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V. M. EHLERS  
PRESIDENT

*International Association of Milk Sanitarians*

# JOURNAL of MILK TECHNOLOGY

Volume 2

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## Editorials

*The opinions and ideas expressed in papers and editorials are those of the respective authors.  
The expressions of the Association are completely recorded in its transactions.*

### Vision for 1939

The year 1938 witnessed considerable progress in the milk field, judging by the accomplishments of producers, distributors, manufacturers, research, and enforcement officials. The year 1939 presents to us, as milk sanitarians, a great opportunity to further the general welfare. Let us concentrate our efforts on cooperating with:

The producer to interest him in improved quality and in new labor-saving equipment;

The manufacturer to produce new and superior products with less effort;

The distributor to improve processing, safe-guard his products, reduce delivery costs and expand his existing market;

The researcher by presenting to him our problems and encouraging him to find new uses for milk and milk products;

The general public to interest the citizenship in increasing daily milk consumption and becoming more fully aware of milk's value as a food; and

The milk enforcement officials to standardize regulatory procedure and to unify our efforts.

To attain our objectives for 1939, we must adopt a cooperative attitude to all groups engaged in any phase of the milk field. It is of paramount importance that efforts should be put forth to build state or area organizations where none exist. Such organizations should be encouraged to affiliate with the International Association of Milk Sanitarians whose objectives are to qualify the sanitarian to be more than an enforcement official, to be in fact a promotor of welfare that affects all of the people.

V. M. EHLERS, *President.*

## Some Weak Links in Milk Sanitation

The splendid progress that has been made in the engineering, laboratory examination, and administrative control of milk sanitation as public health measures shows no signs of abatement.

In the field of milk plant engineering, Leete's discussion of milk plant equipment of the future (1), Scharer's (2) and Roger's (3) findings of faulty plant operations as revealed by the use of the phosphatase test, and Shrader's (4) review of new developments in equipment as exhibited at the Dairy Industries Exposition indicate some points where improvements are desirable, and also some new ground that has been gained. All of this indicates that milk and milk products plant equipment of all types and for all purposes is still in the developmental stage. It would be helpful if there existed for each type of equipment a list of the necessary items to be designed and applied for enhancing their satisfactory or "acceptable" construction and performance, covering both the sanitary and efficiency aspects. This is a function of milk sanitarians and technologists, and can be accomplished by cooperative action with the manufacturers of equipment and supplies.

Improvements have been made in the bacteriological technic as indicated in the work on the composition of media and the temperature of incubation. But where can a laboratorian purchase a bacteriological incubator whose performance is dependable in the light of the work of Breed and his associates (5) on the variations in temperature during load? Many laboratorians use a miscellaneous assortment of modifications of the Babcock technic for the determination of fat in ice cream as shown by Robertson (6). In spite of the real usefulness of the phosphatase test for the determination of the improper pasteurization of milk, workers do not yet agree as to its limitations, its applications, nor even its technic, indicating that more laboratory research is needed to evaluate the several available methods.

The great advances in strengthening some of the above-mentioned links in the chain of measures for adequately safe-guarding milk supplies have tended to divert our attention from other important items involved in a well administered program. We are doing an increasingly effective job of examining the incoming milk, processing it, and regulating its handling, but there is a tendency to lose sight of the condition of the cow—the source of the milk. For example, it is impressive how much is written about mastitis in contradistinction to how relatively little is really known and intelligently done about it.

An important factor in another phase of the industry is the milk bottle. We have no automatic control of the cleanliness and safety of every milk bottle. We depend on average results—the very situation which condemned milk-in-the-bottle pasteurization. Yet the returned empty milk bottle is brought into the dairy from an unknown treatment (or mistreatment) in all kinds of households. Every single drop of milk is required to be adequately pasteurized, but the bottle itself that holds twenty thousand drops (about a quart) may or may not be adequately cleansed and

sterilized before refilling. Who knows its condition? The extensive sanitary investigations of the manufacturers of fiber milk containers reported by Tanner (7) should serve both as an incentive and as a warning to the glass bottle industry, the purchasers and users, and the bottle-washer manufacturers.

Added to this weakness is that of the possibly questionable health of the operator at the filling and capping machine. Discharges from the nose and throat are sprayed over equipment and products during talking, and profusely so during "clearing the throat", coughing, and sneezing. Often there is no protection of the open, washed bottles or the uncapped bottles of milk from such infection. Disease conveyed by infected individual bottles of milk is difficult to trace because of the extremely limited epidemiological data available. But the total amount of such isolated, untraced, single cases may exceed the number of cases in a traced milk-borne outbreak.

Collaboration between the sanitarians, milk producers, equipment manufacturers, processors, and distributors should solve every one of these difficulties. The dairy industry expects the leaders of public opinion—such as physicians, dentists, nutritionists, teachers, and other such public-minded workers—to urge greater consumption of milk. It cannot be expected that such approval of dairy products will be given as unconditionally as when these foods are adequately safe-guarded. Great advances have been made. Refinements are now in order.

### REFERENCES

1. *J. Milk Tech.* 1 (2), 38 (1938).
2. *J. Dairy Science* 21, 21 (1938).
3. *J. Milk Tech.*, this issue, page 21.
4. *Ibid.*, 1 (7), 35 (1938).
5. *Amer. J. Pub. Health* 22, 745 (1932); 24, 477 (1934). *N. Y. Agr. Exper. Sta. Tech. Bul.* 213 (1933).
6. *Twenty-fifth Ann. Rept. Internat. Assoc. Milk Sanitarians*, p. 283 (1936).
7. *J. Milk Tech.*, this issue, page 4.

J. H. S.

## The Present Status of the Paper Milk Container\*

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Success of a single service container for fluid milk is influenced by many factors such as cost, convenience, freedom from health hazards, and improvement over containers which have been used before. All of these factors will not be considered at this time for several have been discussed in other places. The States of California and Maryland have regulations which control cleansing and re-use of used food containers of various sorts. These problems were appreciated very soon after milk was distributed in bottles, for attempts were made to introduce a container which would not have to be returned to the dairy. Many of the factors involved have been reviewed by Tanner (1) who also reviewed some of the earlier history of paper milk containers.

Paraffined paper-board containers for milk are being used on a large scale in certain urban centers. They have been approved by the Ministry of Health of Great Britain, and local authorities may also approve cartons submitted to them for use both with ordinary milk and milk sold under special conditions. In the United States, the Council on Foods of the American Medical Association has voted to accept milk distributed in one of the single service type of containers. The Council voted to permit the display of the seal of acceptance on one container when accompanied by a statement identifying the product which stands accepted. The American Association of Medical Milk Commissions has also approved the closure on one of these types of paper milk containers. The fact that this com-

mission has approved one type for certified milk is strong indication that the container is satisfactory. Certified milk is the most carefully produced and protected milk in America.

Paper milk containers<sup>†</sup> are made of heavy paper board heavily coated with paraffin wax. Bacteriological problems involved in their manufacture may be discussed under three headings: 1. the bacterial content of the paper-board; 2. the effect of hot paraffin wax on microorganisms in and on this board; 3. the bacteriological condition of the container just before filling at the dairy. Under these three headings will be presented some of the information which has been collected during development and perfection of one bottle now in wide use in the New York Metropolitan district.

While single service paper containers have not been widely used for milk prior to about 1935, milk has been in contact with paper caps for many years. Rice (2, 3) studied this question in an attempt to explain fluctuating bacterial counts in pasteurized milk. Caps were found to contribute bacteria to milk. The bacteria involved were found, however, of no public health significance. Rice found that the bacteria originated in the paper plant. None were pathogenic, as would be expected. Various paper cartons have been used for ice cream, butter, and cottage cheese for many years. If paper containers are objectionable in the dairy industry, this fact would have been discovered years ago. Even fluid milk has been distributed for many years in containers which have been closed with paper caps.

### MICROBIOLOGY OF PAPER BOARD

One of the first problems was to determine the bacteriological condition of paper which is used. Few data were available in the literature on bacteria in paper

until the present paper container for milk was perfected. Most of the work which has been done on microbiology of paper concerned deterioration of papers of record by bacteria or other agents. The effect of certain microorganisms which might interfere with manufacturing procedures was also studied.

Sanborn (4) pointed out that pulp systems and in particular ground-wood stock provided an ideal environment for development of a diverse flora of microorganisms. Whether many of these forms survive the steps in paper-making depends somewhat on their ability to resist unfavorable agents and on the bactericidal value of certain procedures used by the paper-maker. Sanborn emphasized factors which would allow large numbers of microorganisms to appear in finished paper. In a later paper he (5) again showed that the bacterial content of paper could be lowered by certain plant practices, most of which involved more sanitary practice in the paper mill.

Prucha (6) found pulp in the bleaching vats to be sterile. Washing, dilution, and treatment with size resulted in a plate count of 400 organisms per gram. Strips of paper, two by two inches, removed just as it left the drying rolls and after disintegration in water gave no colonies. The paper was reported to be practically sterile on its surface. Prucha considered the standard, adopted at the First Conference on Sanitation of Paper Milk Containers at Geneva, N. Y., July 12, 1937, of not over 500 per gram, to be very liberal. He believed that it was possible to make practically sterile paper-board, if proper attention is given in the paper mill to certain steps which influence bacterial development.

According to Sanborn (7) the sanitary status of a mill depends on the degree to which it achieves strict microbiological control. He offered experimental data, as did Prucha (6), to show that close relationship existed between vat contamination and bacterial counts on finished paper. These observations indicate that paper mills which intend to market their product for use in the food industry must

face the necessity of using the bacteriologists' standard of cleanliness. Sanborn (5) mentioned the following principles which must be observed in mills making paper-board to be used for paper milk containers:

1. Properly constructed and operated paper mill in which modern ideas of sanitation are enforced.
2. Use of pure virgin wood pulp or other materials of equal quality.
3. Water from a supply of unimpeachable bacteriological quality.
4. Careful protection of paper from contamination after leaving the paper-making machine.

The procedures for making paper-board used by a typical mill would yield a product practically free from microorganisms. The high temperatures attained on the drying rolls of approximately 121.1° C. (250° F.) destroys all pathogenic bacteria and most of the non-pathogenic species. This has been demonstrated by thousands of counts on paper-board as well as by results of experiments in paper making plants. In these experiments Wheaton (8) sprayed heavy suspensions of bacteria onto the paper web just before it passed over the drying rolls. The organisms used were *Erythrobacillus prodigiosus*, *Escherichia coli*, *Staphylococcus aureus*, a yellow sarcina from a paper milk container, and an aerobic spore former. Heavy suspensions were used. *Escherichia coli* suspension had 510,000,000 per c.c. Only the aerobic spore former survived the temperature of the rolls. Prucha (6) found paper to be practically sterile when it came from the drying rolls. If properly handled it may be kept so, and thus becomes a factor in better methods of packaging foods.

The paper-board used in making paper milk containers is made from pure virgin wood pulp. It is not made from waste paper. This fact indicates that bacteriological problems relating to the pulp and finished paper-board are minor ones and probably of little public health significance. This pulp is made into paper in clean paper mills by procedures involving chlorination, cooking, and drying on rolls

\* Read at the 27th Annual Meeting of the International Association of Milk Sanitarians, Cleveland, Ohio, October 19-21, 1938.

† Some of the statements in this paper are based on the author's experience with one type of paper container. They may not, therefore, hold for other

at temperatures which destroy practically all of the bacteria. Only a few spore formers remain. As the paper comes from the machines, it is cut and collected in large rolls which are wrapped and sealed before being taken to the factory to be made into milk containers. When it is placed on the machine which cuts it into the body and end blanks of the carton, the edges are well trimmed and several layers are discarded before such blanks are cut. These blanks are cemented together by a thermoplastic cement applied at a temperature of 204.4° C. (400° F.). The containers are then completely immersed in hot paraffin wax for upwards of 20 seconds.

#### SAMPLING TECHNIC

The bacteriological content of thousands of paper containers has been determined, and the paper-board used in the container with which the speaker has been connected is constantly controlled. Each run of paper is sampled at the beginning and then every six hours until finished. A punch press provided with die has been developed with which samples may be taken without possibility of contamination. The samples are collected in a sterilized tight container in which they are taken to the laboratory. It is believed that every possible precaution must be taken to prevent contamination both in collecting the sample and in disintegrating it for bacteriological analysis. More attention must be given to methods for determining the number of microorganisms in paper. Lack of standard procedures makes it difficult for different laboratories to secure comparable results.

Disintegration of the sample has been accomplished in our earlier work by placing 10 gms. of the sample in 1000 c.c. of sterile water in a motor-driven disintegrator. This consists of a steel cell about 4 inches in diameter and 6 inches high provided with a screw cap. Through a bushing in the cap, a shaft provided with blades thoroughly disintegrates the paper sample. With such a device there is no opportunity for contam-

ination with air. Sanborn (9) has recently described the apparatus which he has used. This consists of a motor-driven shaft operated essentially as described for the apparatus just described. Prucha also uses a modified "mixer" for the same purpose.

A better disintegrator is now being developed. This consists of a stainless steel capsule containing stell balls. This may be provided with the desired quantity of water, sealed and sterilized. After the paper sample has been added, the capsule is again sealed and rotated end over end on a special machine in such a manner that the balls thoroughly disintegrate the paper. In this machine thorough disintegration of the paper sample is being accomplished in a short time.

The question may properly be raised whether a sterile paper should be required. It has been shown beyond reasonable doubt that pathogenic bacteria cannot survive the conditions which exist on a modern paper-making machine. The only bacteria which do survive are harmless saprophytes in small numbers of no sanitary significance. Our work has shown that paper-board used for making paper milk containers is "sterile" as the term is generally used in the dairy industry. This point is discussed later in this paper. In some cases, the milk which is put into a bottle will contribute many more bacteria to the bottle than the bottle will contribute to the milk.

The counts which we are securing on paper samples collected as described above are far below the standard which has been tentatively accepted. The average count on thousands of samples is approximately 60 bacteria per gram. Once in a while a sample will give a count considerably above the standard but such instances are rare. Just what causes them is not known. Some 10 percent of the samples have shown no microorganisms at all.

#### STANDARDS

What bacteriological standards shall be adopted for paper board to be used in paper containers? This question can be answered only after careful bacteriological

examination of pulp being made into paper, and the final product. This has been done by Prucha (10), Sanborn (7) and Wheaton. Sanborn stated that container board taken at any stage prior to moisture-proofing should not have a count exceeding 500 colonies per gram of disintegrated board. Effective observance of certain sanitary procedures in the paper mill could make it possible to manufacture a product with less than 100 bacteria per gram. The Second Conference on Sanitation of Paper Milk Containers at Geneva, N. Y., May 2, 1938, recommended that at no time should container-board have more than 500 bacteria per gram. This was considered to be a lenient standard. Where milk came directly in contact with unwaxed surfaces, the standard was placed at 100 bacteria per gram. Whether any standard which is adopted should place a hard and fast maximum is questionable. Some tolerance is usually permitted in most public health work where non-pathogenic bacteria are concerned. Perhaps a more reasonable standard should read that not over 90 or 95 percent of samples should contain over 500 bacteria per gram. Such tolerances are used in controlling the amount of lead on sprayed apples, in water analysis, and in other cases.

