because of having become sour, tainted, rancid, unclean, or otherwise unfit for human food during processing and storage.

### LOCAL OR INTRASTATE INSPECTION OF MEATS

It is estimated that at least 35 million meat animals are slaughtered annually in plants that are not under Bureau inspection. This does not include animals killed on the farm for use by farm families. The number of such slaughtering establishments is not known accurately. However, it was estimated in 1946<sup>5</sup> that there were 365 such plants which slaughtered more than 2 million pounds of meat each per year, 4,300 which slaughtered between 300,000 and 2 million pounds each, and 22,000 butchers who slaughtered less than 300,000 pounds each.

Only five states have mandatory meat inspection laws.<sup>7</sup> Hundreds of cities in the remaining 43 states have passed meat ordinances and carry on some form of meat inspection program. However, there is no uniformity as to requirements of the ordinances or methods or degree of enforcement.

From the writer's observations, the majority of the local or intrastate meat plants are as backward as milk plants were 25 years ago. Most of them need major structural, equipment, and methods improvements even to approach the sanitary standards which are common in other types of food processing establishments. Local meat inspection, where it is being done, is all too often as archaic as the meat plants.

The condition of most of the smallest and of many of the medium size meat plants cannot be adequately described. Liquid wastes including blood are frequently delivered without treatment to the surface of the ground or into a small stream. Condemned and inedible parts and offal are quite frequently fed raw to hogs in the vicinity of the

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smaller plants where insufficient killing is done to justify, in the opinion of the operator, installation of rendering equipment. Even when rendering equipment is provided, all too frequently it is not cleaned, or parts and viscera are permitted partly to putrefy before they are rendered so that extremely objectionable odors are released during the rendering and when the tank is opened for emptying. Entirely too little attention is paid to fly breeding and fly control. Many of the smallest plants do not have refrigeration for this highly perishable food. Some even do not have running water or an ample quantity, and many do not have hot water or steam boilers to permit effective cleaning of the plant and equipment.

GENERAL

Meat plants lend themselves to standardization of construction, equipment, and operational procedures about as readily as do milk plants or any other food preparation and manufacturing establishment, except that special attention must be given to disposal of offal and liquid wastes. The basic structural features needed conform in general to well-known and proven types in use in other food establishments. The equipment needed varies with the type of animals slaughtered and the kind and amount of processing done; but within the natural divisions determined by these, it too is reasonably standardized. Equipment for various operations can be bought in sizes to fit reasonably well any size plant or operation except for the smallest. All of the above are well known to experienced meat inspectors.

However, meat plants differ from all other types of food establishments in the type and amount of inspection required to assure a product which is safe for use as human food. The safety of meats and meat products depends on two basic factors:

1. The meat must have been slaugh-

tered and processed in a plant conforming to good sanitation standards. It must receive prompt and effective refrigeration and clean handling throughout its storage for meat is frequently stored for months in a spoilable condition. As a matter of fact, cured meats require as much as six or seven weeks in the curing process alone and must be protected from contamination and spoilage during the entire process.

2. Only meat which is free of human pathogens and which contains only healthy, parasite-free tissues should be sold. To determine this, it is necessary to have an experienced veterinary meat inspector examine every animal for general condition and symptoms of disease while it is still alive and on foot. He must also be present throughout each slaughtering period to examine each carcass and its viscera, organs, and separated parts such as heads, while they can still be identified with the carcass from which they came. The various diseases of food animals produce gross changes or lesions in various parts of the body. The appearance of these lesions as well as their location enable an experienced inspector to determine not only the specific disease present but the parts of the body or carcass affected. This in turn determines how much of the carcass should be condemned. The parts most frequently showing changes from disease are the lymph glands, liver, kidneys, spleen, lungs, surfaces of the body cavities, intestines, and the tongue and head. This examination must be done immediately after slaughter while the parts can be identified with their carcass, and before any trimming is done which might remove evidence of disease. Naturally, it must be done for every animal slaughtered. Because of the above, periodic inspections at weekly or monthly intervals, as usually employed for other types of food establishments, will not suffice where effective meat inspection is desired.

The inspector should also be present

during much of the processing such as sausage manufacture and smoking of meats and meat food products. Both the meat scraps and other ingredients used in the manufacture of sausage must be checked on as to state of preservation, cleanliness, quality, kind, and amount. The temperature used in processing products such as bologna, weiners, or precooked ham which may be eaten without further cooking must be sufficient to destroy trichinae as well as salmonella and other organisms which may be present.

ORGANIZATION NEEDS FOR EFFECTIVE MEAT INSPECTION

Meat plants are the only nation-wide food plants for which reasonably uniform requirements and inspection have not been required by health departments. The U. S. Bureau of Animal Industry demonstrated some fifty years ago that meat plants can be constructed and operated so as to conform with strict but practical standards of sanitation. It also demonstrated that trained veterinary inspectors, properly organized and working under capable supervision, can detect diseased animals and parts even in the largest plants where slaughtering is quite rapid. This was before any national standards for other types of food establishments were accepted. Despite this, meat plants, except for those under Bureau inspection and in a few cities and states, are for the most part the most poorly constructed, equipped, operated, and inspected food plants in the country. There appears to be three main causes for this:

1. *Administrative.* The first blame must be placed on the public health administrators particularly at state and possibly the national levels. They must first decide to have effective meat inspection and must provide the organization necessary for its inauguration. Possibly because the U. S. Bureau of Animal Industry is required to inspect all meats entering interstate

commerce, the U. S. Public Health Service has not made studies or devoted attention to the public health phases of meat plants and meats in the past. This may account in part for the lack of interest at the state level. Recent reports indicate that the Veterinary Division of the U. S. Public Health Service has started a cooperative project of meat inspection with one state health department. This should be expanded to include at least one demonstration state in each of several different sections of the country.

State public health administrators have not provided qualified supervisory personnel. City and county administrators rarely provide enough funds for effective meat inspection. As a matter of fact, most city meat inspection programs are financed entirely through payment of inspection fees, an undesirable system abandoned long ago for other types of foods. In addition, in many of the cities under 50,000 population, meat inspection is a part time job. In a few of these, a veterinarian is employed full time but is assigned so many other duties such as milk and food inspection and rabies control, that he cannot stay at the meat plant throughout slaughtering. In others, he may be expected to do inspections at two or more plants which are permitted to slaughter simultaneously. In a majority of the smaller cities where meat inspection is attempted, a practicing veterinarian is employed part time as meat inspector. His pay is frequently whatever he collects from the inspection fees established by the meat ordinance.

When we add to the above the fact that no supervision or direction is given, and that the prevailing wage for this highly technical work is frequently about that for a nontechnically trained sanitarian, there appears to be no cause for surprise at the frequent ineffectiveness of the program.

Public health administrators learned many years ago that unsupervised, part

time, public health workers were not effective. Yet the condition in meat inspection all too often is comparable to the part time city health officers of 30 years or more ago. Why administrators retain a method in meat inspection abandoned many years ago as ineffective for all other public health programs is not evident.

2. *Public.* Enforcement of laws and regulations can be only as effective as the public demands or will support. Intelligent public support can be secured only by public education. This has not been utilized for meat inspection except in a very few local instances. The educational efforts which have been devoted to milk sanitation, venereal disease control, or any of a number of other public health programs would create a definite demand by most consumers for clean, wholesome, inspected meat.

3. *Meat Inspectors.* The veterinary meat inspector has usually been blamed when meat inspection programs were ineffective. In those instances where adequate facilities and time for effective inspection have been provided, he should be blamed if the job is not done properly, provided he is an experienced inspector. A degree in veterinary medicine no more signifies efficiency in meat inspection than does a degree in medicine assure an efficient health officer. Veterinarians need experience and further training on meat inspection before they are placed in charge of a program for a city or even for a single plant. They also need supervision and direction as is given to other types of public health workers.

The veterinary meat inspector who has more than one meat plant in which to do inspection, or so many other duties that he cannot do the inspection effectively, as well as the practicing veterinarian employed part time on meat inspection deserves criticism. But this criticism should be from his own profession for accepting employment under conditions which will not permit

him to do the job in a professional manner, rather than from the health administrators who are responsible for setting up such unworkable conditions.

#### DISCUSSION

A great deal of work and time will be needed to work out the details for a practical and effective local meat inspection program. In addition, supervisory personnel really qualified in meat inspection who devote their full time and thought to the program will be necessary. Since the U. S. Bureau of Animal Industry has no legal authorization for inspection of meat plants selling only intrastate, nor for cooperative action with states on such programs, the U. S. Public Health Service which has both legal authorization and funds for assisting states in solving their public health problems should take the lead and both encourage and assist several states in working out a satisfactory program. From the experience gained in these states, a practical program should be outlined by the Public Health Service for recommendation to other states, and advisory assistance should be provided as is now being done in practically all other public health problems and programs. The Veterinary Division of the Public Health Service now has public health veterinarians assigned and working with a number of states on veterinary public health problems, some of more or less local or regional significance. Since meat slaughtering and processing is nation-wide, and intrastate establishments furnish about one-third of the commercial meat supply, this is certainly of nation-wide significance.

After a proven, practical program is worked out, the states should be encouraged to start long range meat inspection programs. Such programs cannot be inaugurated or completed in a short time. Milk, food, sanitation, and other programs have been actively carried on for years and still have not

been completed in any state. But they have achieved beneficial results. The same can be produced in meat plants when the public health administrators and officials decide to provide the necessary funds, qualified personnel, and direction for a meat inspection program.

The above discussion does not imply that meat inspection should be provided for all of the 26,000 meat plants in the United States. At a majority of those now in operation, inspection of meats definitely should not be provided for they are so poor structurally and from any sanitary standpoint that even though diseased carcasses and parts were condemned, the animals could not be slaughtered, or meats handled and processed in a sufficiently clean manner to justify being stamped to signify safety or fitness for human food. Besides, a great many of these plants slaughter too few animals to justify the expenditure that would be necessary for effective inspection.