#### UNPARAFFINED BOTTLES

Many examinations have also been made on microorganisms in unparaffined containers. Those with normal contamination as well as those which were sprayed with test bacteria have been used. Such bottles have been examined in different ways. They have been rinsed with a sterile medium and this medium incubated in the bottle and they have been examined according to the rinse procedure described in Standard Methods of Milk Analysis. We have observed that bacteria die out on card-board which is held under atmosphere conditions at room temperature.

#### PARAFFINING

The completely assembled bottle is passed through a hot paraffin bath in order to water-proof the paper and con-

tribute to its sterility. When paraffin waxed-paper milk containers were first brought to the attention of bacteriologists and health officers, it was believed that application of the hot wax would sterilize the paper or container. It is known that the paraffin treatment given the paper milk container removes practically all of the viable bacteria. The heat in the paraffin bath has appreciable sterilizing value, and accomplishes for the milk container what steam retort treatment accomplishes for canned foods. Both treatments establish conditions which protect the health of the consumer. It would be ridiculous to demand that the containers be absolutely sterile. A similar position has already been assumed in the dairy industry. The Committee on Milk Supply of the Public Health Engineering Section of the American Public Health Association and the committee on Milk Sanitation, Conference of State Sanitary Engineers in their joint report entitled "When Are Milk Containers and Equipment Clean and Sterile" (Holmquist, Chairman 1934) stated: "The word 'sterilization' as used here and as it has been used for years in dairy work means bactericidal treatment resulting in the devitalization of milk-borne pathogens and the further material reduction of all bacteria." The bacteriological results of paraffining paper-board in making the paper milk container are well within this definition. The Public Health Service Milk Ordinance and Code: 1936, after specifying the procedures that shall be used for washing and sterilizing glass milk bottles, places a standard of efficiency for this effort at not more than 1 bacterial colony per c.c. capacity of the bottle. This is presumably their conception of "sterility". If glass bottles are allowed almost 1000 bacteria per bottle, certainly there can be little argument against a container of which some 80 percent yield no colonies whatever and the remainder average about 2 colonies per container. It has been argued that bacteria might be liberated to the milk from the paper board through the paraffin. Considering the low count

of the finished container, is it reasonable to expect that sufficient bacteria could be surrendered to the milk to raise the count in excess of the generally accepted standard of one colony per c.c. capacity? Results published by Rice (11) answer this question. He packed milk in both paper and glass containers and found that the bacterial counts obtained from milk in paper containers was lower in every way than corresponding counts made on milk in glass bottles. Rice was not satisfied with the degree of sterilization that was achieved in the glass bottles.

After the container with which I am associated has been fabricated, it is completely covered with a coating of paraffin wax. The carton is completely submerged in and filled with hot paraffin ranging in temperature from 74°—82° C. (165°—180° F.). It is then drained in an atmosphere of filtered air proven by frequent bacteriological examination to be free from bacteria. After application of the wax and draining, the bottles pass through filtered air at a temperature of 10° C. (50° F.). A jet of cold air is also delivered to the interior of the carton. Then the bottle is sealed by the cap with which each has been provided.

The paraffin is transported from the refinery to the factory in the liquid state in stainless steel tank trucks. In the factory it is stored in stainless steel tanks in which it is kept liquid at 66° C. (150° F.) until used. As it is pumped to the paraffining machines, it is filtered. In this manner a water-proofing material of unquestioned purity is secured.

Study of the behavior of hot paraffin oil and wax on microorganisms dates back as early as 1898 when its use was advocated as a medium for sterilization. It was proposed at this early date that surgical and dental instruments could be sterilized by heating in paraffin. The effect of solid paraffin on a surface like paper has not received much study. Whatever role solid paraffin played was due undoubtedly to ability to prevent reinfection after foods had been sterilized by other procedures. It has been used for many years for this purpose.

Paraffin-wax has been one of the commonest water-proofing materials for food containers. It is harmless to health. Practically all of the paper milk containers now in use are water-proofed in this manner. Besides any effect on microorganisms which might be noticeable, the wax serves several other useful purposes.

1. Contributes greatly to strength and rigidity.
2. Helps to secure an hermetical seal.
3. Improves the appearance of the bottle by causing a more brilliant finish.
4. Inert to attack by ordinary bacteria.
5. Free from color, taste, and odor.
6. Available with various melting points.
7. Practically free from microorganisms.
8. Good water-proofing surface.
9. Prevents microorganisms from entering the milk.
10. Contributes to a sterile container.

#### MICROBIOLOGY OF PARAFFIN TREATMENT

It is this last function of the paraffin wax, its contribution to bacteriologically sound container, that is being discussed in this paper. Results of experiments by Fogue and Reclus (12), Anderson (13), Kieffer (14), Conradi (15), Dreyer and Walker (16), Bartlett and Kinne (17), Bullock (18), Davis and Rosen (19) and Dirksa (20) are in general agreement. Hot paraffin wax at temperatures which may be used in the food industry has a detrimental effect on bacteria and reduces the number of viable cells to practically zero.

Prucha (10) did considerable work on the germicidal effects of paraffination of paper board. His work was carried out both in the milk plant and in the laboratory. Prucha heavily inoculated his containers with *Serratia marcescens* (*Bacillus prodigiosus*). In the first experiments the containers were dipped into a suspension of bacteria having a plate count of from 200 to 300 millions cells per c.c. When treated in this manner, each one took up about 7 c.c. of the suspension. From this, Prucha inferred that about one and a half millions cells were deposited on each container. As soon as the containers were dry, they were paraffined and sealed.

In order to determine the number of microorganisms surviving this treatment, Prucha introduced 25 c.c. of melted nutrient agar into each container and shook the container so that the medium washed the interior of the bottle. The medium then ran to the bottom of the container where it solidified making an agar plate. The container was incubated for 4 days at room temperature. Most of the containers were found to be sterile but a few, from 1 to 10 percent, showed presence of living bacteria. This was true for temperatures of paraffination of 76.6° C. (170° F.), 82.2° C. (180° F.), and 87.8° C. (190° F.). When the containers were dipped into a bacterial suspension with but 2 million cells per c.c., none showed the presence of viable cells of the test organism. The number used in the last mentioned experiment was much larger than would be expected in containers in commercial practice.

Prucha continued his work by rubbing the interior of the container with a hand dipped in a heavy bacterial suspension. This method was said to deposit about 5000 bacterial cells in each container. Two suspensions were used containing 250 million and 2 million cells per c.c. These containers were paraffined at the temperatures mentioned above. Again those inoculated with the lighter suspension were all negative.

The work in the laboratory was carried out at first with paper strips 1/2 x 2 1/2 inches in size. They were dipped into a bacterial suspension and dried, after which they were paraffined at temperatures of 71.1° C. (160° F.), 76.7° C. (170° F.), 82.2° C. (180° F.), 87.8° C. (190° F.), 93.3° C. (200° F.), and 100° C. (212° F.). Six strips of inoculated paper were used at each step. The results of this experiment showed that starting with 82.2° C. (180° F.) appreciable destruction of bacteria was evident. Only a few strips were positive for the test organism; these had been heated for only 20 seconds. It is apparent from Prucha's work that an exposure of at least 30 seconds at 82.2° C. (180° F.) gives virtually a sterile con-

dition, even when each strip carried as many as 5,000,000 bacterial cells. Prucha also heated strips of paper heavily seeded with *Escherichia coli* in water. The temperatures used were only 54.4° C. (130° F.), 60° C. (140° F.), 62.8° C. (145° F.), and 65.6° C. (150° F.). All strips were sterile after heating at 65.6° C. (150° F.) even for 10 seconds.

The first conference on sanitation of paper milk containers held in the New York Agricultural Experiment Station, Geneva, New York on July 12, 1937 (Breed, 21) adopted specific recommendations for the handling of paraffin. No specific temperature was mentioned. It was agreed that paraffin for coating, impregnation, or storage be handled in such a manner that flavors or odors would not be contributed to the milk. These recommendations were not changed at the second conference on sanitation of paper milk containers held at the same place May 2, 1938 (Breed, 22).

In order to secure information on any subject such as the one under discussion, the experiments should be carried out under conditions as closely identical with those in practice as possible. In this case thermal death times of bacteria should be determined in hot paraffin on paper board similar to that used in making the paper milk containers.

#### EXPERIMENTS WITH STRIPS OF PAPER BOARD

Some of the early work on this paper was conducted with short strips of paper-board which could be heavily inoculated by various microorganisms. After drying, these strips were subjected to hot paraffin wax at various temperatures for definite periods of time. After removal from the paraffin, they were placed in sterile test media in order to determine the presence of viable organisms. Quantitative data could not be secured with this technic. Only what might be called "absolute thermal death times" could be determined. That is the time required for destruction of all living organisms on the strips. This technic was used in our laboratory as early as 1934 by Mr. Berry and

his associates. It was later also used by Dr. Wheaton and several others in a very intensive study on the effect of paraffin on microorganisms. In some of our early work, these strips were heavily inoculated with *Escherichia coli*, adopted as a test organism because it is not pathogenic and has about the same resistance to heat as do members of the typhoid-dysentery group. Furthermore, its resistance to heat is higher than for most of the pathogens. A concentration of 540 million cells of the strain used in this work survived 15 minutes and was killed in 20 minutes at 60° C. (140° F.) in phosphate buffered water.

After drying they were heated in both water and paraffin. None of those which were heated in water carried living cells after heating at a temperature of 75.5° C. (168° F.). Those which were heated in paraffin at 75.5° C. (168° F.) and 87.2° C. (190° F.), for 12, 15, 17, 20, and 25 seconds showed almost uniform presence of viable cells of *Escherichia coli*. These results are in general agreement with those reported by others and secured in other experiments by the present authors.

Further experiments were carried out by Wheaton in 1935, 1936, and 1937 with this same technic. He again used strips of paper board 1/2 x 2 inches in size, heavily inoculated with heavy suspensions of *Escherichia coli* by dipping in the suspension and drying. These strips were then paraffined at 87.2° C. (190° F.) and 75.5° C. (168° F.) for about 7 seconds. While heating in water killed all of the bacteria on the strips, paraffining at the above temperatures for from 12 to 25 seconds did not result in complete destruction of all living cells. In fact the longest paraffin treatment of 25 seconds did not yield much better results than the shortest period of 12 seconds. It should be emphasized, however, that while a few viable organisms survive, the great majority of them were destroyed, a fact which must not be overlooked in weighing the benefits of water-proofing with paraffin wax.

The experiments described above place a load on the paraffination process quite out of proportion to that which exists in practice. The numbers of bacteria with which the strips were inoculated are many thousands of times greater than are found on the paper-board from which containers are made.

Throughout all of our work on strips of paper-board and unparaffined containers, the inability of the test organisms to survive a long time on paper has been evident. We have found it necessary to proceed with paraffination as soon as possible after inoculation for many cells die. This might indicate desirability of holding paper in storage before it is paraffined.

Results of experiments with this technic are open to question. The ratio of cut edge to surface area is too large. Inoculation of paper strips by dipping into a heavy suspension of microorganisms causes many bacteria to enter the paper at the cut edges. These may not be subjected to the effects of hot paraffin and even be protected from the heat by the layers of paper. When the paraffined paper is transferred to culture media these organisms in small numbers are surrendered to the medium. This is easily demonstrated when such strips are placed in sterile Petri dishes and covered with a sterile culture medium. The growth practically always appears around the edges of the paper strip. Another difficulty is the slow penetration of heat into paper.

#### EXPERIMENTS WITH INOCULATED UNPARAFFINED CONTAINERS

Various types of experiments have been made on the effect of paraffination on microorganisms in containers themselves. This is the best method of studying the problem for it gives conditions as close to the practical as possible.

Our first experiments involved a study of containers which had been made from paper-board heavily inoculated with *Escherichia coli*. This organism was selected because it has some standing as an indicator of pollution in foods and milk and because some strains have been found to survive times and temperatures used in

pasteurization. The strain used in this work in a concentration of 540 million cells per c.c. survived 15 minutes but was killed in 20 minutes at 60° C. (140° F.).

In some of our preliminary experiments, sheets of paper-board were inoculated with two concentrations of cells from this culture. The board was dipped into a heavy suspension of the cells. This paper was then made into containers and paraffined at 71.1° C. (160° F.) for 6 seconds. Only in one case of a container in a group of nine paraffined by this method for 6 minutes were living cells of *Escherichia coli* found. It could not survive short exposures to hot paraffin at temperatures which are now used in the making of containers even with the very heavy inoculation which was used.

The situation, however, was different with *Staphylococcus aureus*. Assembled, unparaffined, half-pint containers after heavy inoculation with the organism showed a small percentage with viable cells. I shall not go into the details of these experiments. It should be pointed out also that *Staphylococcus aureus* is one of the most heat resistant non-spore-forming bacteria which is known and also that the containers received inoculations in our work which were much greater than is present on paper board used in commercial manufacture.

Having passed containers which had been heavily inoculated with *Staphylococcus aureus* through the paraffin bath, and believing that the bath might have been heavily seeded with organisms which would be contributed to containers passed through the bath, subsequently a series of uninoculated containers were paraffined. All of our work on this question indicated that there is no pick-up in the paraffin bath even though a few containers containing large numbers were passed through it.

Some have maintained that the temperature of the paraffin bath should be as high as possible, at least 85° C. (185° F.), in order to secure maximum destruction of bacteria. While this temperature may give slightly better bacteriological re-

sults, they are not significantly different from those secured at lower temperatures. The slightly greater destruction of bacteria at 85° C. (185° F.) than at 71.1° C. (160° F.) is more than offset by the lighter coat of paraffin which is applied at 85° C. (185° F.). Since the function of the paraffin is to waterproof the container and give a surface which is impervious to bacteria, a heavier coat may be desirable. It may be stated that the differences in results secured on bottles paraffined at 71.1° C. (160° F.) and 82.1° C. (180° F.) are practically nil. In some of our experiments, lower counts were secured from bottles paraffined at 71.1° C. (160° F.) than from bottles paraffined at 82.1° C. (180° F.). It is well to stress the fact that paraffination is done under aseptic conditions. The machines are provided with air filters so that bacteria cannot enter from the outside.

The question may now be raised as to how to measure the effect of hot paraffin wax on bacteria. What organism shall be used and in what concentrations? The test organisms which have been used have been *Serratia marcescens*, selected because it is more easily detected and probably not because of any special importance in the dairy industry, *Staphylococcus aureus*, and *Escherichia coli*. The last mentioned species has been used in studies of other problems in the dairy industry. Practically all experiments with pure cultures have been carried out with very heavy suspensions of cells. The inoculation has been much heavier than it would ever be in practice. In the future, more attention should be given to attempts to bring the number of bacteria in the inoculum to that which would ordinarily be expected under actual practice.

#### PARAFFINING IN MILK PLANT

Depending on whether they are paraffined in the milk plant or in the factory, containers may be separated into two groups. One type is shaped and paraffined in the dairy just before it is filled with milk. Other types are paraffined in the factory and then shipped to the dairy. The relative merits of these containers

are obvious to anyone who studies them. Whether one or the other is used will be determined largely by conditions which obtain in the plant of a milk dealer who is considering adoption of a single service container. On this point I would like to quote Sanborn (23): "For example, in specifying that containers shall be paraffined in the milk plant, where they are filled with milk, the ruling fails to take into account two significant facts, namely that considerable defective paraffining takes place in milk plants where machines may not come under competent supervision, and that premade containers are frequently more nearly free from organisms than some of the containers that are filled immediately after paraffining." The factory-fabricated container and the milk-plant fabricated container should probably be considered as individuals and regulations adopted for each. Both are good packages for milk.

It has been suggested that paper milk containers which are made in a factory and shipped to a milk plant should be given some bactericidal treatment. This might be indicated for containers which are open but for those which are closed and shipped in sealed packages, it would seem to be unnecessary. Especially is this reasonable for those which are practically sterile. Such a treatment might increase the bacterial load. Certainly it would necessitate more machinery in the dairy when the trend today is to reduce it as much as possible.

#### BACTERIOLOGICAL CONDITION OF THE FINISHED CONTAINER

The bacteriological condition of the finished container is influenced by all of the factors mentioned earlier in this paper. Several papers have already been published which report results of bacteriological examination of finished containers. Sanborn (24) reported that average plate counts for all types of containers which he examined was usually less than 50 per container. The average count of one type of container made from board which was practically bacteria-free was only 4 per container. The container which gave the

highest count yielded on the average only 50 colonies per container. Tanner (25) reported that over 90 percent of the containers examined with the rinse test yielded no colonies. Of those which did, many yielded only one or two colonies. Since then nearly 8000 containers of one type have been examined; 80 percent of these gave no colonies whatever. The 20 percent which were not sterile yielded generally an average of 2 colonies per bottle. Rice (26) reported results of an extensive investigation on the bacterial content of one type of paper container for fluid milk which he described as "an open conical type." Counts were not made on them. He pointed out the superiority of factory-sealed paper milk containers over those which were shipped unsealed. Results of another comprehensive investigation of another type of one service paper milk container were published by Rice in 1938. This bottle was said to compare favorably with the glass bottle as a receptacle for milk. From the public health angle it was said to be superior for there was no chance of spreading communicable diseases from household to household.

#### TYPES OF BACTERIA FOUND

The types of bacteria which have been found in paper containers are of no sanitary significance. In one series of bottles examined in the author's laboratory, 5 c.c. of rinse water from each of 1200 bottles gave no evidence of the presence of *Escherichia coli*. Since then no attention has been paid to this organism. The organism has never been found. Most of the bacteria are aerobic spore formers; a few white staphylococci and sarcinae make up the rest of the flora. The type of bacteria yielded by paper containers are of no significance. Those which have been identified are closely related to members of the subtilis-mesentericus group.

#### METHODS OF EXAMINATION OF CONTAINERS

Where it became necessary to select bacteriological procedures for examination of paper containers, bacteriologists naturally

resorted to methods which had been developed for the glass bottle. A method was published for this purpose in the 6th edition (1934) of Standard Methods of Milk Analysis published by the American Public Health Association. This method consisted of rinsing the bottle with 100 c.c. of sterile water and plating 2 one c.c. portions and 2 0.1 c.c. portions in plain agar. Such a procedure was probably satisfactory for glass bottles, many of which have high counts. Owing to low counts secured with paper containers and to the fact that many yielded no bacteria, another procedure had to be devised. Wheaton suggested rinsing the container with 10 c.c. of sterile water and plating the entire amount. This method was finally used by many of those studying the problem. Owing to the fact that it was impossible to secure all of the 10 c.c. for plating, Fitzgerald suggested rinsing with 20 c.c. and plating 10 c.c. A constant quantity would always be secured for plating. This is the general procedure now in our work and in other laboratories where cooperative work is being done. Plating is done either by distributing the 10 cc. of rinse water evenly in three regular-size Petri dishes 90 mm. in diameter or in one large Petri dish 150 mm. in diameter. Use of the latter dish obviates much manipulation of the sample.

Standard methods for examination of containers implies that "standards" showing the maximum number of microorganisms which will be allowed, will also be established. There seems to be little reason why they should be different for paper and glass since it is the number of microorganisms in the bottle which is of interest. At least two containers which are now being used are practically sterile.

#### MUNICIPAL REGULATIONS AND CONTROL

The problem just now for health officers is to establish systems for control of these new packages. Some cities have already passed ordinances; some of them were adopted without sufficient information. Examples among the first to be adopted are those of the Cities of Baltimore and Reading. These ordinances

specified that "the best obtainable white spruce pulp" should be used. There is little basis for limiting a manufacturer to white spruce pulp because other spruces and even other species of trees could supply a pulp just as satisfactory as the white spruce. Furthermore, this ordinance recognized adhesives prepared from starch and ignored the excellent thermoplastics which are used today by one or two paper container manufacturers. The former may be heavily loaded with bacteria as shown by numerous investigators (Clark and Tanner, 1937) while the latter are sterile. The Baltimore ordinance also specified that the containers must be paraffined in the dairy, an unnecessary requirement, as discussed elsewhere. Those who are trying to establish regulations for paper milk containers would be greatly aided by observing the manufacturing of the container.

The significance of any proposed advance in public health may best be appraised by comparison with the effort which it proposes to replace. A joint report of the Committee on Milk Supply of the Public Health Engineering Section of the American Public Health Association and the Committee on Milk Sanitation, Conference of State Sanitary Engineers (Holmquist Chairman, 27) indicated that the two most important items in protecting milk from cow to consumer were pasteurization of the milk, and sterilization of milk containers and equipment with which milk comes in contact. These committees stated that efficiency of cleaning and of sterilization were matters of particular concern to health officials. A year later the same committees (Holmquist Chairman, 28) reported their observations on procedures for cleaning and sterilizing glass bottles. These committees were justified in their concern over the condition of glass milk bottles, if results of an investigation on the bacterial content of glass milk bottles reported by Layson, Brannon, and Huffer (29) reflect the average condition of these containers. Those which they examined came from 25 different typical milk plants in Illinois.



Only 26 percent yielded no bacteria with a rinse test; 14.5 percent yielded gas-forming bacteria. The rest of the bottles yielded counts from a few hundred to so many that the colonies could not be counted. They stated that in any case of 12 bottles, two or three might be sterile while some of the others might contain as many as 180,000 to 200,000.

The sum and substance of these observations can only be that paper containers can avoid many of these problems. They are freer from bacteria and do not show the presence of gas-producing bacteria. In addition to advantages which are supported by scientific data are those from the general or aesthetic viewpoints. Packaging of milk in single service containers is in keeping with the trend in merchandizing of food-stuffs of various kinds.

The Public Health Service Milk Ordinance and Code requires that all containers, equipment, and other utensils used in handling, storage, and transportation of milk or milk products shall between each usage be subjected to an approved bactericidal process with steam, hot water, or chlorine. The members of the Public Health Service Milk Sanitation Advisory Board apparently recognized these problems for a container which must be used over and over.

#### OTHER PROBLEMS

Various problems which introduction of paper milk containers introduces, have been mentioned. Some of them are:

1. Increased cost of packaging milk.

When all factors are considered, it is possible to package milk in paper and compete on an even basis with glass. Bartlett (30) showed this after studying the subject in Boston and New York.

2. Fear of expense of replacing old machinery in a milk plant.

This is not a public health problem in the strict sense and need not be discussed here.

3. Impossible to see the cream line.

This is not an important point. At least it is not as important as it was once.

Adequate supervision of milk distribution has fixed the fat content of milk. The homemaker no longer has to observe the cream line as an index of quality. Further, increasing tendency to homogenize milk has negated this objection.

4. Paraffined paper containers might be porous and permit absorption of milk.

This point has been raised but data on it are uncertain. Perhaps it has been suggested largely by loss of shape of containers which have been held for much longer time than would be the normal holding period for fluid milk. Bacteriological data are now needed.

#### SUMMARY

The paper milk container is an acceptable package for distribution of fluid milk and is a real achievement in the field of public health. Examination of thousands of containers of one type has revealed a low number of viable bacteria and an entire absence of pathogens. *Escherichia coli* has never been found even though generous quantities of rinse water were examined. As Sanborn (7) has said, there is no reason why a paper container made from clean, chemical or mechanical pulp, should not be suitable for perishable foods. Careful methods of manufacture will result in a paper milk container with a very low count—so low that it is unreasonable to question its desirability from the sanitary standpoint. The counts which are being secured on some makes of containers are low and have never yielded gas-producing bacteria. Pathogenic bacteria cannot survive the various procedures which are involved. The advantages of a single service container are obvious.

#### REFERENCES

1. Tanner, F. W., Public Health Aspects of Fiber or Paper Containers for Milk. *Dairy Inds.* 3 (4) and 3 (5) 125-128; 165-170 (1938).
2. Rice, J. W., Bottle Caps as a Source of Contamination of Milk. *Penn. Assoc. Dairy and Milk Insp. Ann. Rept.*, 1930.

3. Rice, J. W., The Source and Control of Bacteria in Milk Bottle Caps. *Penn. Assoc. Dairy and Milk Insp. Ann. Rept.*, 1932.
4. Sanborn, J. R., Development and control of micro-organisms in a pulp and paper mill system. *J. Bact.* 26; 373 (1933).
5. Sanborn, J. R., Paper Mill Sanitation in Relation to the Manufacture of Food Wraps and Containers. *J. Bact.* 34; 347 (1937).
6. Prucha, M. J., Certain Sanitary Aspects of the Use of Paper Milk Containers. *Dairy Manuf. Conf., Manual* 203-210.
7. Sanborn, J. R., Reasonable Sanitary Standards for Paper Products. *Paper Trade J.*, 1-6 (1938).
8. Wheaton, E., Lueck, R. H., and Tanner, F. W., Observations on Problems Relating to the Paper Milk Bottle. *J. Milk Tech.* 1 (3) 11 (1938); also private communications.
9. Sanborn, J. R., Disintegration of paper board for bacteriological examination. *Amer. J. Pub. Health* 28, 576-578 (1938).
10. Prucha, M. J., Certain Sanitary Aspects of the Use of Paper Milk Containers. *J. Milk Tech.* 1 (2) 4-9 (1938).
11. Rice, J. W., Recent Studies of the Bacterial Content of Paper Milk Containers. *Penn. Assoc. Dairy Sanitarians, 14th Ann. Rept.*, 1938.
12. Forgue and Reclus, *Traite de Therapeutique Chirurgicale.* (1898).
13. Anderson, Ein neuer Oelsterilisateur. *Dtsch. zahnaril. Monatschr.* 1907-1908.
14. Kieffer, Asepsis und antiseptis die Zahnheilkunde. (1907).
15. Conradi, H., Ein neues Sterilisierungsverfahren. *Dtsch. Med. Woch.* 35, 1015-1016 (1909).
16. Dreyer, G. and Walker, E. W., Resistance of Microorganisms Suspended in Glycerine or Oil to the Sterilizing Action of Heat. *J. Path. & Bact.* 17, 142-143 (1912-13).
17. Bartlett, C. J. and Kinne, F. B., Resistance of Microorganisms Suspended in Glycerine or Oil to the Sterilizing Action of Heat. *Cent. f. Bakt. Bd. I*, (59), 358 (1914).
18. Bullock, H., The Resistance of Spores to Heating in Anhydrous Fluids Such as Glycerine. *J. Hyg.* 13, 168 (1914).
19. Davis, D. M. and Rosen, R., Surface Sterilization of Tissue for Bacterial Studies. *J. Inf. Dis.* 21, 327 (1917).
20. Dirksa, C., Ein Beitrag zur Losung der Sterilisationsfrage komplizierter zahnartzlichter Instrumente. *Ztrbl. f. Bakt. Ab.* 1 (87) 387. (1922).
21. Breed, Robert S., Conference on sanitation of paper milk containers. *The Milk Sanitarian* 6 (9), 11 (1937).
22. Breed, Robert S., Second conference on sanitation of paper milk container. *J. Milk Tech.*, 1 (5) 47-53 (1938).
23. Sanborn, J. R., Microbiological control in the manufacture of paper wraps and containers for foods. *Ind. Eng. Chem.* 29, 949 (1937).
- Sanborn, J. R., Sanitary Aspects of paper milk containers. *15th Ann. Rept., N. Y. State Assoc. Dairy & Milk Insp.*, 177 (1937).
24. Sanborn, J. R., Proposed standards for paper milk container. *J. Milk Tech.* 1 (2) 41 (1938).
- , Suitable Paper Wrappers and Containers for foods. *Amer. J. Pub. Health* 28 (5) 571-575 (1938).
25. Tanner, F. W., Microbial Flora of Paper Containers. *Amer. J. Pub. Health* 28, 587-592 (1938).
26. Rice, J. W., The Bacterial Content of Paper Milk Bottles. *Penn. Assn. Dairy & Milk Insp., Ann. Rept.*, 1936.
27. Holmquist, C. A., Chairman, When are milk containers and equipment clean and sterile? *Amer. Pub. Health Assn. Yearbook* (1933-1934), 72-74. *Suppl. to Amer. J. Pub. Health* 24 (1934).
28. Holmquist, C. A., Chairman, Milk Supply. *American Pub. Health Assn. Yearbook* (1934-1935); *Suppl. to Amer. J. Pub. Health* 25 (1935).
29. Layson, S. V., Brannon, J. M., et al. Results of Bacterial Survey of Milk Jugs and Milk Bottles. *Milk Plant Monthly* xxv, 34-36 (1936).
30. Bartlett, R. W., Increasing the efficiency of milk distribution. *Dairy Manuf. Conf. Manual*, 211-218 (1937); also in *Amer. Produce Review*, 380-382 (1938).

## Application of the Rapid Phosphatase Test to Dairy By-Products\*

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During the past year, the modified rapid phosphatase test has been applied to the testing of certain dairy by-products in an effort to determine the suitability of this method in judging the efficiency of pasteurization of such products. The study is admittedly incomplete but the results thus far obtained are here presented so that interest may be further stimulated in this type of investigation. Certain changes in the published technique (1) of the rapid phosphatase test have been made which allow easier manipulation of the method and further simplify the preparation of the necessary solutions. Since a discussion of our results depends upon the technique used, we present herewith some of the changes in the method.