#### SUMMARY

1. Veterinary inspection of meats and meat slaughtering plants has direct public health and definite epicurean significance. The latter is an important secondary reason for inspection of any human food.
2. About two-thirds of the meat entering commercial trade channels in the United States receives effective inspection under the U. S. Bureau of Animal Industry. This is required for all meat from cattle, goats, sheep, and swine which enters interstate commerce.
3. The remaining third of the meat is slaughtered in some 26,000 establishments most of which are woefully inadequate as to structure, equipment,

methods, and general sanitation. At most of these, no meat inspection at all is done, or the inspection is not done effectively.

4. The principal causes for lack of inspection at so many plants and for the ineffective inspection are:

- a. Lack of interest on the part of public health administrators at all levels, but particularly at national and state levels.
  - b. Lack of administrative policies for meat inspection which were found necessary long ago for all other public health programs.
  - c. Failure to provide necessary supervision, direction, and training for meat inspectors.
  - d. Acceptance by veterinarians of employment as meat inspectors under conditions which make it impossible to do effective inspection.
5. The U. S. Public Health Service should take the lead in working out a practical program of local or state meat inspection, and should then encourage the states to inaugurate such programs.

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## THE VALUE OF THE COLIFORM COUNT IN THE ROUTINE EXAMINATION OF MILK AND DAIRY PRODUCTS

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Standard Methods for the Examination of Dairy Products define the coliform group of organisms as: "All aerobic and facultative anaerobic, Gram-negative, non-spore forming bacteria which ferment lactose with gas formation".<sup>1</sup> The two genera of bacteria ordinarily associated with the coliform group are the *Aerobacter* and *Escherichia*. In the *Aerobacter* genus the organisms commonly dealt with are the several varieties of the *aerogenes* and *cloacae* species; while in the *Escherichia* genus the organisms commonly dealt with are the several varieties of the *coli*, *freundii*, and *intermedium* species. *E. coli* is the only member of the coliform group which is a normal inhabitant of the intestinal tract of vertebrates while the other organisms of this group may or may not be present in the alimentary canal.

Because each species of the coliform group can sometimes be found in the alimentary canal of vertebrates, their presence might indicate evidence of fecal contamination of milk supplies, with the possibility that *Salmonella* and *Shigella* organisms might be present. A study of the statistics accrued in this laboratory over a period of two years presents evidence which we feel shows that the coliform count in pasteurized milk could be used as a criterion in determining the sanitary condition of milk for approving and condemning milk supplies.

### EXPERIMENTAL

The data presented in this paper were obtained from the coliform and

standard plate counts run weekly upon raw and pasteurized samples of milk submitted by the Illinois State Training School for Boys, St. Charles, Illinois, over a period of two years from April 1946 to April 1948. Each sample of pasteurized milk was submitted with its corresponding sample of raw milk. The milk was pasteurized at 142° F for thirty minutes in a Creamery Vat Pasteurizer of 150 gallons capacity. Phosphatase tests were made upon both the raw and pasteurized samples as an indicator of pasteurization.

Standard tryptone-glucose-extract-milk agar was used in determining the standard plate count. The plates were prepared according to standard methods of examining dairy products and were incubated at 37° C for 48 hours.<sup>2</sup> Desoxycholate agar was used in determining the coliform count. The plates were prepared according to standard methods and were incubated at 37° C for 24 hours.<sup>3</sup>

The statistics used were obtained from the results of counts upon 267 samples of raw milk and 267 samples of pasteurized milk.

Table 1 shows the distribution of the coliform counts in raw milk in relation to the standard plate counts and demonstrates that from our source of raw milk there was no correlation between the standard plate count and the coliform count. Out of the 267 samples of raw milk examined 75 percent had a plate count of 100,000 or under and 25 percent had a plate count over 100,000 to one million. No coliform organisms were found in 11.9 percent of the milk samples having a standard

TABLE 1  
DISTRIBUTION OF THE COLIFORM COUNT IN RAW MILK IN RELATION TO THE STANDARD PLATE COUNT

Standard Plate Count	Coliform Count	Number of Samples
Up to 25,000	0	5
	1 to 25	31
	26 to 50	23
	51 to 100	19
	101 to 200	8
	201 to 300	1
26,000 to 50,000	0	1
	1 to 25	0
	26 to 50	5
	51 to 100	8
	101 to 200	9
	201 to 300	14
51,000 to 75,000	Over 300	1
	0	8
	1 to 25	3
	26 to 50	4
	51 to 100	8
	101 to 200	7
76,000 to 100,000	201 to 300	3
	Over 300	3
	0	11
	1 to 25	4
	26 to 50	2
	51 to 100	3
Over 100,000	101 to 200	7
	201 to 300	1
	Over 300	3
	0	20
	1 to 25	12
	26 to 50	4
51 to 100	6	
101 to 200	12	
201 to 300	3	
Over 300	6	

plate count of 100,000 or less, while 30.3 percent of those over 100,000 contained no coliform organisms.

Table 2 shows the distribution of the coliform counts in pasteurized milk in relation to the standard plate count. Out of the 267 samples of pasteurized milk examined 95.1 percent had a standard plate count of 30,000 or less and 4.9 percent had a standard plate

count over 30,000 up to 100,000. No coliform organisms were found in 90.1 percent of the milk samples having a plate count of 30,000 or less. Ninety-eight and one-tenth percent of the samples having an acceptable standard plate count showed from 0 to 5 coliform organisms.

### DISCUSSION

We found no correlation between the standard plate count and the coliform count in raw milk. A low plate count did not necessarily mean that raw milk would have a low coliform count or a high standard plate count did not indicate a high coliform count. In other words the standard plate count did not give any indication of the coliform count. Because there was no paralleling of this relationship as shown by our results, we feel that the standard plate count can not always be used as an indication of the sanitary conditions under which the raw milk was produced. The results obtained in this laboratory show that the coliform count would be a more consistent indication of the sanitary conditions under which the raw milk was produced than the standard plate count.

TABLE 2

DISTRIBUTION OF THE COLIFORM COUNT IN PASTEURIZED MILK IN RELATION TO THE STANDARD PLATE COUNT

Standard Plate Count	Coliform Count	Number of Samples	Percent
Up to 30,000	0	229	90.1
	1	15	6.0
	2 to 5	5	2.0
	6 to 10	3	1.1
	11 to 20	1	0.4
	Over 20	1	0.4
Over 30,000	0	11	
	1	1	
	2 to 5	0	
	6 to 10	0	
	11 to 20	0	
	Over 20	1	

\* Deceased, June 15, 1950.

The results of the pasteurized milk showed that when milk is properly pasteurized, irrespective of the coliform count of the raw milk, 98 percent of the samples would have a coliform count from 0 to 5 and 90 percent of the samples would contain no coliform organisms.

The above results would seem to indicate that a maximum limit of 5 coliform organisms per ml could be set for Grade A pasteurized milk, because even raw milk having relatively high coliform counts will have coliform counts at this level after pasteurization. Pasteurized milk having higher coliform counts than 5 per ml would strongly indicate in our estimation that a possible contamination after pasteurization had taken place due to unsanitary handling. Contamination of this sort would most likely be of human origin and would indicate fecal contamination with all the dangers of enteric infections.

We feel, however, that the desoxycholate agar plate method for coliform counts has many limitations and the limit of 5 coliform organisms per ml would be applicable only to this method. Methods of determining the coliform count in milk utilizing larger inoculations of the sample might show the need for a higher limit than five

### CONCLUSIONS

1. The standard plate count in milk does not give any indication of the coliform count.
2. The coliform count is a better indication of the sanitary quality of milk than the standard plate count.
3. Ninety-eight percent of properly pasteurized samples of milk will show less than 5 coliform organisms per ml regardless of the standard plate count and the coliform count of the raw milk when the desoxycholate agar plate method is used.
4. We believe that a coliform count of 0 to 5 per ml could be used as a standard for Grade A pasteurized milk when the desoxycholate agar plate method is used.
5. Because pasteurized milk having a low plate count still could contain an undesirable number of coliform organisms we believe that a coliform count should be done routinely upon all samples of pasteurized milk.

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## SOME OBSERVATIONS ON BACTERIA ISOLATED FROM MILK THAT GROW WITHIN A PSYCHROPHILIC TEMPERATURE RANGE

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Psychrophilic microorganisms are defined as those organisms which have an optimum temperature range of 5 C to 25 C. These microorganisms are often associated with spoilage of foods held at refrigerator temperatures.

The possibility of mesophilic bacteria, with an optimum temperature range of 25 C to 45 C, becoming adapted to growth at the lower temperatures must be accepted. Therefore both psychrophilic and adaptive mesophilic bacteria may initiate undesirable biochemical changes in milk held at cold-storage temperatures.

### REVIEW OF LITERATURE

Bacterial counts on refrigerated samples of milk have been made by several different groups of workers. In a study made by Palmer and McCutcheon<sup>4</sup> in 1930 it was observed that in samples of pasteurized milk held at 42° F for 6 hours decreases in count occurred in 80 percent of the samples and increases in 13 percent of the samples. Out of 105 samples held for 24 hours at 42° F, 68 percent showed a decrease and 32 percent an increase. Ice cream held at 10° C on which bacterial plate counts were made on alternate days, demonstrated a progressive increase in bacteria as was shown by Weinzirl and Girdeman.<sup>2</sup> Leete<sup>3</sup> called attention to the fact that in all investigations involving growth determinations as a measure of storage conditions, care must be taken to account for variation in refrigerator temperatures with

the same refrigerator. Daily and hourly changes in temperature by several degrees were noted in a group of dairies investigated by Leete in 1931. In 1942 Motte and Mazer<sup>6</sup> studied the fluctuations in daily plate counts on certified pasteurized milk, Grade A pasteurized milk, and Grade B pasteurized milk from various sources. Duplicate samples were made of each sample and stored in a refrigerator at 40° F for 5 days. After 3 days some samples of both Grade A and Grade B milk had bacterial counts which were over the regulation maximum. The regulation maximum was not given. After 4 days the pasteurized certified milk conformed to the standards.