Because of the difficulties encountered by many in the preparation of the recommended saturated basic lead acetate solution, we have substituted a 50 percent lead acetate  $Pb(C_2H_3O_2)_2 \cdot 3H_2O$  solution with appropriate changes in the amount of buffering agents to accommodate this more readily prepared solution. The buffering agent remains the same as previously suggested (28.427 grams of Borax C.P. and 3.27 grams of NaOH per liter), but twice as much is used in preparing the buffered substrate.

The removal of residual phenol from the disodium phenyl phosphate by tedious washing with ethyl ether is replaced by making a concentrated solution of the disodium phenyl phosphate, buffering to a pH of 9.6, developing the indophenol color by means of a few drops of the BQC reagent, and extracting the color with normal butyl alcohol. Certain commercial brands of this phosphate con-

tain considerable amounts of impurities and frequently show excessive amounts of free phenol which may require several extractions with butyl alcohol for complete removal of the phenol from the concentrated solution. Other brands of the phosphate have been found to be quite acid, necessitating the addition of larger amounts of buffer, both in the cleaning process and in the subsequent preparation of the buffered substrate, in order to maintain the correct pH conditions. While butyl alcohol will extract the phenol from the water solution, the indophenol has a greater solubility in the alcohol and its color serves as an indication of the completeness of the phenol extraction. A suitable disodium phenyl phosphate is available under the name "Phen-Free." This salt in large, well defined crystals, is remarkably free of phenol and contains an almost negligible amount of di-basic sodium phosphate and sodium diphenyl phosphate. Using this compound, a concentration of  $\frac{1}{2}$  gram per liter has been found sufficient. Dissolve the required amount for a liter in about 5 cc. of distilled water in a small test tube (10x100 mm.). Add about  $\frac{1}{2}$  cc. of the buffer, shake well, and add a few drops of the BQC reagent, allow five minutes for development of coloration, and extract the indophenol by shaking about 2 cc. of normal butyl alcohol. When the alcohol layer has separated completely, remove the alcohol layer with a medicine dropper and discard. Add the remainder of the solution to 100 cc. of the buffer and dilute to a liter. The solution is phenol free and will be stable for a considerable period if kept under refrigeration. Saturation of the solution with chloroform aids in preventing the decomposition of the solution with the

resulting formation of free phenol. However, it is advisable to conduct a blank determination on this reagent daily. The preparation of smaller quantities of the solution, sufficient for immediate needs, is a more practical measure.

### COLOR STANDARDS

The series of phenol standards previously prescribed (1) because of their comparative instability have been replaced by the following inorganic standards prepared by Junior Chemist Max Cohan:

In each instance dissolve the indicated amount of reagent in 1 percent HCl and dilute to 100cc. with 1 percent HCl.

Color solution, Blue—30.0 grams Copper Sulfate ( $CuSO_4 \cdot 5H_2O$ ).

Color solution, Red—5.959 grams Cobalt Chloride ( $CoCl_2 \cdot 6H_2O$ ).

Color solution, Yellow—4.505 grams Ferric Chloride ( $FeCl_3 \cdot 6H_2O$ ).

The ferric chloride solution should be standardized.

Prepare color standards by combining the quantities indicated in the table below and diluting to 5 cc. with distilled water.

Units	COLOR SOLUTIONS		
	Blue cc.	Red cc.	Yellow cc.
1	0.2	0.35	0.5
2	0.5	0.6	0.55
3.5	0.5	0.5	0.25
5	1.0	0.75	0.5
7.5	1.5	0.75	0.25
10	2.0	1.0	0.25

Another method of making a color standard is to employ a 0.002 percent solution of sodium sulfocarbolate. Five cc. of this solution when treated with the buffering agent and BQC solution is equivalent to 2 units. This solution should be prepared weekly. The unit of phosphatase is the enzyme activity which under conditions of the test will produce the color equivalent of 1 gamma (.001 mg.) of phenol per 5 cc. of filtrate or 0.2 ppm. of phenol.

The color standards for the field test have been published previously (2). Tablets are available for ready preparation of the buffered substrate, the buffer, and the

BQC solution. Marked improvement has been noted in the manufacture of these tablets. The substrate tablets now available contain little if any free phenol, and as a matter of interest, one brand which was tested withstood exposure to 130° F. for one thousand hours with the development of only a small amount of phenol.

There being so little, if any residual phenol present in these tablets, the cleaning step may be omitted, but the precaution should be adopted of testing the tablets from time to time wherever there is a possibility of their decomposition due to heat or moisture. The solutions, whether prepared from the tablets or reagents, may be used on either the laboratory method or the rapid field test.

With little change in the manipulative technique, both the laboratory and the field tests can be applied to the examination of by-products for efficient pasteurization. The laboratory technique will be discussed first.

### METHOD—LABORATORY TEST

Transfer 1 cc. of the prepared sample to a test tube using a clean pipette for each sample. Add 10 cc. of the buffered substrate. Shake well, and place in a water bath at 95°—115° F. (35°—46° C.) for an hour. Greater sensitivity is obtained when the incubation temperature is above 100° F. (37.5° C.). After incubation, place tubes in boiling water for five minutes and then cool in ice water. Add 0.1 cc. of lead acetate solution. Shake well, allow to stand for a minute or two until the solids and fat coagulate and separate sharply. (In some cases it may be necessary to add more lead acetate solution to complete the precipitation and to allow clear filtration). Filter through ordinary qualitative paper. To 5 cc. of the clear filtrate, add 0.25 cc. of the borate buffer. Should the solution become turbid on the addition of the buffer, it may be clarified by the addition of a few drops of a 10 percent sodium pyrophosphate solution. Add two drops of the BQC solution. Shake gently. After fifteen minutes, compare with the color standards, and report as phosphatase units. Commercially pas-

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teurized milk correctly processed at 143° F. for 30 minutes will yield less than two units of phosphatase.

A two or four hour incubation period results in a marked increase in sensitivity or color gain per unit of enzyme.

#### CREAM

There is some evidence to support the contention that a greater amount of phosphatase will be found in cream separated following pasteurization of the milk than if said separation precedes the pasteurization. A possible explanation of this observation can be attributed to the fact that the inactivation of the enzyme being a logarithmic function of the time and temperature, the enzyme is not completely inactivated by pasteurizing milk at 143° F. for thirty minutes, and that this small residual amount of enzyme is subsequently concentrated in the cream layer. This hypothesis suggests the complete admixture of any sample before testing. Raw cream contains several times the amount of the phosphatase found in raw milk, and so the standardization or contamination of the pasteurized product with a raw cream is even more readily detected than if raw milk be used. Cream heated at 160° F. for 15 seconds (so called short-time or high-short pasteurization) will show considerable phosphatase. Chocolate drink, Vitamin D milk, condensed milk, goat milk, and the like need no special treatment other than a control determination (see *infra* under ice cream).

#### BUTTER

The application of the technique to the testing of butter and cheese requires change only in the preparation of the sample. In the case of butter, melt the sample at a temperature below 100° F. (37.8° C.), centrifuge so as to effect a complete separation of the water serum, and pipette 1 cc. of this serum directly into 10 cc. of the buffer substrate. The rest of the procedure is the same as for milk and cream except that less lead acetate solution is required for precipitation. In testing the melted uncentrifuged butter, difficulty was encountered in obtaining uniform distribution of the en-

zyme and therefore the above procedure was resorted to. The enzyme is concentrated in this water serum and so yields higher results than the corresponding melted butter. This also allows more accurate detection of slight additions of raw products. Only slight phosphatase activity was detected in butter made from "holder" pasteurized cream, but high activity was detected even after prolonged periods of storage in butter made from raw cream and proportionately in butter made from raw and pasteurized cream, and cream held at 141° F. for thirty minutes. Butter made from "flash" pasteurized cream exhibited considerable phosphatase activity.

Some brands of commercially processed butters consistently show no phosphatase activity. A larger percentage showed 1 to 5 units, and a surprising number showed from 5 to 500 units. These butters were all labeled "made from pure pasteurized cream," or the equivalent designation. In a series of experimental determinations, an apparent change in the enzyme activity of butter serum was noted and is being further investigated. Several butter cultures were tested and found to introduce little if any phosphatase. Since no standard for phosphatase activity in pasteurized butter has yet been established, all samples yielding 5 units or more are further investigated.

Brown and Parfitt (3) have made the interesting observation that when butter samples were subjected to the keeping quality test for 10 days, there was a greater percentage of drop in score in those samples which originally showed a positive phosphatase test.

#### CHEESE

Because of the acidity and high protein content of cheese, it is expedient to neutralize and dilute the sample in the following fashion: Triturate 20 grams of the cheese with 20 cc. of the buffer and 20 cc. of water. Use a 1 cc. portion and proceed as before. The enzyme was detected even after 18 months of storage with undiminished activity in cheese made from raw milk, but was absent in cheese made from pasteurized milk including

both the holding and a short-time high-temperature methods. Cheese produced from milk or cream treated at sub-standard temperatures or sub-standard holding intervals gave positive phosphatase values.

Generally speaking, the so-called "pasteurized process" packaged cheese gave negative values. Positive phosphatase values were most frequently obtained from the following cheeses: Cream, Liederkranz, Roquefort, Camembert, Cheddar and Cottage. The ripening process does not seem to produce appreciable amounts of enzyme, and the persistence of the phosphatase in the finished product can probably be attributed to the use of raw or improperly pasteurized milk or cream.

Relish cream cheese and several other types of cheese may give colored filtrates which interfere with the evaluation of the indophenol. In such instances, extract the indophenol with butyl alcohol, and compare with a known negative test similarly treated. Where contamination with a "phenolic" product is suspected, a control determination is made by substituting buffered water for the buffered filtrate. No incubation is needed. A positive test in this control indicates presence of a phenolic product since there is no substrate present to support enzyme hydrolysis.

Mold, yeast and bacteria growth have been deliberately promoted in a series of samples now under observation to determine the extent of production of phosphatase or phenols by such treatment.

#### ICE CREAM

Processing temperatures generally recommended or required in mix pasteurization are considerably higher than those required for milk and cream pasteurization (New York City Sanitary Code requires processing at 155° F. for 30 minutes when any raw products are used in the mix). Many plants employ a temperature and holding interval considerably in excess of the requirements. Consequently, the mix should show no phosphatase activity. When a positive result is obtained, the test should be repeated with the substitution of buffered water for the buffered substrate. Since there is no substrate present to support enzyme

hydrolysis, no incubation is necessary in this "control" and any indophenol color produced in it may be attributed to interfering substances such as excessive amounts of vanilla powder, vanillin, coumarin, coloring matter, fruit syrups, or synthetic fruit flavors which may introduce phenols or phenol-like products.

When the incubated test produces a blue color greater than that of the "control", phosphatase is present, and indicates inefficient pasteurization or contamination with a raw or improperly pasteurized product.

Aside from faulty equipment, the two main factors generally responsible for positive phosphatase tests in ice cream mix are: failure of the human element, leading to inadequate manual temperature control or total or partial omission of the holding period; and the practice of standardization following pasteurization.

This standardization may have been accomplished through the use of a raw or improperly pasteurized product. Such products should of course be tested for pasteurization before being so utilized. In one instance, a condensed milk used in this way had itself been standardized with a raw milk.

While the recommended 60 minute incubation period will readily detect the presence of as little as 0.1 percent raw product, longer incubation is required for greater sensitivity and to show a small deficiency in holding time whenever a temperature of 145° F. or greater is used since the inactivation or destruction of the enzyme is a function of both the time and temperature treatment.

In testing ice cream, each flavor should be tested individually with nuts and fruits strained out. Fresh pack fruits, raw fruits, or unroasted nut meats may introduce both the enzyme and a phenol-like substance ascertained by heating a portion at a relatively high temperature and comparing its resultant test and control with that of an unheated portion.

#### THE SIMPLIFIED SHORT TEST OR RAPID FIELD TEST

Generally speaking and particularly where laboratory facilities are not avail-

able or where a determination must be made within 10 to 15 minutes, the short test (or rapid field test) using the normal butyl alcohol procedure (2) may be substituted for the laboratory test. Interfering substances are again evaluated by substitution of buffered water for the buffered substrate. Increasing the incubation period from ten to twenty minutes results in increased sensitivity.

The simple, readily mastered technic must be followed very closely, especially at first, and the published precautions strictly adhered to, if accurate results are desired. Solutions should be freshly prepared or stored under refrigeration if they are to be stored 24 hours or longer. Tablets are obtainable to allow frequent and inexpensive preparation of the necessary reagents. An aged substrate solution may decompose with the formation of free phenol and thus give a false positive test, while an aged BQC solution may lose its sensitivity and yield a false negative test. It is strongly recommended that the solutions be prepared daily for greatest accuracy, and that for practical purposes where the requirements for pasteurization are 143° F. for 30 minutes, no sample of milk or cream be classified as improperly pasteurized unless it yield

**Measurement of sanitary ventilation.** W. F. Wells and M. W. Wells. *Amer. J. Pub. Health* 28, Mar., 343 (1938).

During coughing and sneezing, minute droplets containing microorganisms may be ejected into the air. The moisture evaporates, and the solid residues drift in the air currents like particles of cigarette smoke, and may pass from one person to another.

Sanitary ventilation may be defined as the rate at which microorganisms are vented, or as the proportional air replacement which would remove the equivalent number of microorganisms eliminated by any other means.

Methods of measurement consist of atomizing test organisms (*B. coli* is named) into the air, and determining the bacterial concentration in an air centrifuge. Two technics for calculating the ventilation rate are (1) the determination of the rate at which the organisms are removed (details given), and (2) the

an indophenol color greater than the two unit field test standards (2). When the extraction method is properly conducted in a test tube of recommended size (about 10 x 100 mm.) by allowing the bubbles to subside after each inversion before re-inverting the tube, the alcohol will separate in a clear layer at least  $\frac{3}{4}$  inch in length.

Distilled water is recommended for making the substrate solution since tap water may contain interfering amounts of chlorine or phenol.

While such extreme attention to detail seems unusual, it has been found that adverse criticism of the method in most instances has been traceable to the lack of consideration of these and other published precautions.

Results achieved by the New York City Department of Health on more than 100,000 samples, and the experience of many others, agree that, for practical purposes, the short test compares very favorably with the one hour laboratory technique.

#### REFERENCES

1. Scharer, H., *J. Dairy Science* 21, 21 (1938)
2. Scharer, H., *J. Milk Techn.* 1 (5) 35 (1938).
3. Brown, W. H. and Parfitt, E. H., *J. Dairy Science* 21, 149 (1938).

equalization of the rates of removal and addition by which the former becomes known by reason of knowledge of the number of organisms added.

Direct irradiation of the room is a far more effective procedure in special cases (as for example, operating rooms) when the eyes of the occupants can be protected. Photographs of the actual bacterial concentrations with and without exposure to the ultraviolet light show the marked bacterial effect of the light.

Partial irradiation is more generally applicable where the space without the eye zone is directly irradiated, and there is adequate air movement. A light barrier in the form of an ultraviolet light screen can protect a cubicle from invasion by microorganisms or vice versa.

*Digest:* The bacterial content of air in a confined space can be kept very low by controlled ventilation, and by ultraviolet light screens.

J. H. SHRADER.

## The Practical Application of the Phosphatase Test\*

D. M. Roger

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Two or three years ago we should have been extremely doubtful of a statement to the effect that it was possible, by means of a simple test, to detect under-pasteurization of dairy products to the extent of:

- (1) Five minutes short holding.
- (2) A drop of 1 degree in holding temperatures (from 143° F.-142° F.).
- (3) The addition of 0.1 percent of raw milk to efficiently pasteurized milk.

However, work done recently (1, 2, 3, 4, 7) has proved that this is possible. This means that an effective method has been given to us for the control of pasteurized dairy products, probably one of the most valuable tests ever given to the dairy industry. This test is possible because Nature has included in mixed supplies of cows milk (raw) the enzyme phosphatase which has the property of splitting or hydrolyzing certain phosphoric esters. When milk is heated to pasteurizing temperatures the phosphatase is progressively inactivated as the time of holding progresses so that at the end of the holding period the enzyme is almost completely inactivated. A measurement of the activity of phosphatase in pasteurized mixed milk has been shown to be a measurement of the efficiency of pasteurization (1, 2, 3, 4, 7). When milk containing this enzyme is brought in contact with a certain phosphoric ester (di-sodium phenyl phosphate) under certain conditions, it has the ability to split off or liberate free phenol (carbolic acid). The amount of phenol liberated under these conditions is therefore dependent

on the activity of the enzyme. In efficiently pasteurized milk, the phosphatase present will liberate only a certain limited amount of phenol under the same conditions. The test therefore resolves itself into a determination of free phenol. This is achieved by the addition of a reagent which reacts with the liberated phenol producing a blue color.

Efficiently pasteurized milk will show only a slight phosphatase activity, liberate only a little phenol, and produce only a slight blue color with the reagent.

Improperly pasteurized milk will show a greater phosphatase activity, consequently will liberate more phenol, giving a deeper blue color with the reagent.

There are several laboratory tests (1, 2, 3) to determine the activity of phosphatase in milk designated as pasteurized but these tests are too complicated and time consuming for practical plant work where results must be obtained quickly. Fortunately a very simple test, as simple as the Babcock test for butterfat, has been developed which has proved to be quick and surprisingly accurate. This test is known as the Scharer Rapid Field Test. It employs Gibbs reagent (8) for the determination of free phenol. The procedure for Scharer's Rapid Field Test (5) is as follows:

#### THE IMPROVED RAPID FIELD TEST FOR EFFICIENCY OF PASTEURIZATION\*

"The buffered substrate (white) tablet contains the phenyl phosphoric ester, magnesium to catalyze the enzyme reaction and adequate buffer to make 50 cc. of the buffered substrate solution—sufficient for ten tests. The BQC (yellow) tablet contains 2.6 dibromoquinone-chlomoimide and a stabilizer—sufficient for 30 or more tests. These tablets should be kept under refrigeration if possible.

\* Read at the joint session of the International Association of Milk Sanitarians and the International Association of Milk Dealers, Cleveland, Ohio, Oct. 19, 1938.

\* From *J. Milk Technol.* 1 (5) 35 (1938).

The buffered substrate tablets available commercially may develop varying amounts of phenol under certain conditions of storage such as exposure to light or heat. Since the extraction technique is extremely sensitive, it is necessary to work with a phenol-free substrate; therefore the following procedure is utilized to remove any phenol and is recommended in all cases:

Crush buffered substrate tablet in test tube, dissolve in 5 ml. of distilled water. Add 2 drops of BQC solution. Allow five minutes for color development, then extract the indophenol with 2-2.5 ml. of normal butyl alcohol. Allow to stand until alcohol layer has separated at top of tube. Remove alcohol layer with medicine dropper and discard. Dilute remainder of solution to 50 ml. This solution is then phenol free. Dissolve the BQC tablet in 5 ml. of 95% ethyl or methyl alcohol. **DO NOT USE A DENATURED ALCOHOL.** Transfer to dropping bottle delivering 50 drops per ml.

#### METHOD

Add 1/2 ml. of sample to 5 ml. of buffered substrate. Shake briefly. Incubate for ten minutes in a water bath at 98° F. (36.7° C.). (If no water bath be available, incubate in pocket for somewhat longer period.) Remove from bath, add 6 drops of BQC solution. Shake well immediately. After five minutes compare color with opaque standards.

Properly pasteurized milk will be a gray or brown.

Properly pasteurized cream will be a gray or white. Raw milk or cream will be an intense blue. The appearance of any blue is indicative of improper pasteurization; the degree of intensity of color being proportional to the seriousness of the condition.

If only a trace of blue is found it may be hard to distinguish. In this event add 2 ml. of normal butyl alcohol (neutral). Invert the test tube SLOWLY at least ten times and allow to stand. Rapid inversion will result in an emulsion being formed but if correctly performed the alcohol will separate clearly and will have extracted the indophenol formed by the test.

The appearance of any blue or blue green in this alcohol layer is indicative of improper

pasteurization. In the absence of a properly pasteurized milk to be used as a control a boiled milk may be substituted.

The test has been standardized with milk pasteurized—under laboratory conditions satisfying legal requirements—namely, a preheating period of one to five minutes and a holding period of exactly 30 minutes at 143° F. Under commercial conditions varying time of preheating and filling and emptying of tanks should be taken into consideration.

#### CAUTION

All equipment should be thoroughly washed and rinsed before reuse. Avoid the use of phenolic resin bottle closures anywhere in the test. The BQC reagent is sufficiently sensitive to demonstrate the leaching of phenol from the resin by water.

Both solutions decompose with age and should be stored under refrigeration or prepared shortly before use.

A reagent blank should be made by adding 3 drops of BQC to 5 ml. of substrate. If a blue color results the substrate solution should be discarded. If the butyl alcohol procedure is utilized this reagent blank should be extracted with the alcohol.

#### THE TEST IN PLANT PRACTICE

All our pasteurizing plant test rooms are equipped to perform this test as a regular routine procedure. During the past year we have performed more than 18,000 tests by this method or slight modifications of it. No cases have been encountered in our work where interfering substances, in the milk itself, have caused positive tests.

#### SOME CAUSES OF FALSE POSITIVE TESTS

- (1) The equipment used in preparing the sample was contaminated with raw milk.
- (2) The tablets for preparing the test solutions were too old or had not been kept in the ice box.
- (3) One case was found where some one had dipped a pipette, contaminated with raw milk, into the bottle of

distilled water used for making up the substrate.

- (4) Another case was found where some one had contaminated the distilled water with sulphuric acid from a storage battery hydrometer. While this did not cause a positive test it did interfere by retarding the development of the indophenol blue.
- (5) Some reagent bottle closures have been encountered that liberated phenol.

#### SOME CAUSES OF ERRATIC TESTS

- (1) Insufficient mixing of milk with substrate at start of test.
- (2) Insufficient shaking after the (BQC) indicator has been added.
- (3) Not waiting full five minutes after the (BQC) indicator has been added before extracting the color.
- (4) Insufficient shaking when extracting with the alcohol.

Where the test has been used intelligently it has proved to be most reliable.

Most of the work that we have done has involved the use of amyl alcohol\* (boiling range 126 - 132° C.) as a solvent in place of normal butyl alcohol. While the amyl alcohol does not extract quite so much of the indophenol blue, it has the advantage of not extracting so much of the yellow color from high color milk and cream. This yellow color does interfere with tests on slightly un-

der-pasteurized products; however, for most practical purposes butyl alcohol is satisfactory.

Since many of our plant testers work by artificial light it is important to develop a distinct color contrast between a pasteurized milk test and a test on a sample that is slightly under pasteurized. To accomplish this we have equipped our plants with small water bath incubators and have increased the time of incubation from ten to thirty minutes. The data in Table 1 indicates the color gain achieved by this increased incubation:

It will be noted in Table 1 that pasteurized samples show little increase in color due to increased incubation but slightly under pasteurized samples show considerable gain.

The following table shows the results of tests on some samples of "B" Raw Milk pasteurized under laboratory conditions.

#### APPLICATION OF THE TEST

Where discrepancies in pasteurization have occurred, this test has proved invaluable because results may be obtained quickly and the trouble more easily traced. The dramatic effect of being able to produce a blue color from a test on an under pasteurized milk and show it directly to the operator is far more effective than volumes of laboratory reports.

#### SOME CAUSES OF HIGH PHOSPHATASE TESTS

It is generally believed that high phos-

TABLE 1

	Incubation	
	10 Mins.	30 Mins
Milk held 25 mins. at 142° F. ....	B	B
30 " " " " .....	5+	5++
35 " " " " .....	2+	5
25 " " 143° " .....	2+	5+
30 " " " " .....	2-	2
35 " " " " .....	2-	2

\* Different color standards are required when amyl alcohol is used.  
NOTE: The color standards used in Table 1 are those described by Scharer (5). Normal butyl alcohol was used for extraction. B indicates that the blue color was easily detected without extraction. (With experience one is able to pick out even smaller discrepancies without the alcoholic extraction).

phatase tests may be attributed to one or more of three faults:

- (1) Short Holding
- (2) Low Holding Temperatures
- (3) Added Raw Milk or Cream

#### SHORT HOLDING

- (1) A fast recorder clock indicating full holding time but causing milk to be released ahead of time.
- (2) Leaky outlet valves allowing partly pasteurized milk to leak into the pasteurized milk line.
- (3) Deliberate short holding.
- (4) In a multiple holder system where the valves are hand operated it is possible for the operator to become confused and release a holder before the full holding period has elapsed or to run raw milk into a holder that contains pasteurized milk.

#### LOW TEMPERATURE OF PASTEURIZATION (Indicated on Recorder Chart)

- (1) In holders only partly filled, the milk may not retain the temperature.
- (2) Holders cold at the start of the run and the first milk insufficiently heated to compensate for this.
- (3) Holders in exposed positions may lose temperature.
- (4) Holders poorly insulated.
- (5) A layer of low temperature milk may form at the bottom of a poorly insulated or exposed holder, if an agitator is not used. This condition may not show on the recorder if the recorder bulb is set in the holder above this layer, but if the recorder

bulb is set in the outlet pipe this condition will be indicated by a gradual rise of the recorder pen rather than the abrupt verticle rise obtained when all the milk in the holder is uniform in temperature.

#### LOW TEMPERATURE OF PASTEURIZATION (Not Indicated on Recorder Chart)

- (1) Recorders reading high.
- (2) In one case it was found that while the recorder proved to be correct when tested in a pail of water, it gave a reading of 5° F. high when connected in the holder. This was due to the fact that the recorder cable passed through the heating medium (steam) in the jacket surrounding the holder.
- (3) A layer of low temperature milk that will form due to poor insulation and improper agitation may not be recorded if the recorder bulb is in the holder.
- (4) If holders are not equipped with foam heaters or very efficiently insulated covers, the foam is apt to show a very high phosphatase activity.

If pasteurized milk, that is off due to causes No. 3 and No. 4, is mixed in a large storage tank and diluted with efficiently pasteurized milk, it is probable that the milk from the filler will show no high tests, but should the milk level in the storage tank drop so that the milk just runs through the tank without dilution and on into the filler, then intermittent high tests may be expected.

TABLE 2

Time of Holding	Temperature of Holding ± 0.2° F.				
	141° F.	142° F.	143° F.	144° F.	145° F.
20 Minutes	B	B	B	5	2---
25 "	B	B	5+	2	2---
30 "	B	5++	2-	2	2---
35 "	B	5+	2-	2---	2---
40 "	B	5	2---	2---	2---

NOTE: These samples were incubated thirty minutes at 98° F. The same color standards were used as in Table 1. Normal butyl alcohol was used for extraction of this indophenol blue.

#### ADDED RAW MILK

- (1) Defective valves or valves carelessly left open allowing unpasteurized milk to leak into the holders during the holding period.
- (2) Adding raw or improperly pasteurized milk or cream to pasteurized cream to standardize further the fat content.
- (3) Leaks in a milk-to-milk section of the regenerator due to improper pressure (6).
- (4) Contamination after pasteurization by passing the milk through equipment containing, even a little, raw milk.

#### SUMMARY

Some 18,000 tests that we have performed in the field and laboratory during the past year have proved the Scharer Rapid Field Test to be reliable when used intelligently.

The test is simple involving the minimum of equipment.

When this test is used as a routine procedure, the operators appreciate how easily even the slightest inefficiency in pasteurizing may be detected.

#### ACKNOWLEDGMENT

The writer wishes to acknowledge his indebtedness to A. J. Powers, Director of Laboratories, and D. H. Warren, Assistant Director, for many valuable suggestions when reviewing this manuscript;

also those workers whose findings have been drawn upon to compile the list of causes for inefficient pasteurization.

#### REFERENCES

- (1) Kay, H. D. and Graham, W. R. The Phosphatase Test for Pasteurized Milk. *J. Dairy Research* 6, 101-203 (1935).
- (2) Gilcreas, F. W. and Davis, W. S. The Practical Value of the Phosphatase Test in Determining the Efficiency of Pasteurization. *Annual Proc. Internat. Assoc. Milk Dealers*. 34-54 (1937).
- (3) Scharer, H. A Rapid Phosphomonoesterase Test for Control of Dairy Pasteurization. *J. Dairy Science*. 21, (1), 21-34 (1938).
- (4) Storrs, A. B. and Burgwald, L. H. The Phosphatase Test. *J. Milk Technology*, 1 (2) 18-35 (1938).
- (5) Scharer, H. Improvements in the Rapid Phosphatase Test for the Detection of Improper Pasteurization of Milk and Its Products. *J. Milk Technology*, 1 (5) 35-38 (1938).
- (6) Fuchs, A. W. Contamination of Pasteurized Milk by Improper Relative Pressures in Regenerators. *J. Milk Technology*, 1 (5) 9-16 (1938).
- (7) Tracy, P. H. and Hahn, A. J. Determining the Efficiency of Milk Pasteurization. *Annual Proc. Internat. Assoc. Milk Dealers*, Lab. Section. 57-71, (1937).
- (8) Gibbs, H. D. Phenol Tests III. The Indophenol Test. *J. Biol. Chem.* 72, 649 (1927).