Prescott, Bates, and Needle<sup>5</sup> also demonstrated that foods refrigerated discontinuously show a slow increase in numbers of organisms at refrigerator temperatures.

Since various types of microorganisms are known to survive the pasteurization process, the ability of the psychrophilic bacteria to survive pasteurization temperatures is questioned. Many studies have been made on the thermotolerant characteristics of microorganisms encountered in the dairy industry. In an investigation made by Hucker<sup>1</sup> on the thermotolerant cocci, it was shown that the number of bacteria resisting pasteurization was effected only slightly by the temperature at which milk had been stored previous to pasteurization at 142° F for 30 minutes. The type of microflora was influenced by previous storage temperatures. Of 180 strains of cocci resisting

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pasteurization, *Streptococcus thermophilus* predominated. *Streptococcus faecium*, *Streptococcus liquefaciens*, and *Micrococcus epidermidis* were also encountered as thermophilic microorganisms. In addition to this Hucker also found that pasteurized milk held at 20° C for 4 hours prior to pasteurization contained large numbers of *Streptococcus thermophilus*. The same milk held at 10° C for 4 hours before pasteurization contained a greater variety of bacteria after pasteurization. Waisman and Petazze<sup>10</sup> reported that when samples of freshly-pasteurized milk were stored at 23° C for 2, 6, 12, and 24 hours, all samples showed unpleasant tastes and odors after 12 hours. This was attributed to the survival of thermophilic microorganisms during pasteurization. An investigation which differs somewhat from the previously mentioned studies was made by McKenzie and Morrison.<sup>12</sup> The workers studied the occurrence of thermophilic bacteria in raw milk rather than pasteurized. They concluded that micrococci, sarcina, streptococci and corynebacteria most commonly survived experimental pasteurization at 145° F-150° F for 30 minutes.

In a study on the temperature relationships of the more common milk streptococci, Sherman and Stark<sup>6</sup> reported that *Streptococcus fecalis* and *Streptococcus liquefaciens* will grow as low as 10° C. Both of these microorganisms survived pasteurization temperatures. Morris<sup>8</sup> observed numerous samples of milk in which bacterial numbers increased almost as rapidly at incubation temperatures of 4° C as at room temperatures. These psychrophilic bacteria were proved to be of the *Pseudomonas* genus. It has been shown by Dahlberg<sup>13</sup> that the coliform bacteria in pasteurized market milk held at refrigerator temperatures increased more rapidly in numbers than did the total count. After 4 days storage at 45° to 50° F, the coliform bacteria constituted 5 percent of the total count. It

was also demonstrated that this group of bacteria grew more rapidly in warm weather than in cool weather. *Serratia marcescens* was implicated in spoilage of butter at storage temperatures as was reported by Hammer and Long.<sup>7</sup> Frazier and Rupp<sup>11</sup> observed that *Bacillus mesentericus* and *Bacillus vulgaris* will also grow at refrigerator temperatures.

EXPERIMENTAL

1. Methods and Materials

Bacterial Counts

The standard agar plate count was used throughout this study as suggested in *Standard Methods for the Examination of Dairy Products*.

Temperature and Time of Incubation

Psychrophilic cultures were incubated at approximately 10° C in a mechanical household refrigerator for varying time intervals. The plates and cultures which were incubated at 20° C were placed in a mechanically-refrigerated room. Room temperatures used for incubation throughout these studies ranged from 25° C to 27° C. The exact temperature is given with the procedure of the experiment.

Culture Media Used

With the exception of the experimental work done on specific biochemical activities of the psychrophilic cultures, standard tryptone-glucose extract medium was used throughout this investigation.

Isolation of Psychrophilic bacteria from Raw and Pasteurized Milk

Pure cultures of psychrophilic organisms were obtained from the dilution plates and transferred to tryptose glucose agar slants. The cultures were checked microscopically, and for their ability to grow on culture media within the cryophilic temperature range. Fifteen cultures were selected for detailed study.

TABLE 1  
COMPARISON OF THE NUMBER OF PSYCHROPHILIC ORGANISMS IN RAW AND PASTEURIZED MILKS AT 10° C.

No. of Samples	Average bacterial counts in raw milk		
	24 hours	48 hours	72 hours
36	3	85	1500
33	Bacterial counts in pasteurized milk		
	1	42	320

Stock Cultures of Psychrophilic Bacteria

Stock cultures, the psychrophilic microorganisms isolated from milk were carried on tryptone-glucose extract agar slants. They were stored at 10° C and transferred every third day.

EXPERIMENTAL RESULTS

Psychrophilic bacteria in raw and pasteurized milk

Standard plate counts were prepared on the samples of both raw and pasteurized milk immediately after they were obtained from the University Dairy. The plates were incubated at 10° C for 24, 48, and 72 hours. The results are shown in table 1.

The number of psychrophilic colonies which appeared after 24 hours incubation was lower in the pasteurized samples than in the raw milk samples. As the time of incubation was increased the number of colonies on plates of

both the raw and pasteurized samples increased. After 72 hours there was a greater number of psychrophilic colonies on the plates containing raw milk than on those with pasteurized milk. Those colonies which appeared after 72 hours incubation could have been either slow growing psychrophiles or mesophilic bacteria which had become adapted to growth at 10° C during the 72-hour incubation period.

Thermophilic properties of psychrophilic bacteria isolated from raw and pasteurized milks

Fifteen flasks of sterile milk were inoculated respectively with the 15 pure cultures isolated. Each inoculated flask of milk was laboratory pasteurized at 145° F for 30 minutes. Total bacterial counts were made on the milk before and after pasteurization and the plates incubated at 10° C for 48 hours.

The results are recorded in table 2.

TABLE 2  
REDUCTION IN NUMBER OF ORGANISMS PER MILLILITER AFTER LABORATORY PASTEURIZATION

Sample No.	Organisms per ml before pasteurization	Organisms per ml after pasteurization	Percent reduction
6 B(a)	11,000	65	99.4
10 X	25,400	10,500	58.6
18 X	13,700	175	98.7
10 E(a)	6,400	250	96.0
10 G(a)	4,500	100	97.7
10 D	16,000	30	99.9
10 E(b)	8,900	2,550	71.3
X 5	54,000	12,500	76.8
X 16	23,200	0	100.0
6 B(a)	103,000	98,000	4.8
X	560,000	12,600	97.8
10 G	26,500	12	98.8
X 17	18,700	10,200	50.8
X 9	120,000	158,000	24.0
10 F	86,000	35,200	59.0

Most of the cultures demonstrated heat lability. Eight of the 15 had a percent reduction in numbers of bacteria of 90 or over. Five had reductions of 50 percent to 90 percent. The remaining two cultures showed greater thermoduric characteristics than the others, one having a 24.0 percent reduction and the other a 4.8 percent reduction. A control was run on skim milk and found to be sterile.

The temperature at which there was the most rapid increase in number of cells was considered to be the optimum growth temperature. The results are recorded in table 3.

DISCUSSION

In the samples of milk tested there were more psychrophilic bacteria found in raw milk than in pasteurized milk. Since only two of the 15 samples tested for thermoduric properties proved to be strongly thermoduric, the pasteurization process was largely responsible for the reduction in the number of psychrophiles. Pasteurization is known to cut down the variety of types of bacteria to leave only a few types which predominate. For this reason the raw milk having a greater variety of microflora than the pasteurized would be more apt to contain psychrophiles even

TABLE 3

OPTIMUM GROWTH TEMPERATURES OF PSYCHROPHILIC CULTURES

Sample	10 C	20 C	26 C	37 C
6 B(b)	3+	2+	1+	—
10 X	3+	2+	2+	—
18 X	1+	2+	3+	±
10 E(a)	2+	3+	1+	—
10 C(a)	3+	1+	1+	—
10 D	2+	3+	1+	—
10 E(b)	3+	1+	1+	—
X 5	1+	2+	3+	±
X 16	1+	3+	2+	—
6 B(a)	2+	3+	2+	—
X	3+	1+	1+	—
10 G	1+	2+	3+	±
X 17	3+	2+	1+	—
X 9	3+	2+	1+	—
10 F	2+	3+	1+	—

Key:  
 — Decrease in numbers.  
 ± Questionable increase in numbers.  
 1+ Slight increase in numbers.  
 2+ Moderate increase in numbers.  
 3+ Greatest increase in numbers.

Seven of the 15 samples had an optimum growth temperature closer to 10° C than 20° C. Five had an optimum growth temperature close to 20° C, and three grew best at room temperature. Those flasks which were incubated at 35° C showed in three samples a questionable increase. The increase, if any, was so small that it could have been well within the limits of experimental error.

though the two samples had approximately the same initial bacterial count. The colonies which appeared on the plates after 72 hours incubation quite possibly were adaptive mesophiles.

Storage of milk at 10° C tended to increase the number of psychrophilic bacteria. The probability of the adaptive mesophiles beginning to grow after 3-5 days incubation at 10° C must not be overlooked. The total bacteria

counts for the three grades of milk studied, increased in proportion to the initial number of bacteria in the sample and the temperature, at which the sample was stored.