An experimental series on the contamination of milk caps and milk truck sacks. R. V. Stone. *The Sanitarian* 1, June, 6 (1938).

Tests, to determine the safety value of the double cap, were made on both of the caps, on the contamination factor present in the common burlap sack used in trucks to cover and cool the milk load, and on the effect of detergents and chlorine compounds to reduce the sack contamination.

The inner cap of double capped bottles averaged per cap 0.1% of the average count on the single caps; or in other words, the latter averaged 929 times the bacterial load of the protected closure. Washing the sacks and treating them with chlorine reduced their contaminating load of organisms about 90%.

*Digest:* Protected milk caps carry only a small bacterial load.

J. H. SHRADER.

## Homogenized Milk\*

C. J. Babcock

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Control officials are frequently confronted with the question as to the merits of new products and how such products should be processed and handled. Strictly speaking, homogenized milk is not a new product. It is, however, new to some localities, and present trends indicate that in the near future it will be found in nearly every milk market of the United States. Reports from dairies from all sections of the United States have contained evidence of very encouraging progress with homogenized milk. Many of these report not only increased sales of homogenized milk but increased *per capita* consumption of milk and milk products. A survey of the milk plants of the United States is being conducted by the Bureau of Dairy Industry. At present 260 plants have responded to the questionnaire. Of these 260 plants, 30 plants in 15 States are featuring homogenized milk. The percentage of the milk handled by these plants which is homogenized ranges from 1/2 of 1 percent to 100 percent. Of these 30 plants, only 7 reported that the sale of homogenized milk is not increasing.

This widespread introduction of homogenized milk and the reports of increased sales are bound to increase the number of milk plants featuring homogenized milk. As our control agencies are also service agencies, it will be necessary for them to be in a position not only to control the processing and handling of homogenized milk, but to act in an advisory capacity regarding its handling and processing. Let us therefore briefly review some of the

defects which frequently occur in homogenized milk and their remedies.

### DEFECTS

Lipase, a fat-splitting enzyme which causes milk to become rancid, is present in all milk. Ordinarily it is not present in sufficient quantity to give trouble in our regular milk supplies. However, unless this enzyme is destroyed either before or immediately after the fat globules are broken up by homogenization, it goes to work on the finely divided fat particles, and we have rancid milk within two hours. For this reason, homogenization cannot be applied to raw milk. Also, in preparing pasteurized homogenized milk, this point must always be kept in mind. We must not allow a lag period of any duration between homogenization and pasteurization, for if we do the flavor of the product deteriorates.

Another source of trouble with homogenized milk are the leucocytes or white corpuscles. These are present in all milk, but, like the enzyme lipase, give no trouble with our regular milk supplies. This is because they are carried up with the fat globules and held in the cream layer. This is clearly shown by the fact that if we take samples from the top, middle, and bottom of bottles of pasteurized milk 3 hours after bottling, we find that the top contains over 6 million leucocytes per ml. as compared to approximately 128 thousand at the middle and at the bottom. After 24 hours, we find nearly 9 million per ml. at the top and approximately 92 thousand at the bottom. In other words, there are millions of leucocytes in the cream layer but nobody can see them so no one is particularly concerned about them.

### CLARIFICATION

What happens when we homogenize milk? We have no rising fat globules to

carry the leucocytes to the top and no cream layer to hold them. The cells, which apparently have a slightly higher specific gravity than the milk, therefore settle to the bottom. The leucocyte counts from the top and bottom of the bottle are the reverse of those found in unhomogenized milk. The result is a ring of greyish, slimy sediment in the bottom of the bottle. If we could select milk with a leucocyte count below 100,000 per ml., we should have no trouble with sediment in homogenized milk. Mixed milk as received at milk plants frequently does not have a leucocyte count this low. As the leucocytes are too small to be removed by strainers or filters, the only known method of preventing sediment in homogenized milk at the present time is by clarification. Clarification removes a high percentage of the leucocytes from the milk and prevents the formation of sediment.

There is some question as to whether clarification should take place before or after homogenization. Some investigators claim that a higher percentage of leucocytes are removed if clarification follows homogenization. Personally, I am in favor of clarifying before homogenizing. Clarification at this time prevents sediment and lessens the lag period between homogenization and pasteurization, which has been mentioned as necessary if we are to insure a product of good flavor. In brief, I consider the proper sequence to follow in the production of homogenized milk to be: Clarification, homogenization, pasteurization. Milk can be homogenized after pasteurization, but unless the homogenization takes place at the pasteurizing temperature we again frequently encounter abnormal flavors. The flavor encountered is not the rancid flavor obtained with raw milk, but usually an oxidized or bitter flavor. However, I do not feel that we should consider homogenization after pasteurization. In the preparation of any milk product, the pasteurizer should come as near the end of the process as possible in order to prevent the contamination of the pasteurized milk.

### HOMOGENIZATION CONDITIONS

Having discussed the necessary steps in the processing of homogenized milk, the next consideration is the temperature and pressure of homogenization. There is no relation between either the temperature or pressure of homogenization and the formation of sediment. This of course, applies only when the pressure is sufficiently great to accomplish our purpose, *i. e.*, a sufficiently complete emulsion to prevent the rising of the fat globules. Roughly speaking, the higher the temperature, the lower the pressure required to obtain the same results. In regard to temperature, it is best to homogenize at or near the pasteurizing temperature. By so doing we guard against the action of lipase and thereby have another safeguard against the development of abnormal flavors. As for the pressure, when using the pasteurizing temperature, there seems to be no advantage in going above 2,000 to 2,500 pounds. This pressure and temperature will give a milk which when placed in a quart bottle for 24 hours will have a fat content at the top of the bottle practically the same as at the bottom of the bottle. Therefore we can safely recommend homogenizing at a pressure of not less than 2,000 pounds at a temperature of approximately 140° F.

Comparing this procedure with what is actually taking place in the industry, the survey revealed that of the 30 plants featuring homogenized milk, 22 homogenized at a pressure of 2,000 pounds or more, and the same number homogenized at a temperature of 140° F. or higher. The homogenizing pressure ranged from 1,000 pounds to 3,500 pounds, and the temperature from 95° to 150° F. Of the 30 plants, 17 were clarifying, 14 before homogenizing and 3 after homogenizing; and 2 were filtering. I am not attempting any long distance trouble shooting, but it is interesting to note that of the 7 plants which stated that the sale of homogenized milk was not increasing, 4 were not using a clarifier and 1 was homogenizing at 1,500 pounds at 95° F.

\* Read at the 27th Annual Meeting of the International Association of Milk Sanitarians, Cleveland, Ohio, October 19-21, 1938.

## SANITATION

In dealing with homogenized milk, it should always be remembered that homogenization is not a process that improves the sanitary quality of milk. Furthermore, it should be remembered that homogenization is a process by which both the flavor and appearance of milk can be easily destroyed. Therefore, the first step in the production of homogenized milk should be to start with a high-quality milk, a milk not only of low bacterial count but a milk of good flavor.

In speaking of bacterial counts, the homogenizer has always been considered as a source of increased counts. This can easily be true. The homogenizer is not the simplest equipment to keep clean, and unless it is kept clean it is a serious source of contamination. It also breaks up the colonies of bacteria in the milk and thereby gives a higher plate count. Those dairies, however, which are handling pasteurized homogenized milk and are taking proper care of the homogenizer and following homogenization by proper pasteurization, report that the bacterial count of the pasteurized homogenized milk has been, on the average, practically the same as that of their pasteurized unhomogenized milk. Such being the case in actual practice, it appears evident that if we meet with high bacterial counts in homogenized milk, the source of the trouble will likely be improper care of the homogenizer, improper pasteurization, or both.

## ADVANTAGES CLAIMED

I shall not take time to discuss the objections to homogenized milk. They are practically all based on the fact that the milk does not have a cream layer. I do, however, wish to mention briefly some of its advantages. Undoubtedly one of its greatest advantages is the even distribution of fat throughout the bottle. This is probably of greater value in milk for schools and restaurants than in the home. This is well illustrated by a survey conducted by Ernest Kelly (5). He found that of milk served to school children in half-pint bottles, an average of 5.6 percent

of the milk was left in the bottle. This in itself would be insignificant but when we consider that this 5.6 percent of the milk represented nearly 16 percent of the fat in the 4-percent milk served, it throws a little different light on the loss.

Another advantage claimed for homogenized milk is improved palatability. Practically all surveys and experiments conducted with homogenized milk have resulted in a preference shown for pasteurized homogenized milk over pasteurized milk. Without going into detail, I might say that these surveys and experiments as a whole have been more decisively in favor of homogenized milk than the one conducted by the author (2) in the Bureau of Dairy Industry, in which 62.1 percent of the opinions showed no prejudice against homogenized milk and the preferences for homogenized milk and unhomogenized milk were practically equal.

The third advantage given for homogenized milk is that it is more digestible. This claim was originally based on the fact that the fat is finely divided and therefore more readily attacked by the digestive juices. It is now known that homogenization, in addition to breaking up the fat globules, softens the curd of milk. Undoubtedly in the future, the claim that homogenized milk is more digestible will be largely based on its soft-curd characteristic.

## SOFT CURD PROPERTIES

The production of soft-curd milk by homogenization is comparatively new. Weisberg, Johnson, and McCollum (10), Wallace (8), and Washburn (9), were among the first to report that homogenization changes the character of the curd of milk. Hill (4) reports, however, that the results of his work do not justify the use of the homogenizer in the production of soft-curd milk. More recent work by Doan and Welch (3), Tracy (7), Theophilus, Hansen, and Spencer (6), Wolman (11), and others, has shown that soft-curd milk can be produced by homogenization. The author, using raw milk with an average curd tension of 50.2

grams, by pasteurizing at 143° F. for 30 minutes and then homogenizing at the pasteurizing temperature at a pressure of 2,500 pounds, reduced the average curd tension to 16.7 grams. It should be mentioned that these curd-tension measurements were made by using an HCl pepsin solution as a coagulant. This coagulant gives slightly lower curd-tension readings than are obtained by using the Hill coagulant. Experimental work, however, indicates that such a coagulant more closely simulates gastric conditions than does Hill's calcium chloride-pepsin coagulant.

## DIGESTIBILITY

Few reports of experimental work have been published regarding the comparative digestibility of homogenized and unhomogenized milk. Wolman (11) reports that adequately homogenized milk makes an excellent foundation for infant feeding. The curds obtained are always much finer than the curds of formulas derived from identical specimens of milk pasteurized but not homogenized. Similar results were obtained by Anthony (1) in regurgitation experiments on homogenized and other milks. Doan and Welch (3) have shown that milk with small soft curds is more quickly digested by gastric juices, and it would appear that a large percentage of the work which has been done on the digestibility of soft-curd milk would also apply to homogenized milk.

It is impossible to measure accurately digestion in vitro because stomach conditions cannot be accurately simulated. Indications of digestibility, however, can be obtained. Without going into detail as to procedure except to state that a N/10-HCl-pepsin solution was used as a digestant, the digestion took place at a pH of approximately 3.5 at a tempera-

ture of 37° C., and the digestion was measured by formol titration, the author obtained the results as shown in Table 1 and graphs 1 and 2. Boiled milk, which has long been recognized as a soft-curd milk, has been included in order to show the similarity between boiled and homogenized milk.

Table 1 and graphs 1 and 2 show that both boiled and pasteurized homogenized milks digest more rapidly than raw milk. This is especially true during the first 15 minutes of digestion, when 76.5 percent and 56.5 percent more digestion took place with the boiled and the pasteurized homogenized milks respectively than with the raw milks from which they were prepared. It should also be noted that while the boiled milk showed a greater percentage increase in digestion during the first 15 minutes than occurred with the pasteurized homogenized milk, the percentage of increase in digestion did not drop off as rapidly for the homogenized milk as it did for the boiled milk. At the end of 2 hours, 15.4 percent more digestion had taken place in the homogenized milk than in the raw milk, whereas only 10.3 percent more digestion took place with the boiled milk than with the raw milk. At the end of 5 hours, digestion was practically the same for the raw and processed milks. These results indicate that both boiled milk and pasteurized homogenized milk are more quickly but not more completely digested than raw milk. Incomplete data indicate that a similar but less pronounced trend takes place when the pasteurized milk is homogenized at 1,000 pounds pressure instead of 2,500 pounds pressure.

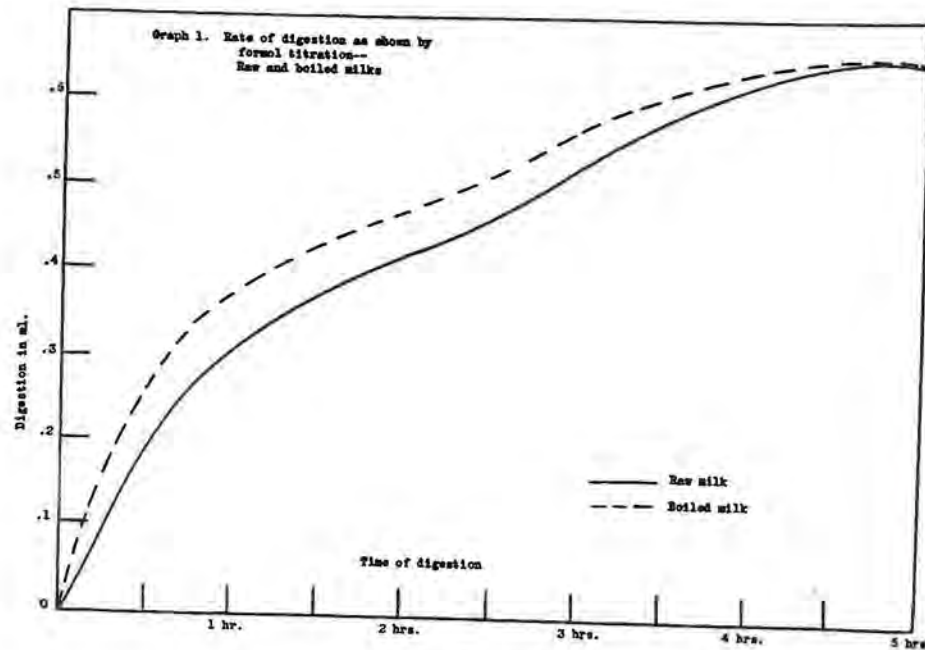
## CONTROL CONSIDERATIONS

This work indicates that control officials may well foster the development and

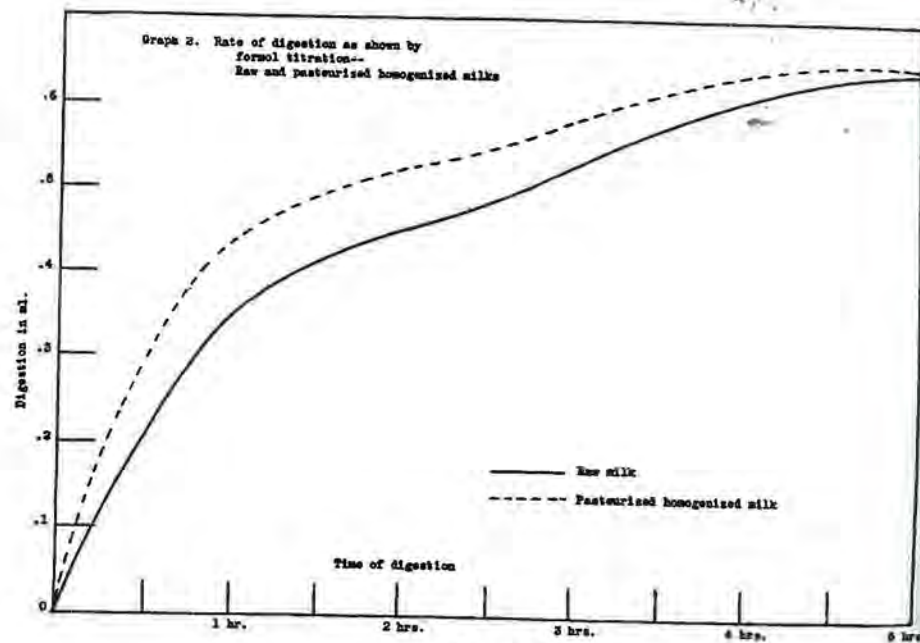
TABLE 1.  
Percentage increase in amount of digestion of milk boiled 5 minutes and pasteurized milk homogenized at 2,500 pounds over raw milk.