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 4-2800

## REPORT OF COMMITTEE ON APPLIED LABORATORY METHODS \*

At the 1949 Annual Meeting a report on laboratory and field trials of quaternaries and detergent-sanitizers was presented by a member of our Committee on Applied Laboratory Methods, Dr. F. W. Barber. This report of our Committee has recently been published in the September-October 1950 *Journal of Milk and Food Technology*. Although no additional work on this subject was undertaken by your committee since the 1949 Annual Meeting, some members of our committee have been actively engaged in related investigations. One member, Dr. P. R. El-likier, reported on The Application of Quaternary Ammonium Compounds in Dairy Sanitation, in the May-June *Journal of Milk and Food Technology*, and on Cleaning and Bactericidal Values of Detergent Sanitizers, in the July-August issue.

Members of the Association may be interested to know that the proceedings of a conference held last year on "Mechanism and Evaluation of Antiseptics" by the New York Academy of Sciences, one section of which was devoted to Surface-Active Agents, was published recently as Volume 53, Article 1, *Annals of the New York Academy of Sciences*. Another recent publication containing some material of interest on detergents and chemical sanitizing agents is the *Official Proceedings of the 36th Mid-Year Meeting of the Chemical Specialties Manufacturers Association*, June 1950, published as a supplement to the periodical, *Soap and Sanitary Chemicals*.

Last year your Committee on Applied Laboratory Methods arranged for a comparative study by twelve cooper-

ating laboratories of plating agars proposed as substitutes for the present APHA Standard Methods agar containing skim milk. At the 1949 Annual Meeting a brief report was made, outlining this study and commenting on the trend of results, but details could not be presented until completion of a statistical analysis. This analysis was made possible by the cooperation of the staff of the Bureau of Records and Statistics of the New York City Department of Health. A report incorporating these data, prepared by Miss Vivian Pessin, Senior Statistician of the New York City Department of Health, and the Chairman of your Committee on Applied Laboratory Methods, L. A. Black, appears on the program for this 1950 Annual Meeting, and will be submitted for later publication in the *Journal of Milk and Food Technology*.

Last year your committee arranged for nine laboratories to cooperate in a comparative study of stains proposed as substitutes for the present APHA Standard Methods stain for the direct microscopic examination of milk. At the 1949 meeting a brief report was made outlining this study, but again, detailed results could not be presented pending a statistical analysis of the data obtained. Dr. J. C. Olson, Jr., a member of the Committee on Applied Laboratory Methods, has made such an analysis and a report incorporating this data appears on the program for this 1950 Annual Meeting and will be submitted for later publication in the *Journal of Milk and Food Technology*.

Although no additional projects were undertaken last year by the Committee on Applied Laboratory Methods, several matters have been under considera-

## MODERN METHODS OF MASTITIS TREATMENT CAUSE TROUBLE IN THE MANUFACTURE OF FERMENTED DAIRY PRODUCTS

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MODERN methods of mastitis treatment and control include the use of penicillin, streptomycin and aureomycin, as well as various sulfa drugs. Continued use of these materials proves that they are a useful and important part of a mastitis control program. During the summer and fall of 1949, however, the improper handling of the milk from cows treated with these materials was found to be responsible for the manufacture of a number of lots of poor quality cheese. A condition of no-acid production during the process of cheddar cheese making at the University of Idaho was traced to the milk from an occasional cow treated for a mastitis condition. Penicillin, used in the treatment, was found to be responsible.

In the fall of 1949, reports from various sources indicated that similar conditions existed throughout the State of Idaho, as well as in other countries. Katznelson and Hood<sup>2</sup> of Canada report that acid production was completely inhibited by 0.5 unit of penicillin per ml of milk when 3 percent starter was used. Under the same conditions the Canadians report strong inhibition by 0.1 unit and moderate inhibition by 0.05 unit of penicillin per ml of milk. On the basis of milk production from a cow treated by infusing 75,000 units of penicillin into one quarter of the udder, Krienke<sup>3</sup> re-

ported that slow acid production would result when the milk from one treated quarter was mixed with the milk from 20 untreated cows. Krienke<sup>3</sup>, Hunter<sup>1</sup>, and Katznelson and Hood<sup>2</sup> reported that ordinary pasteurization temperatures had no effect on penicillin activity.

In the light of these reports and Idaho's experience with this condition, experiments were set up to determine the length of time required, after treatment with these antibiotic substances, for the milk to return to normal condition. Normal milk was considered as milk which would permit the growth of lactic acid forming bacteria.

A study was also made to determine the amount of milk from treated quarters required to prevent the growth of lactic acid bacteria when mixed with normal milk, as well as the effect of the various antibiotic substances in the milk from treated quarters on lactic acid starters secured from different sources. Experiments were also set up to determine the effect of these antibiotic materials in the milk from treated quarters, when the cream is used for the manufacture of cultured butter and the milk for the manufacture of milk powders. As milk powders are often reconstituted for the manufacture of cottage cheese and buttermilks, this step seemed appropriate.

The cows used in these experiments were selected from the university herd. They were first treated by infusing 200,000 units of penicillin in 40 ml of a 10 percent solution of sulfamerazine into one quarter of the udder of each of four cows. The combined

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milk from all four quarters of each cow was collected at each milking after treatment. The milk from each milking was pasteurized at 143° F for 30 minutes, cooled to 88° F, and 2 percent of starter added. Rennet was added at the rate of 12 ml per 100 pounds of milk. After the milk was coagulated the curd was cut and the titratable acidity of the whey determined. Cheese was then made by the regular method, using a cooking temperature of 102° F. Acidity tests on the whey were made every hour after cutting, for six hours, the time ordinarily required for the cheesemaking process, and again the following morning. The cheese curd was not pressed but left overnight in the containers in which it was made, along with a small amount of whey. During this later holding period the temperature of the cheese was gradually reduced from 102° F to 72° F.

In these first experiments, cheese was made from the milk from each cow for six consecutive milkings after treatment. The highest acid production at the end of six hours was as follows: The first milking after treatment, 0.13; the second milking, 0.14; the third milking, 0.17; and the fourth milking, 0.18 of 1 percent. The fifth and sixth milkings after treatment were only slightly below the control samples, which usually had a titratable acidity at the end of six hours of between 0.43 and 0.46 of 1 percent.

Inasmuch as the penicillin used in the first experiment was dissolved in a 10 percent solution of sulfamerazine, the sulfamerazine might have had an influence on the results. Thus, the same cows were treated again in one quarter of the udder, this time at four successive milkings with 100,000 units of penicillin in physiological salt solution. Milk samples were again taken, starting with the first milking (approximately 12 hours) after the last treatment. The samples were handled in the same manner as in the first series and the results obtained were almost identical. It was not until the fifth and

sixth milking that the milk was back to normal, as determined by its ability to support the growth of lactic acid bacteria.

With these preliminary results in mind, two cows were then treated in one quarter with 100,000 units of penicillin in a physiological salt solution. In this case, however, the milk from the treated quarters was kept separate from the milk from untreated quarters. The milk was collected in this manner for the first two milkings after treatment. The samples were then each divided into eight separate portions, sterilized, and inoculated with 1 percent of starter. Eight starters, each obtained from a different source, were used, with one sample of milk from the treated quarters and one sample from the untreated quarters being inoculated with each starter. The samples were incubated overnight, and titratable acidities determined the following morning.

Little or no acid development took place in the milk from the treated quarters, regardless of the starter used. Milk from the untreated quarters developed acidities of 0.83 to 0.88 of 1 percent, which is comparable to the acidity of milk from untreated cows, when handled in the same manner. Following this experiment with penicillin, treatments of one quarter of a cow were then carried out, using each of the following materials: (a) One gram of streptomycin in saline solution, (b) 200 milligrams of aureomycin in saline solution, (c) 7.56 grams of sulfanilamide in 20 ml of oil, or (d) 25 ml of a 10 percent solution of sulfamerazine. Again the milk from the treated quarters was kept separate from the milk from the untreated quarters. The samples were again divided into eight equal portions and treated as outlined above. Again no acid developed in the milks from treated quarters, irrespective of the treatment or starter used. Normal acid production developed in all cases in the milk from the untreated quarters.

These results show that there is no diffusion of the drugs from one quarter of the udder to the other. Also that all of the drugs which were used had the same inhibitory effect on all eight starters, regardless of their source.

To determine the amount of milk from untreated quarters necessary to give a sufficient dilution to overcome the restrictive effect of these drugs in the cheesemaking process, cows were again treated with penicillin, streptomycin, aureomycin, sulfanilamide, and sulfamerazine. The solutions were prepared in each case as outlined above. The milk from the treated quarter was collected at each milking after treatment. It was then pasteurized and mixed with pasteurized milk from untreated cows in amounts varying from 1 to 50 percent. The mixed milk was then made into cheese by the process previously outlined. Titratable acidities of the whey were made every hour after cutting the curd for a six-hour period and again after the cheese had incubated overnight.

Figure I shows the titratable acidity of cheese whey six hours after cutting when the milk contained 1.0 percent milk from a quarter treated with streptomycin.

These results show that streptomycin has a restrictive action on acid production for six milkings when as little as 1 percent of the milk is from the treated quarters.

Under the same conditions, penicillin shows a restrictive action for the first three milkings. The acidity developed when 1 percent of the milk was from the treated quarter was 0.215 at the first milking, 0.24 at the second and 0.35 at the third. At the fourth milking the quantity of penicillin present appeared to have a slight stimulating action, as samples containing as much as 3 percent of milk from the treated quarters developed higher acidities than did the control samples.

Experiments with aureomycin showed that 1 percent of the milk from the treated quarters had a restrictive action

for the first two milkings after treatment. At the third milking, 50 percent or more of the milk from treated quarters was necessary to restrict acid production. When aureomycin is used a slight orange color is observed the first milking after treatment, but does not persist beyond the first milking.

Experiments with sulfamerazine showed that 1 percent of the milk from treated quarters restricted acid production for two milkings, while sulfanilamide, under the same conditions, had a restrictive effect for five milkings after treatment. However, both of the milk samples containing sulfamerazine and sulfanilamide, when incubated in a small amount of whey overnight, showed a normal titratable acidity the following morning. This indicates a bacteriostatic action which is eventually overcome. However, it is questionable if a desirable flavored fermented dairy product could be made under these conditions.

In the study of the effect of various antibiotic substances on the manufacture of cultured butter and dried milk solids, the treatment with five antibiotics was repeated. The milk from the treated quarters was collected, transferred to large separating funnels, and allowed to stand overnight at 40° F. The separated milk was then drawn off at the bottom, leaving only the cream. Both the milk and cream were then pasteurized at 165° F for 30 minutes, cooled to 40° F, and held until ready for use.

For buttermaking, mixtures were prepared from good sweet cream separated from pasteurized surplus market milk and 0.25 percent to 100 percent of the gravity separated cream from treated quarters. The mixture was pasteurized at 195° F for 30 minutes, cooled to 72° F, then inoculated with 2 percent of starter, and incubated overnight (16 hours). The following morning the titratable acidities of the cream were determined. The results are given in table 1.

These results show that if 1 percent

TABLE 1

TITRATABLE ACIDITY (CALCULATED AS LACTIC ACID) OF CREAM 16 HOURS AFTER INOCULATION WITH 2 PERCENT STARTER WHEN THE CREAM CONTAINS VARYING PERCENTAGES OF CREAM FROM QUARTERS TREATED WITH VARIOUS DRUGS

Percent cream from treated cows added to normal cream	Treatment				
	Penicillin 200,000 units in 40 ml	Streptomycin 1 gram in 40 ml	Sulfanilamide 40 ml	Sulfamerazine 40 ml	Aureomycin
0.25 percent	0.67*	0.65	0.56	0.62	0.65
0.50 percent	0.46	0.67	0.48	0.56	0.64
1.00 percent	0.33	0.41	0.48	0.52	0.64
2.00 percent	0.26	0.32	0.48	0.52	0.63
100.00 Penicillin cream	0.24	0.22	0.34	0.50	0.27
Normal cream	0.72	0.72	0.64	0.63	0.63

\* Average of three determinations. Variation between triplicate determinations not more than 0.05 percent.

of the cream is from quarters treated with penicillin or streptomycin, difficulty would no doubt be experienced in the manufacture of cultured butter. The sulfanilamide, sulfamerazine, and aureomycin treatment probably would not seriously interfere, as it is doubtful if as much as 2 percent of the cream would ever be from the treated quarters.

The skim milk that remained when the milk from the treated quarters was separated, was mixed with skim milk from untreated quarters. One part of the skim milk from the treated quarters was mixed with 99 parts of the skim milk from untreated quarters. The mixture was then preheated to 175° F, condensed to 35 percent total solids, and spray dried.

The milk powder was then reconstituted by adding 10 grams of the powder to 90 ml distilled water. The reconstituted milk was pasteurized at 195° F for 30 minutes, cooled to 72° F, and inoculated with 2 percent of starter. The samples were incubated overnight at 74° F and the titratable acidities determined the following morning. The results are shown in Figure II.

These results show that penicillin and streptomycin are not destroyed by the process of condensing and drying, and still show strong inhibiting ability in the reconstituted milk. Sulfanilamide, sulfamerazine, and aureomycin

evidently are changed in some way, as there is shown a stimulating effect on acid production in these samples.

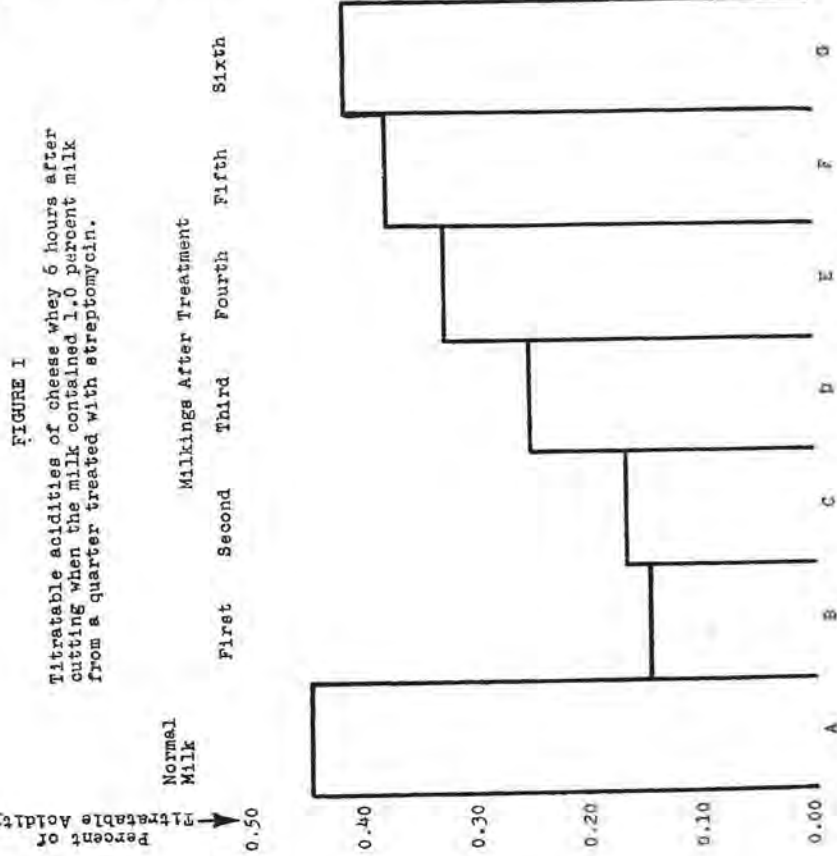
SUMMARY

Penicillin, streptomycin, aureomycin, sulfanilamide, and sulfamerazine, when used as a treatment for mastitis, are given off in the milk in sufficient amounts to restrict the growth of lactic acid bacteria. If as little as 1 percent of the milk is from a treated quarter, acid production will be restricted for from four to six milkings after treatment.

There is no diffusion of the drugs from the treated quarters to the untreated quarters. It is, therefore, recommended that the milk from the treated quarters be discarded or fed to livestock, for three days or six milkings after treatment.

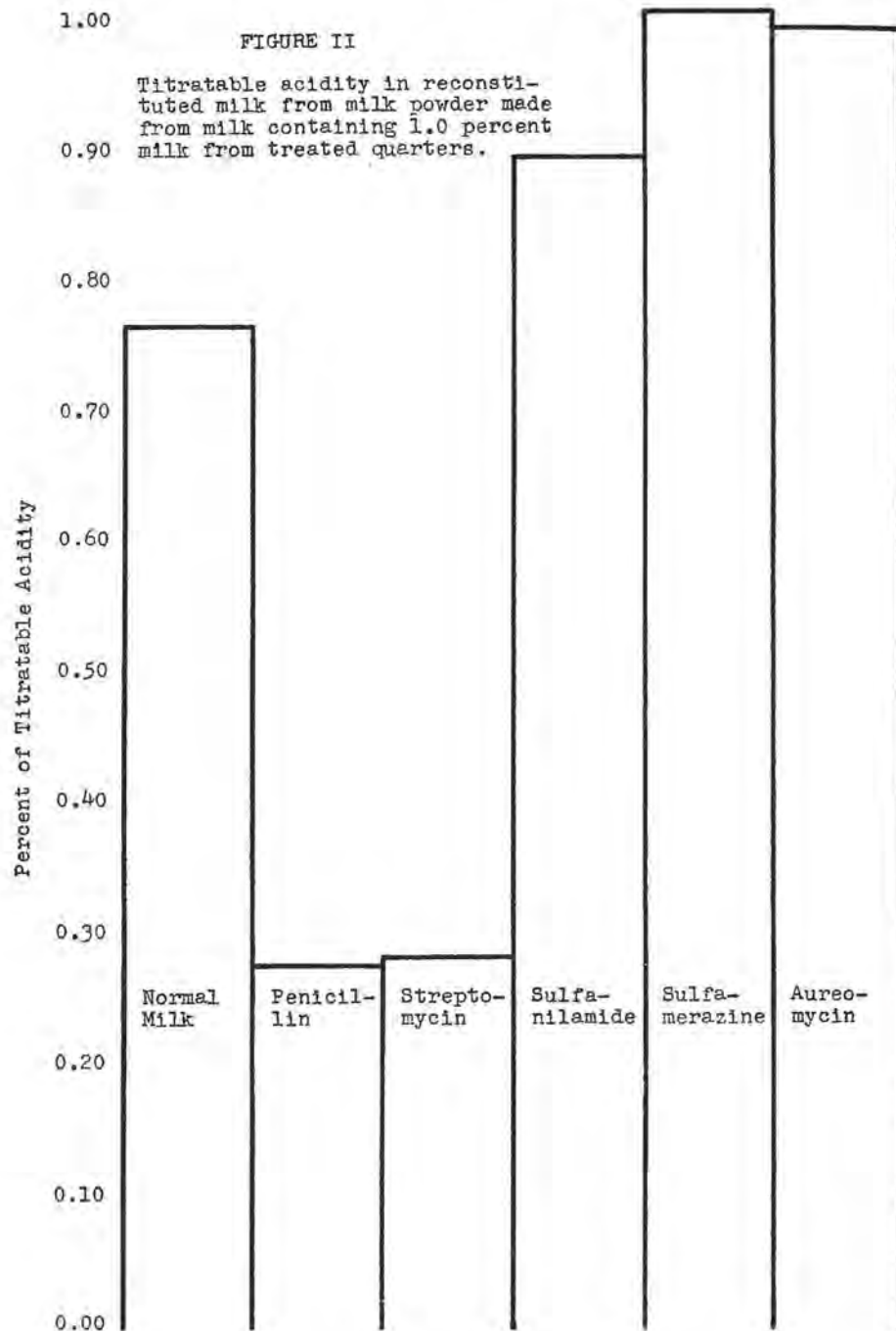
The drugs given off in the milk following treatment were found to restrict the growth of eight starters, each secured from a different source.