Character of milk	Percentage increase over raw milk							
	Digestion time in hours							
	1/4	1/2	3/4	1	2	3	4	5
Boiled .....	76.5	34.8	25.0	17.2	10.3	8.5	1.6	0.4
Homogenized .....	56.5	40.5	30.1	22.2	15.4	9.3	4.9	0.8





GRAPH 1



GRAPH 2

distribution of homogenized milk. The uniform distribution of fat, the soft-curd characteristic, and the apparently quicker or easier digestibility of homogenized milk places it in a position to be of great assistance in the feeding of infants, children, and sick adults. Furthermore, the improved palatability reported for properly prepared homogenized milk may make it an important factor in increasing the *per capita* consumption of milk.

While undoubtedly more research work is needed to fully bring out all the effects of homogenization on milk, we now know how to produce a palatable product. If the product is to become an important factor in the dairy industry and to the health of the nation, we must have standards for homogenized milk. As stated before, from all appearances the dairy industry is going to promote this product. Control officials will find it beneficial to themselves and to the industry if they establish standards while the product is in its infancy. The placing of an improperly processed product on the market is a serious handicap to the product. It is easier for control officials to guide the industry into proper methods than it will be to reform the industry if it should get started on improper methods. I therefore feel that as soon as homogenized milk appears on a market it should be required that it be:

1. Properly labelled.
2. Free from sediment.
3. Adequately homogenized as shown by no apparent rising of the cream upon standing for 48 hours.
4. Pasteurized after homogenization.

Commenting further on these requirements, I should suggest, owing to the fact that we have no cream layer, that the label be permitted to carry the information that the milk contains not less than a certain percent of fat. Regarding adequate homogenization, the time is undoubtedly not far distant when it can be required that this be shown by curd tension measurements. However, until agreement is reached as to the method of determining the curd tension as well as what curd tension should be required, this is impractical. At the present, I favor

determining adequate homogenization according to the definition of homogenized milk suggested by the Technical Committee of the Dairy Industries Supply Association. This definition would require that homogenized milk, upon standing for 48 hours, have a fat content at the top of the bottle not more than 5 percent greater than the fat content of the original milk. Such a requirement would not complicate enforcement work and at the same time would insure adequate homogenization and a low curd tension.

## LITERATURE CITED

- (1) Anthony, G. E. Local study on soft curd milk. *Bulletin of the Genesee County Medical Society* 9 (1936).
- (2) Babcock, C. J. The effect of homogenization on certain characteristics of milk. *U. S. Dept. of Agr. Tech. Bull.* 438 (1934).
- (3) Doan, F. J., and Welch, R. C. Soft-curd milk. *Penna. Agr. Expt. Sta. Tech. Bull.* 312. (1934).
- (4) Hill, R. L. A decade and a half of soft-curd milk studies. *Utah Agr. Expt. Sta. Circ.* 101 (1933).
- (5) Kelly, Ernest. Effect of certain factors on amount of fat consumed in milk served to school children. *Internat. Assoc. Dairy and Milk Inspectors, 21st Ann. Rept.* (1932).
- (6) Theophilus, D. R., Hansen, H. C., and Spencer, M. B. Influence of homogenization on the curd tension of milk. *J. Dairy Sci.* 17, 519-524 (1934).
- (7) Tracy, P. H. Certain problems related to the marketing of homogenized milk. *Milk Dealer* 25, 4, 30-32 (1936).
- (8) Wallace, C. Soft-curd milk. *Amer. J. Diseases of Children* 44, 1143-1144 (1932).
- (9) Washburn, R. M. Soft-curd milk. *Milk Dealer* 21, 3, 46-47, 78-84, 1931 (Dec.).
- (10) Weisberg, S. M., Johnson, A. H., and McCollum, E. V. Laboratory studies on the chemistry of soft-curd milk. *J. Dairy Sci.* 16, 225-247 (1933).
- (11) Wolman, Irving J. Soft curd and homogenized milks. *Proceedings 30th Ann. Convention, Lab. Sec., Internat. Assoc. of Milk Dealers* (1937).

## The Relation of Streptococcic Mastitis to Certain Phases of Milk Sanitation\*

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Mastitis is an inflammation of the udder, one result of which may be the production of abnormal milk. A physical examination of the milk does not always indicate the abnormality for not all affected cows produce abnormal milk; and conversely not all animals giving milk of abnormal appearance have mastitis. On this account both a laboratory examination of the milk and a physical examination of the udder are essential for proper diagnosis.

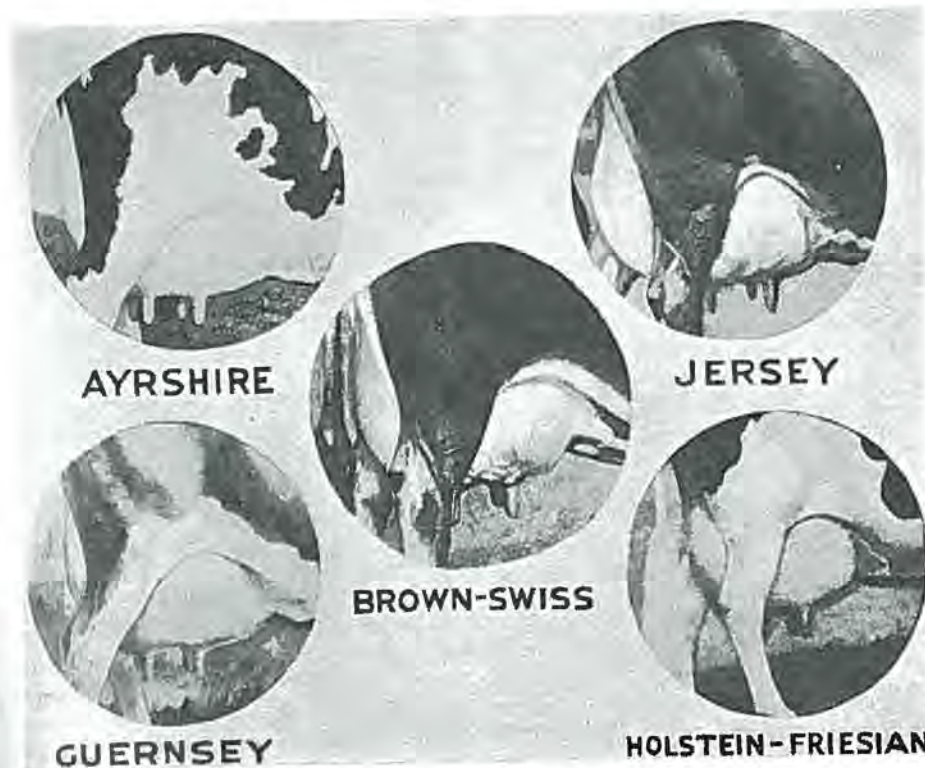
Non-infectious agents such as bruises, injuries of the udder or teat, and chilling may cause mastitis. In some cases, the inflammation subsides, the character of the milk returns to normal as soon as the local condition is corrected, and the injury heals. The non-infectious mastitis, therefore, does not become a herd problem except when streptococcic mastitis is already present in the herd. The injury then permits the easy entry of the bacteria into the udder, resulting in streptococcic mastitis.

The mastitis problem that confronts the average dairy farmer is presented by infectious mastitis. The streptococcus group of bacteria is responsible for approximately 99 percent of the infectious mastitis with *Streptococcus agalactiae* causing about 95 percent of the streptococcic mastitis. The streptococci get into the udder through the teat canal or injury of the udder or teat. Irrespective of whether the streptococci are of human or bovine sources, they are capable of producing the same physical and chemical changes in the milk and bring about the same pathological changes in the udder.

When they are of human origin the streptococcic mastitis becomes extremely important from a public health standpoint.

All cows eliminating the streptococci in their milk must be considered as being infected if a program of prevention and control of mastitis is to be carried out. At first very little alteration may be noticed in the milk or udder, but later definite evidence of changes is encountered. A large number of tests for mastitis have been suggested and used by many people. The value of testing is determined by the accuracy of the test employed, which, in turn, is essential in aiding the dairyman to detect infected cows and to eradicate the disease from his herd. The accuracy of the various tests is indicated in Table 1. It is obvious from these data why the microscopic test of a properly collected and handled milk sample, and a physical examination of the udder, are essential in determining the status of each cow in the herd.

The laboratory or microscopic test should be applied to composite milk samples from each lactating cow in the producing herd to determine the status of each cow. If a rapid survey is to be made of the mastitis situation of a milk shed or dairy to determine the presence or absence of streptococcic mastitis in any herd, the microscopic test may be applied to producer composite milk samples. This procedure is efficient in detecting herds with the infection where the ratio is such that at least one of eighteen cows has streptococcic mastitis. The physical examination of each cow's udder is important from the standpoint of determining the extent of pathological



Photograph—Courtesy Dr. C. W. Turner, University of Missouri

FIGURE 1.

*This illustrates the udders idealized by the various breed associations. It is evident that each breed has its own specific type of udder, but in general all are of good size, symmetrical or balanced, have squarely placed teats of medium size, with the udder well attached below and in the rear. These characteristics indicate the conformation of the ideal udder and should serve as a guide in the selection of cows for dairy purposes.*

change present in the udder. The presence of extensive pathological changes is sufficient evidence to recommend that a cow be removed from the herd for slaughter.

### SIGNIFICANCE OF INFECTION

The economic significance of any dairy cattle disease is in part determined by its incidence among the dairy cattle. Upon checking 2715 cows and 322 herds of a typical milk shed, employing the microscopic test and making a physical examination of each cow's udder, 86 percent of all herds were found to have streptococcic mastitis, and 26.2 percent of all milking

cows were infected with streptococcic mastitis. (Table 2). The incidence of infection was not influenced by the size of the herds, for some large and some small herds were found to have a high percentage of cows infected, and others had a low incidence of infection. The employment of sanitary procedures is essential in a program of mastitis control, and at the same time serve the purpose of aiding in the production of a clean milk of higher quality.

The presence of mastitis streptococci in the udder is not a normal condition; therefore, one might expect variations in the composition of milk as a result of

\* Read before the Twenty-seventh Annual Meeting of the International Association of Milk Sanitarians, Cleveland, Ohio, October 19-21, 1938.



FIGURE 2.

It is not necessary that a dairy cow have an udder that measures up to the ideal for the breed to be classed a normal udder. The above cow has a good normal udder. It is possible to find many udders like this one, and for profitable milk production it is advisable that the cows have normal udders. In addition, notice should be made that the good physical condition of this cow is indicative of her general appearance as a comparison will be made with cows in the advanced stages of mastitis. See Figure 4.

TABLE 1.

The efficiency of various tests in detecting streptococcic mastitis  
(Milk samples must be properly collected)

	Positive cows	Negative cows
	giving positive reaction Percent	giving positive reaction Percent
1. Microscopic test .....	99+	0
2. Blood agar plate .....	90	0
3. Chloride content .....	73	10
4. Physical exam. of udder .....	61	10
5. Hotis test—48 hour reading.....	62.2	12 (79% = suspicious)
24 hour reading .....	52.8	7.2 (72% = suspicious)
6. Leucocyte content .....	50	0.7
7. Thymol test .....	47.8	4.0
8. Physical exam. of milk.....	20	—

The microscopic test is based upon the continued presence of streptococci of more than six elements per chain in the milk of infected cows, and the absence of such streptococci from the milk of non-infected cows. The milk samples must be properly collected and incubated at 37° C. for at least 12 hours prior to microscopic examination for the presence of streptococci. When streptococci are present in the milk, the animal must be considered as being infected if a program of control of streptococcic mastitis is to be carried out, even though the milk and udder appear normal upon physical examination. In some cases the abnormality upon physical examination develops within a few days or weeks while in other cows the abnormalities may not be evident for a year or more but always do occur. The other tests were evaluated using the results of the microscopic test as the standard.



FIGURE 3

Streptococcus infection of the udder injures the secreting tissue, which is then replaced by scar tissue. This replacement is progressive and finally results in altering the appearance of the udder. An unevenness of the udder may develop because of the swelling of one or more quarters or the decrease in size of one or more quarters or both. The changes found in the udder do not indicate the length of time that a cow has been infected; in some cases scar tissue forms rapidly while in others it forms slowly. This udder is affected with chronic mastitis and is characterized by an enlargement of the left front quarter, together with decrease in size of the left rear quarter. Scar tissue is present in both quarters, and streptococci are being eliminated in the milk. The milk producing ability of such an udder is greatly reduced.

TABLE 2.

Summary of the total number of cows tested, percentage of streptococcic mastitis infection among cows tested, and the physical examination of the udders.

	Total Tested	Total Not Infected	Total Infected	Percentage of Infection	Total Udders Indurated	Classification of Indurated Udders by Microscopic Test		
						Infected	Suspicious	Not Infected
Herds .....	322	45	277	86	610	491	10	109
Cows .....	2715	2003	712	26.2				

interference with its normal function. The changes in milk from mastitis affected cows are presented in Table 3, and are important from the stand-point of milk sanitation. The consumer determines the desirability of a product largely by flavor and appearance. A comparison of flavor and quality of milk from non-infected and from the infected cows of the same herd, and of cows from a herd that has been

mastitis free for three years, is made in Table 4.