When cream is separated from the milk and cultured for buttermaking, as little as 1 percent cream from quarters treated with penicillin and streptomycin restricted the growth of the lactic acid bacteria. Sulfanilamide, sulfamerazine, and aureomycin were found to have somewhat less restrictive action in cream prepared for the manufacture of cultured butter.



- A. Percent acidity produced in normal milk in 6 hours. (0.45 percent)
- B. Percent acidity developed in 6 hours after adding 1 percent milk from first milking following treatment with streptomycin. (0.15 percent)
- C. Percent acidity developed in 6 hours after adding 1 percent milk from second milking following streptomycin treatment. (0.17 percent)
- D. Percent acidity developed in 6 hours after adding 1 percent milk from third milking following streptomycin treatment. (0.255 percent)
- E. Percent acidity developed in 6 hours after adding 1 percent milk from fourth milking after treatment with streptomycin. (0.33 percent)
- F. Percent acidity developed in 6 hours after adding 1 percent milk from fifth milking following treatment with streptomycin. (0.38 percent)
- G. Percent acidity developed in 6 hours after adding 1 percent milk from sixth milking after treatment with streptomycin. (0.415 percent)

THE DAIRY RESEARCH INSTITUTE (U.S.)



In milk powder manufactured from milk containing 1 percent milk from treated quarters, reconstituted milk containing milk from penicillin and streptomycin treated quarters restricted the growth of lactic acid bacteria. When the reconstituted milk contained milk from sulfanilamide, sulfamerazine, and aureomycin treated quarters, a definite stimulating effect on the acid produced by lactic acid bacteria was found.

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### Bacteriologist Examination in Public Health Service

A competitive examination for appointment of bacteriologists to the Regular Corps of the United States Public Health Service will be held on February 12, 13, and 14, 1951. Examinations will be held at a number of points throughout the United States, located as centrally as possible in relation to the homes of candidates. Applications must be received no later than January 15, 1951.

The Regular Corps is a commissioned officer corps composed of members of the various medical and scientific professions, appointed in appropriate categories such as medicine, engineering, nursing, and the sciences. Bacteriologists may be appointed to either the Scientist or Sanitarian category, depending upon their qualifications.

Appointments will be made in the grades of Assistant Scientist and Assistant Sanitarian (equivalent to Navy rank of Lieutenant, j.g.) and Senior Assistant Scientist and Senior Assistant Sanitarian (equivalent to Lieutenant). Appointments are permanent in nature and provide opportunities for qualified bacteriologists to pursue their profession as a life career in the research and public health activities. The names of applicants who successfully complete the examination will be placed on a merit roll, from which appointments will be made to fill current and future vacancies.

Requirements: All candidates: United States citizenship. At least 21 years of age.

Assistant Scientist: At least seven years of educational training and professional experience subsequent to high school, including receipt of the doctor's degree in bacteriology

from a university of recognized standing.

Senior Assistant Scientist: At least ten years of educational training and professional experience subsequent to high school, including receipt of the doctor's degree in bacteriology from a university of recognized standing.

Assistant Sanitarian: At least seven years of educational training and professional experience subsequent to high school, including receipt of the master's degree in bacteriology from a university of recognized standing.

Senior Assistant Sanitarian: At least ten years of educational training and professional experience subsequent to high school, including receipt of the master's degree in bacteriology from a university of recognized standing.

Gross pay is identical to that of officers of equivalent rank in the Army and Navy. Entrance pay for Assistant Scientist and Assistant Sanitarian with dependents is \$4,486.56 per annum; for Senior Assistant Scientist and Senior Assistant Sanitarian with dependents is \$5,346. These figures include subsistence and rental allowance.

Retirement pay after 30 years of service or at age 64 is three-fourths of annual basic pay at the time of retirement.

Additional benefits include periodic pay increases, 30 days annual leave, sick leave, full medical care, and the usual privileges extended to officers of the military forces.

Application forms may be obtained by writing to the Surgeon General, United States Public Health Service, Federal Security Agency, Washington 25, D. C., Attention: Division of Commissioned Officers.

Applications received after January 15, 1951, can not be accepted.



# MILK and FOOD SANITATION

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## A STUDY OF THE TANKER SYSTEM OF COLLECTING MARKET MILK

MILK INSPECTION SERVICE, DIVISION OF ENVIRONMENTAL SANITATION  
*Department of Public Health, Oakland, California*

WITH FIVE plants using the tanker system in the summer of 1949 and several others contemplating it, the Division made a thorough study upon which to base future control regulations. Log averages of the market milk SPC\* were tabulated for cans, farm vats, and tankers from August 15 to December 31, 1949. This revealed results as follows: 501 can samples showed 9,500 SPC, 467 farm vat samples with 15,300 SPC and 42.° F temperature, and 157 tanker samples at city plants with 29,000 SPC and 45.5° F temperature. This gave a ratio of SPC of 10:16:30 with an increase of 90 percent SPC between farm vats and tankers on arrival. Another tabulation of milk sampled from farm vats and the same milk again from arriving tankers showed increases from 57 percent to 297 percent for four plants covering 28 shippers and 12 tanker compartments. The weighted averages of the farm vat samples were 28,908 SPC and the tankers 47,234 SPC, or an increase over all of 63.4 percent.

Over the 4½-month period above, the 10 gallon can milk received in all 12 city plants averaged 12,500 SPC with 46.9° F average temperature, all farm vats and tanker SPC as given above. Illegal samples (over 75,000) ranged as follows: 1373 10-gallon can samples 14.9 percent, 467 farm vat

samples, 14.7 percent, and tankers 19.1 percent or tankers produced 29 percent more illegal milk than cans or farm vats.

Rinses and swabs from cleaned (?) farm equipment showed gross variation in SPC and was largely excessive. Often rubber hose were so long—up to 16 feet—that they could not be properly cleaned, the interior was invisible, and fittings were usually not demountable. Already some plants are changing to all steel pipes.

Drivers often assembled or handled milk house pumps or pipes with unwashed hands, wiped thermometers and gauge sticks with hands or dirty rags from their pockets. Vat lids, doors, and tanker covers were generally left open during pumping of milk. Residual milk in pumps and hose was drained into a bucket and dumped into the manhole. Some tanker valves were unprotected with proper dust covers. Pumps carried on tankers were in cabinets near wheels, not dust proof and not cleaned daily, often dirty and odorous.

Many times the clean-up of farm vats and tankers was a "hurry-up" job of a few minutes or less with cold or warm water, many times with a "dual" powder that was supposed to clean and sterilize at once. Steam is rarely used on the larger vats and tankers due to danger of damage. Great difficulty was experienced in thorough coverage of the surfaces, either in washing or sani-

## A COMPARATIVE STUDY OF MILK FROM A REFRIGERATED BULK DISPENSER AND SIMILAR MILK DELIVERED IN SEALED BOTTLES

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THE rising cost of serving food in eating establishments made it necessary for the Director of Food and Housing at Massachusetts Institute of Technology to consider the installation of a double 40 quart refrigerated bulk milk dispenser in the dining service of the New Dormitory at M.I.T. which was opened in September 1949 to house and feed 300 students. As meals were served on a contract basis and as the dining service was essentially a large private boarding house, it was unnecessary to obtain a license from the Cambridge Health Department to serve milk from a refrigerated bulk dispenser.

Since the author serves as Director of Food Sanitation at M.I.T. as well as Professor of Sanitary Science, his professional approval was sought for the proposed installation. The other two large dining services at M.I.T., one at Walker Memorial and the other at the Graduate House, have served milk for years in accordance with the stringent requirements of the Boston Health Department. These regulations require that the milk be properly pasteurized and that it be delivered to the consumer in a sealed container with the pouring lip protected against contamination. It was recognized that this was the safest and most sanitary method of serving milk.

### METHOD OF HANDLING

When offered for sale the bottles of milk are placed in a large pan containing ice water where the level of the

water is maintained just below the pouring lip of the bottle. In consequence, the milk is invariably delivered to the customer at a temperature below 40° F. These two dining services, are in reality large public restaurants. Outsiders as well as students are privileged to eat here and each patron pays in accordance with the amount of food he purchases. The contract plan was not in vogue here, and a license to sell milk in accordance with the regulations of the Cambridge Health Department was therefore required. These regulations are similar to those of Boston. Milk is sold in half-pint bottles at a cost of 10 cents per bottle.

Under the contract plan at the New Dormitory, students were to have the privilege of extra helpings of food including all the milk they desired without extra charge. Since most students consume from two to four large glasses of milk with each meal, i.e., one pint to one quart, it was necessary to purchase clean, safe milk as reasonably as possible to permit the contract plan to operate without financial loss. It was recognized that the milk in use at the M.I.T. dining services would be under periodic laboratory supervision constantly, thus making available knowledge of its bacterial quality. It was also possible to arrange to install a refrigerated bulk milk dispenser\* holding two forty quart cans on an experimental basis for a three months trial period.

Since the containers and the milk

\* Manufactured by the Monitor Process Corporation of Jersey City, N. J.

\* SPC—"Standard Plate Count," from *Standard Methods for the Examination of Dairy Products*, p. 105.

(Continued on page 371)

were to be handled by a large milk distributor in Boston, it was necessary to obtain permission for this purpose from the Boston Health Department. Consent was obtained after the manufacturer agreed to make certain structural changes in the milk cans which increased the ease and thoroughness of cleaning and sterilization. The cans were specially marked and reserved for the exclusive use of the New Dormitory at M.I.T. All the cans were washed and sterilized at the milk plant in Boston (Charlestown) and after being filled with homogenized, pasteurized milk of market quality derived from tuberculin-tested cows, they were sealed and then delivered directly to the walk-in refrigerator at the New Dormitory maintained constantly at 36° F. These cans were installed in the refrigerated dispenser as required. Milk was drawn from the dispenser in clean, sterile open glasses as required. At no time were glasses of milk drawn and exposed to the contaminations of the atmosphere or to the incubating temperatures of the service room.

The bottled milk was also of market grade, but it was not homogenized. It was also derived from tuberculin-tested cows and was pasteurized by the holding method. This milk was delivered twice a day to the dining services at Walker Memorial and the Graduate House. All milk on delivery was placed in separate walk-in refrigerators maintained at 36° F. At Walker Memorial, cases of milk were taken from the walk-in refrigerator as required, brought upstairs (one flight), and the bottles were then immersed in ice water up to the pouring lip when offered for sale. They were kept this way until sold. At the Graduate House cases of milk were likewise brought upstairs (one flight) to the service room, but they were stored for varying periods of time in a poorly refrigerated milk cabinet before the bottles were transferred to a pan of ice water.

## SAMPLING

All the samples taken at the Graduate House were obtained from the pan of ice water. At Walker Memorial, however, all the samples but one, that for February 8, 1950, were obtained from the cases of milk that had been brought upstairs but prior to the transfer of the bottles to the pan of ice water. On February 8, the Walker Memorial sample was taken from the pan of ice water directly.

Samples of milk were collected from the three M.I.T. dining services, usually on Wednesdays and generally at fortnightly intervals. At the beginning of these observations, samples of milk were collected from the New Dormitory at weekly intervals. Each sample was collected aseptically and thoroughly refrigerated until examined in the laboratory. Total counts were made in serial dilutions of 1:10 and 1:100 on Difco's tryptone glucose milk extract agar. Presumptive tests in lactose broth were made simultaneously in the same dilutions and examined after 24 and 48 hours at 37° C for gas formation. All the presumptive tests were negative for each milk throughout the series of observations.

According to the available information, the only difference between the bottled milk and the milk from the dispenser was that the latter was homogenized while the former was not. The quality and age of the milks were the same. The bacterial standard of the Boston Health Department for market milk, homogenized or not, is 20,000 bacteria per ml on TGEM agar at 32° C after 48 hours incubation.

## BACTERIOLOGICAL DATA

Table 1 gives the individual observations for total counts and for temperature on all the samples examined from the three M.I.T. dining services between September 21, 1949, and May 10, 1950. In all, 18 samples were examined from the refrigerated bulk dispenser at the New Dormitory and 16

TABLE 1

TOTAL COUNTS AND TEMPERATURES ON MILKS FROM A REFRIGERATED BULK DISPENSER COMPARED WITH BOTTLED MILKS OF THE SAME GRADE FROM THE SAME SOURCE, SEPTEMBER 1949 TO MAY 1950

Date	New <sup>1</sup> Dormitory	Walker Memorial	Graduate House	New Dorm. 50°F.	Walker Mem. 40°F.	Graduate House 38°F.
Sept. 21, 1949	12,500	9,000	11,500	—	—	—
" 28	17,500	—	—	46	—	—
Oct. 5	16,000	10,000	12,500	50	40	36
" 19	21,000	8,500	24,000	46	44	36
Nov. 2	23,000	14,000	12,500	46	45	40
" 16	10,000	16,500	18,000	50	42	40
" 30	9,500	10,000	10,000	50	46	46
Dec. 14	17,500	8,100	8,000	45	44	36
Jan. 4, 1950	75,000	5,100	9,000	50	49	46
" 11	6,500	—	—	45	—	—
" 18	12,000	7,500	—	45	—	—
Feb. 8	5,500	3,900 <sup>2</sup>	8,500	45	46	46
" 15	6,000	5,500	4,500 <sup>2</sup>	43	38	43
Mar. 1	5,400	6,000	5,500	45	42	40
" 15	6,000	7,500	7,500	46	42	41
Apr. 5	16,000	11,500	6,000	45	45	46
" 26	15,000	27,000	27,500	56	42	40
May 10	35,000	7,500	13,000	48	44	36
			10,000	48	48	41

<sup>1</sup> From refrigerated bulk dispenser.

<sup>2</sup> Feb. 1, 1950.

samples of bottled milk from each of the other two dining services. The statistical analysis of the data for total counts is presented in Table 2 and for temperature, in Table 3.

TABLE 2

STATISTICAL ANALYSIS OF TOTAL COUNTS ON MILK FROM REFRIGERATED BULK DISPENSER  
Compared with Bottled Milks of the Same Grade from the Same Source, Sept. 1949 to May 1950

Range of Total Counts in Groups	Number in Each Group		
	New Dorm.	Walker Mem.	Graduate House
Under 5,000	0	1	1
5,000-9,999	6	9	6
10,000-14,999	3	4	6
15,000-19,999	5	1	1
Over 20,000	4	1	2
Minimum count	5,400	3,900	4,500
Maximum count	75,000	27,000	27,500
Median count	13,800	8,300	10,000
Total samples	18	16	16

It is obvious that the total counts in the milks from the refrigerated bulk dispenser were somewhat higher than those obtained on the bottled milks at

Walker Memorial and the Graduate House. Similarly, the range in total counts for the bulk milk was greater than the range for the bottled milk. Although the median count for the bulk milk was higher than for the bottled milk, it was still very comfortably within the maximum count for this type of milk allowed by the Boston Health Department. Four out of 18 samples of the dispenser milk, or 22.2 percent, had total counts exceeding 20,000 per ml, while 1 out of 16 samples at Walker Memorial or 6.25 percent exceeded this limit and 2 out of 16 samples at the Graduate House, or 12.5 percent exceeded this standard.

It is impossible to determine from the available data whether the higher counts usually obtained on the bulk milk was due to the dispenser or to the milk contained therein, or to other factors. Both types of milk were pasteurized but the milk in the dispenser was homogenized while the bottled milk was not. Although both milks were of the same quality, it is quite possible that homogenization actually

increased the bacterial content of the milk in the bulk dispenser. It will also be observed that the temperature of the milk in the dispenser was invariably somewhat higher than the temperature of the bottled milk. This may also account for the higher counts normally obtained on the milks from the bulk dispenser. Since the temperature at which milk is stored determines in a large measure, its bacterial content, especially after pasteurization, it has been the practice to determine the temperature of each milk on a separate sample after collecting one aseptically for the bacteriological examination. The statistical analysis of the data for temperature at the time the samples were collected is presented in the following table.

TABLE 3

STATISTICAL ANALYSIS OF TEMPERATURES OF MILK FROM A REFRIGERATED BULK MILK DISPENSER

Compared with Bottled Milks of the Same Grade from the Same Source, Sept. 1949 to May 1950

Range of Temperatures, °F	Number in Each Group		
	New Dorm.	Walker Mem.	Graduate House
Under 40	0	1	5
40-45	6	11	7
46-50	11	4	4
Over 50	1	0	0
Minimum temp.	43°F.	38°F.	36°F.
Maximum temp.	56°F.	49°	46°
Median temp.	46°	44°	40°
Total	18	16	16

The data in Table 3 indicate that the bottled milk was invariably colder than the milk in the bulk dispenser. At Walker Memorial and at the Graduate House all of the milks were under 50° F, and only 8 out of 32 samples or 25 percent were at temperatures above 45° F. At the Graduate House where all of the samples were obtained from a pan of ice water, 5 of the 16 samples were under 40° F. At Walker Memorial where the bottled milk is also placed on sale in a pan of ice water but where only one sample was so obtained

for the bacteriological examination, it was this sample only that showed a temperature under 40° F. All the other samples were obtained from cases of milk that had been stored in a walk-in refrigerator at 36° F and brought upstairs for transfer to the pan of ice water. Although the bulk milk is also delivered directly into a walk-in refrigerator at 36° F from which the 40 quart cans are subsequently transferred to the refrigerated dispenser, the temperature of the milk in the dispenser will depend very largely on the time the milk in the can has been stored in the walk-in refrigerator. Since the turn over of the milk in the refrigerated dispenser is quite rapid, the temperature of this milk depends very largely on the temperature which the milk has attained on storage in the walk-in refrigerator. As all of the samples in this study were collected around 10 a.m., shortly after the bulk milk had been delivered to the New Dormitory, and as this milk had not had an opportunity to cool down to 40° F or below in the walk-in refrigerator, it may be concluded that the higher temperatures of the milk in the refrigerated bulk dispenser were due to the higher temperatures at which this milk was delivered and the inadequate storage at 36° F prior to use. The refrigerated bulk dispenser was set to operate at maximum refrigeration throughout the period under consideration.

#### CONCLUSIONS

As a result of the experience at M.I.T. from September 1949 to May 1950, it may be concluded that under the conditions that obtained at the New Dormitory, it is perfectly safe to dispense pasteurized, homogenized milk from a refrigerated bulk dispenser. Furthermore such milk should invariably have a total count under 20,000 per ml in the Boston area. The cans in use should be easily cleaned and sterilized, and there should be a com-

plete absence of cracks and crevices in which milk solids may lodge. Furthermore all joints should be smooth and should be filled with block tin instead of lead. Great care should also be exercised to have all milk delivered to the dining service thoroughly cold, preferably around 40° F and such milk should be delivered directly into a walk-in refrigerator which is maintained below 40° F. The introduction of the cans of milk into the refrigerated bulk dispenser should be performed by an intelligent and experienced person whose aseptic technique is highly satisfactory. It is also important to draw

milk from the dispenser only as required and none of it should be allowed to incubate at the temperature of the atmosphere in the service room for more than 10 minutes. Finally, regular bacteriological examinations and tests for temperature should be made in order to make sure that the milk is cold and of low bacterial content. Under these conditions considerable economy can be effected in the sale of milk in a large dining service without sacrificing the public health protection provided by the sale of milk in individual containers with the pouring lip protected against contamination.

#### REPORT ON APPLIED LABORATORY METHODS

(Continued from page 358)

tion. One of these has arisen as a consequence of the treatment of mastitis by penicillin or other antibiotics. The APHA Committee on *Standard Methods for the Examination of Dairy Products* has been gathering data on a laboratory procedure for the detection of antibiotics in milk.

Your Committee would welcome suggestions at any time from members of this Association on problems involving the application of laboratory methods that seem in need of further investigation.

L. A. BLACK, *Chairman*

#### TANKER SYSTEM OF COLLECTING MARKET MILK

(Continued from page 366)

tizing, untouched areas being common. Surfaces of many tankers were found badly scratched, burred, or dented, making good cleaning difficult or impossible. One tanker had an agitator permanently installed with non-demountable stuffing box and shaft, and very dirty.

The study indicates that the system is largely in an unsettled state as to proper apparatus, installations, and cleaning methods. Formation of an ap-

propriate regulation by control agencies, either state or local, is highly necessary for future guidance of the system, to avoid the errors already in evidence. Tanker collection of market milk has many advantages over the old can method, and now that the status is revealed, improvement can be planned by authorities and industry and greater satisfaction later assured as to arrangement, cleaning of equipment, and protection of the product.

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## ASSOCIATION NEWS

## C. SIDNEY LEETE



The death of C. Sidney Leete on October 8, in Albany, brought a feeling of sincere regret throughout this Department.\*

Since 1930, when he joined the staff of the State Department of Health, Mr. Leete had been active in the work of the Milk and Restaurant Sanitation Section, during most of that period as assistant chief of the Section. Only recently he had been designated as acting chief, succeeding Walter D. Tiedeman.

A native of East Bloomfield, N. Y., Mr. Leete was graduated from Cornell University in 1914 with the degree of Bachelor of Science, having majored in dairy industry and bacteriology.

Following service in World War I, he became associate market milk specialist with the Bureau of Dairy Industry of the U. S. Department of Agriculture, Washington, D. C. In 1920, he served as bacteriologist and field agent for the New Orleans Pure Milk Society, a Medical Milk Commission, supervising certified milk.

During 1921-1929, Mr. Leete again served as associate market milk specialist with the Bureau of Dairy Industry in Washington. He conducted research work on dairy sanitation, milk control and inspection of milk supplies.

For ten years, Mr. Leete served as secretary-treasurer of the International Association of Milk and Food Sanitarians, and at the time of his death was vice-president of that organization. He was also secretary-treasurer of the New York State Association of Milk Sanitarians.

Well versed in his chosen profession, his cordial relationship with his co-workers in the state service, and with other professional workers in the field of milk sanitation, made for him many friends throughout the country.

\* Bulletin, N. Y. State Department of Health, October 11, 1950.

DAIRY TECHNOLOGY CONFERENCES AT UNIVERSITY  
OF ILLINOIS

For those interested in the field of Dairy Technology, a series of one-day conferences have been arranged. The meetings will be held in the Illini Union Building on the campus of the University of Illinois at Urbana.

*Dairy and Food Technologists and Sanitarians, January 9-10*

Panel discussion—Mechanical dishwashing in eating establishments; sanitary and economic aspects of bulk milk dispensers; the National Stream Conservation Program; the control and disposal of dairy wastes; the significance of coliform in milk; the Illinois Brucellosis eradication program.

*Dairy Plant Fieldmen, February 7-8*

Disease control in livestock; quick tests for milk quality; dairy pasture programs; artificial insemination; hauler-fieldman relationships; the fieldman as a salesman of good production methods; general economic outlook for dairy products.

*Milk Plant Operators, March 6-7*

Brown milk bottle; sterile milk; frozen milk; keeping quality of bottled milk; control of coliform organisms in milk; rancidity in milk and cream; fortified skim milk; quaternary ammonium compounds; federal orders.

*Ice Cream Manufacture, April 3-4*

Demonstration of ice cream flavors and novelties. Stabilization of ice cream using Gelatin, CMC, Sodium alginate and emulsifiers. High temperature short time pasteurization of ice cream mixes; diabetic ice cream; mixes for soft ice cream; low fat frozen desserts; testing ice cream for fat.

*Problems of Interest to the Butter Industry, May 1-2*

The purpose of the conference is to discuss pertinent information regarding new developments within the butter industry. Subjects to be included in the conference are: Continuous Churning Methods; Inspection Procedures of the Food and Drug Administration; New Facts Regarding Nutrition Research; Needs of the Armed Forces for Spreads; and a symposium on Merchandising and Packaging Techniques and Procedures.

## INFORMATION

*Programs*

Detailed programs for each conference will be available at a later date. Kindly complete and mail the enclosed postal card indicating the conference in which you are interested and we will send you the detailed program and pre-registration card as they are printed.

**Brucella Ring Test Antigen Prepared by Reduction of a Tetrazolium Salt**  
R. M. Wood, *Science*, Vol.112, p.86 (1950)

Details are given for carrying out the Brucella Ring Test using a tetrazolium salt (4,4'-bis)3,5-diphenyl-2-tetrazolinium-biphenyl dichloride) to stain the Brucella antigen instead of hematoxylin. The tetrazolium salt is reduced by living cells to an

intensely colored violet-blue formazan. Apparently, the reduction takes place inside the cell, and hence the antigenic specificity of the cell surface is not altered. It is reported that lots of antigen prepared over the last two years using the tetrazolium method have all been of uniform color intensity, specificity and sensitivity and have remained stable over prolonged periods under normal conditions of use and storage.

INDUSTRIAL NOTES

Diversey Issues Coliform Booklet

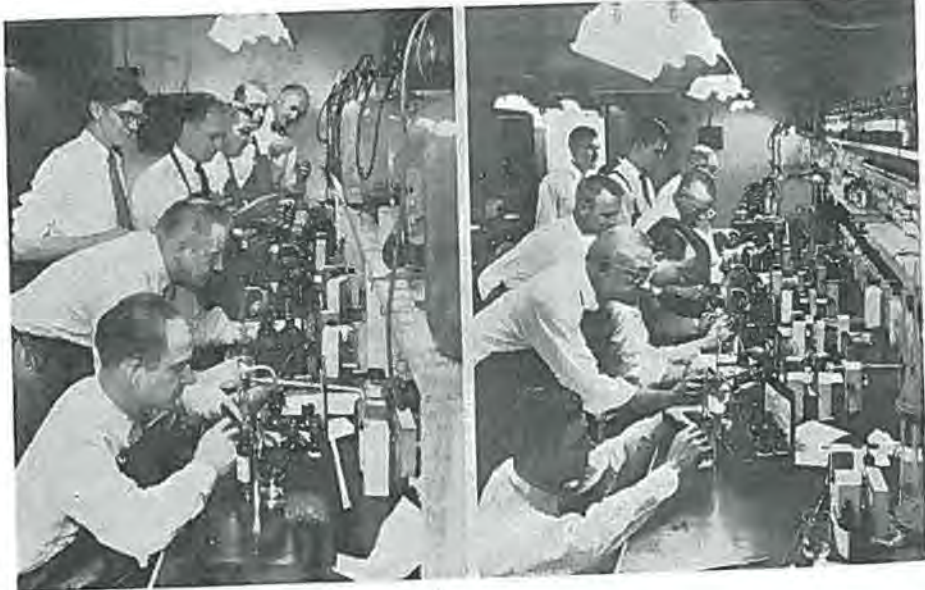
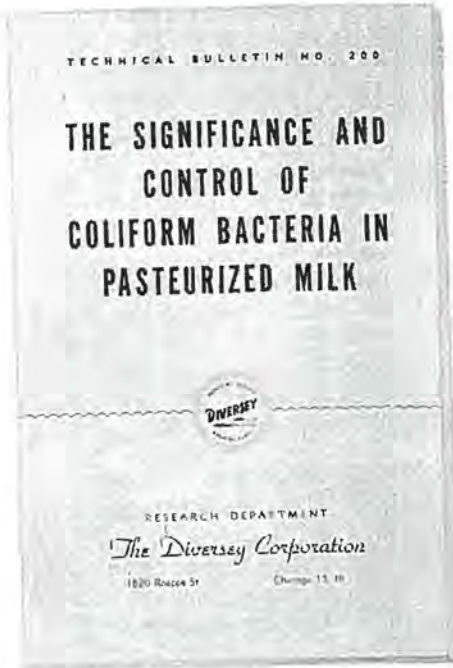
The Diversey Corporation, Chicago, has just published the first of a series of technical booklets covering subjects fundamental to the science and practice of modern dairy plant sanitation.

The booklet on coliforms explains their origin, how they get into milk and how to keep them out so that milk will test regularly below the maximum count permitted.

Copies of the coliform booklet may be obtained by writing to: The Diversey Corporation, 1820 Roscoe Street, Chicago 13, Illinois.

Wyandotte Chemicals Supervisors Attend Food and Beverage Schools

One of a series of schools for Food and Beverage Department Supervisors of Wyandotte Chemicals Corporation is pictured above. Emphasis was placed on control and test devices used by Wyandotte representatives, to get "most miles per dollar" from cleaning and sanitizing materials.



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