Competent milk judges are beginning to associate a salty flavor of milk with mastitis. Investigations covering a period of time during which the milk of infected and non-infected cows was scored, revealed that samples from infected cows were criticized as being salty in 62.7 percent of the cases, samples from the non-

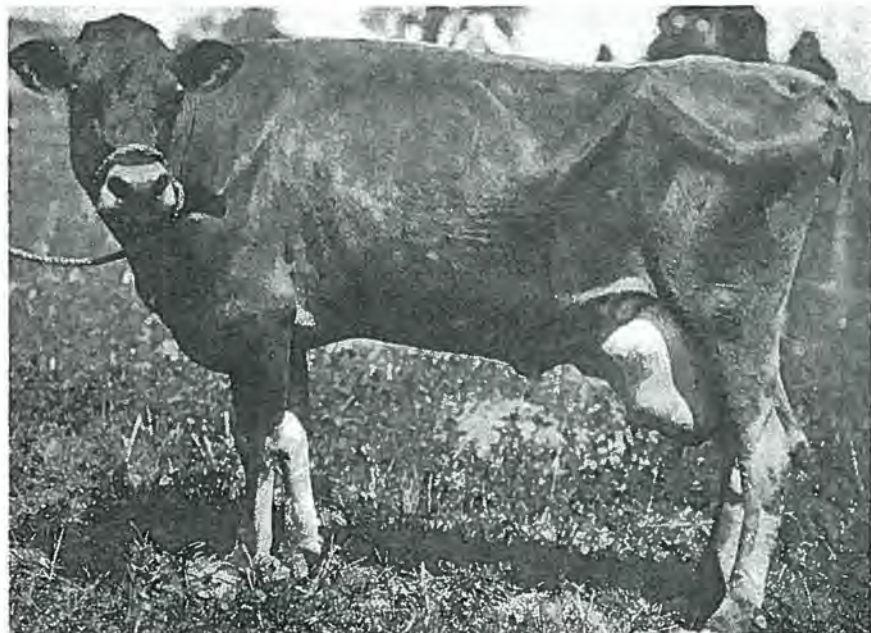


FIGURE 4.

The streptococcus infection may become sufficiently active to break through the outside udder tissues. The open sores become drains for the pus and bacteria found within the infected udder. The cow pictured in Figure 4 was such a case. In this case the open sores were on the opposite side of the udder from that shown in the picture. This not only makes the udder unsuitable for the production of milk and aids in the further spread of the infection in the herd, but the mastitis may change the general physical condition of the cow. The slaughter value of such an animal is greatly decreased, and she usually is sold at a great loss as compared to her value prior to suffering with mastitis.

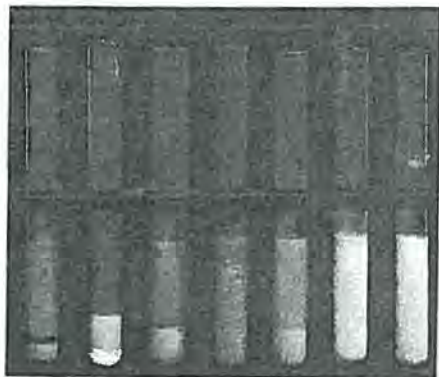


FIGURE 5.

The physical appearance of the milk secreted is not an accurate indicator of the presence or absence of mastitis infection. If abnormal milk, such as is illustrated here, is secreted and an injury of the udder is not responsible, it suggests streptococcal mastitis infection. All of these milk samples, including the normal appearing milk on the extreme right, were obtained from infected cows. This normal appearing milk contained many streptococci and pus cells. It is this abnormal milk that should interest the dairyman sufficiently to check more accurately on the herd condition. A physical examination of the udder and laboratory examination of the milk from each cow should be made as a basis for a proper diagnosis.

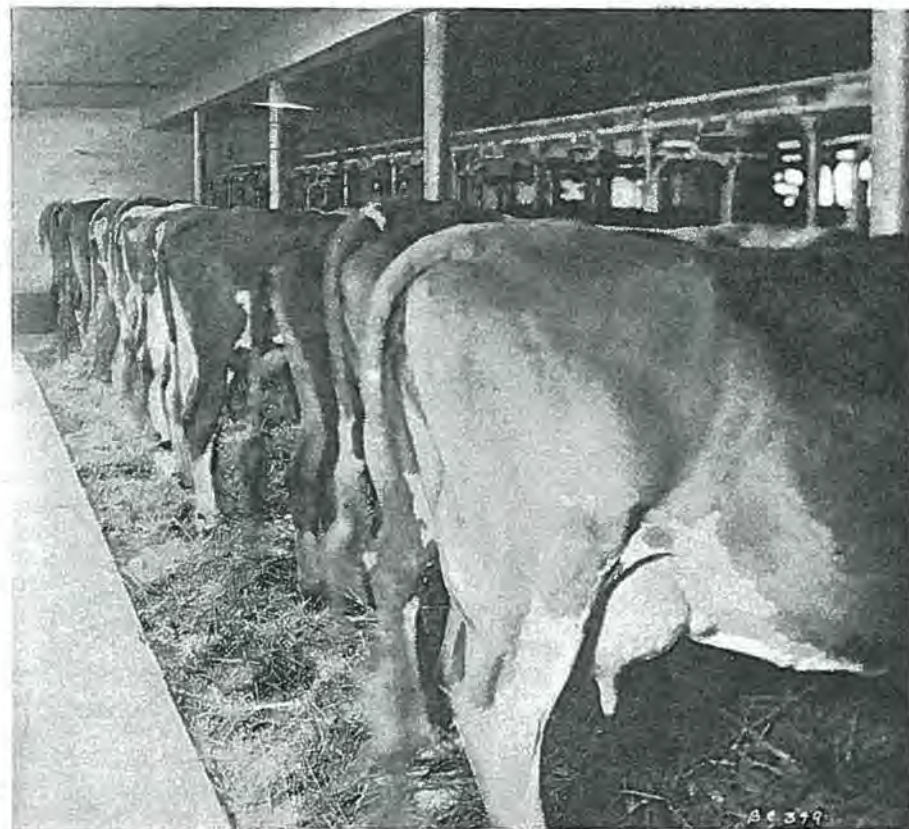


FIGURE 6.

The application of sanitary procedures in handling the dairy herd are valuable in the control of streptococcal mastitis within the herd or in keeping the herd free of streptococcal mastitis if it is free of the disease, and at the same time aid in the production of a clean milk of high quality.

infected cows present in infected herds were so criticized in 22.4 percent of the cases, and only 14.8 percent of the samples from a negative herd were called salty. The cows in the negative herd that gave a salty milk were near the end of their lactation period. These data indicate that not all samples from infected cows were criticized as being salty, but the majority of them were detected as having a salty flavor. Factors, such as stage of lactation, milk yield, and extent of infection undoubtedly have a marked influence on the salty flavor of milk. The intensity of flavor varies directly with the percentage content of chlorides in the milk. These data reveal

the desirability of having mastitis free cows if high quality milk is to be produced.

#### LABORATORY TESTS

The milk sanitarian makes frequent use of certain standard tests and procedures to determine the quality of milk in his territory. Data dealing with the influence of udder infection upon the results of the methylene-blue test, standard plate count, leucocyte count, chloride determination and thymol test, are presented in Table 5. It is significant that 98.5 percent of the milk samples from non-infected cows were of class 1 methylene-blue, with 80.5 percent of those

from cows with only *Brucella abortus* infection of the udder, and only 52.6 percent of the samples from cows with streptococcal mastitis were of the same class, with the remaining samples in classes 2, 3, or 4. In herd composite samples, the presence of as little as 10

TABLE 3

A comparison of the composition of normal and milk from mastitis affected cows as reported by numerous investigators.

Properties	Changes in milk from mastitis affected cows	
	Increase	Decrease
Water	X	.....
Fat	.....	X
Solids not fat	.....	X
Casein	.....	X
Albumin	X	.....
Globulin	X	.....
Lactose	.....	X
Lecithin as percent of fat	X	.....
Ash	.....	X
Chlorides	X	.....
Titratable acidity	.....	X
Acidity (pH)	Above 6.8	.....
Rennet coagulation (time)	X	.....
Catalase	X	.....
Leucocytes	X	.....
Bacteria	X	.....
Sediment	X	.....
Pathogenic streptococci	Present	Absent
Viscosity	.....	X
Cream layer	.....	X
Quantity of milk	.....	X

Taken from report of the Committee on Dairy Farm Methods in *J. Milk Tech.* 1 (3) 51 (1938.)

percent of milk from cows with streptococcal mastitis was sufficient to reduce the methylene-blue quality of the total from class 1 to either class 2, 3, or 4.

The resazurin test is even more sensitive than the methylene-blue reduction test in detecting a reduced quality of milk following udder infection.

The standard plate count demonstrates the same decrease in quality of milk in the presence of udder infection. Since only 5.8 percent of the negative cows have a bacterial count of more than 1000 per cubic centimeter of milk, while 23.4 percent with *Br. abortus* and 68.5 percent of the streptococcus infected cows gave milk with a high count, it is evident that disease free cows produce a milk of higher quality in addition to a safe milk.

The leucocyte count of the milk, chloride determination, and thymol tests are frequently used as rapid tests to check for udder infection, but their results must not be taken as final in this respect, since they detect only 50 percent, 73 percent and 47.8 percent (in the above order of tests) of the infected cows and indicate false positives in 0.7 percent, 10 percent and 4 percent of the cases. These false positive reactions were obtained in the milk of mastitis free cows whose milk was slightly altered in composition with respect to that usually considered as normal milk.

TABLE 4.

A comparison of flavor quality of milk from non-infected and that from infected cows of the same herd and of cows from a non-infected herd, negative for three years (Herd C)

	Non-infected Cows				Herd A	Infected Cows			
	No. of samples scoring		Ave. score*	No. criticized "salty"		No. of samples scoring		Ave. score	No. criticized "salty"
	18-20	21-25				18-20	21-25		
Herd A (11-12 cows; checked 4 wks.)	29	38	20.67	17	Herd A (8 cows)	31	17	19.54	27
Herd B (11-13 cows; checked 4 wks.)	31	76	21.17	22	Herd B (1-3 cows)	11	0	18.1	10
Total samples	60	114	—	39		42	17	—	37
Percent total	34.4	65.6	20.92	22.4		71	29	18.82	62.7

\*Perfect score is 25.  
Herd A was sampled once a week.  
Herd B was sampled twice a week.  
Organoleptic tests were made by Dr. Trout of Michigan State College.

TABLE 5.

Results of examination of milk produced by cows with udder infection and with no udder infection. The data give the percentages of samples occurring in the various classes of the tests employed.

Methylene-blue class	Cow composite samples				Bacteria per cc.			Leucocytes per cc.			Quarter samples*						
	1	2	3	4	Less than 100	100 to 200	200 to 500	500 to 1000	More than 1000	Less than 500 T	500 to 1,000 T	More than 1,000 T	500 T	Less than 0.000 000	More than 0.000 000	Thymol	
																	Chlorides
None	98.5	1.5	0	0	14.4	7.7	26.3	45.8	5.8	95.0	4.3	0.7	90.0	10.0	88.4	7.6	4.0
<i>Brucella</i> only	80.5	14.0	3.4	2.0	15.4	28.5	19.8	12.9	23.4	86.7	11.0	2.3	79.8	20.2	74.5	6.9	19.8
Streptococcal only	52.6	28.0	15.9	3.5	0	0	7.0	24.5	68.5	23.0	27.0	50.0	27.0	73.0	34.5	17.4	47.8

\*If udder is abnormal in any quarter, cow is abnormal.  
(Editor—Thymol is a commercial name for Brom-thymol-blue.)

LANSING CONTROL

The Milk Ordinance of Lansing, Michigan, requires that cows producing grade A, raw milk, be free of tuberculosis, Bang's disease, and streptococcal mastitis. The ruling, including streptococcal mas-

titis, was passed by the Board of Health in April, 1935, and was followed by an educational program rather than strict enforcement. The status of the herds involved can be noted in Table 6. The educational period covering six months in most instances and several years in a very few cases gave the herd owners time to work out their own problems in obtaining herds free of streptococcal mastitis. The dairies concerned cooperated with the City Health Department, and as a result the City of Lansing is unique in that less than 2 percent of its milk supply is raw milk and all of the raw milk is Grade A, produced by cows free of tuberculosis, Bang's disease, and streptococcal mastitis.

SUMMARY

Streptococcal mastitis is very important in a program of milk sanitation, causing approximately a 50 percent reduction in

TABLE 6.

The number of infected and non-infected cows in each herd when the Board of Health ruling went into effect, and 6 months later.

Dairy	Number of milking cows			
	April, 1935		October, 1935	
	Infected	Not infected	Infected	Not infected
1	0	17	0	20
2	1	5	0	6
3	4	14	0	15
4	1	7	0	6
5	16	10	0	13
6	22	12	0	22
7	6	6	0	12
8	3	9	0	15
9	1	6	0	7
10	6	5	0	14
11	10	4	0	11
12	1	8	0	9
13	24	34	0	32
14	17	7	16	16
15	4	0	1	2
16	4	3	3	4
17	9	6	(Is pasteurizing now)	
18	20	0	(Is pasteurizing now)	
19	(Discontinued selling before the herd was tested)			
20	2	11	0	13*
Total	151	165	20	217

\*Discontinued selling milk.

quality as determined by chemical, bacteriological, and organoleptic tests.

The application of sanitary procedures in handling the dairy herd with the view of controlling streptococcal mastitis has additional virtue since the milk produced will be of higher quality than when such procedures are not practiced.

A number of states have made great

strides forward by requiring a physical examination of the udder of each cow that is to produce milk for sale. The city of Lansing, Michigan, has taken one more step, and requires that cows producing Grade A raw milk must be free of streptococcal mastitis in addition to being free of tuberculosis and Bang's disease.

**Permanency of the stainless steels.** V. N. Krivobok. *Metal Progress* 34, Sept., 223 (1938).

No alloy exists which is equally resistant to any and all corrosive media and under any and all conditions. The permanency of the stainless steels is contingent on proper construction and care in service. Microstructural stability of the chromium-nickel steels is enhanced by the addition of columbium or titanium. These diminish the corrosion induced by carbide precipitation. The addition of molybdenum has been found to reduce the pinhole type of corrosion. Such an alloy has the following composition: carbon 0.12% max., chromium 16—20%, nickel 8—14%, molybdenum 2.5—3.5%.

Pinhole corrosion is induced or accelerated by the severe straining of the metal, such as a deep scratch or the cold shearing of the edges, but besides this, the reason for this local selective attack is not known. The mechanical properties of the molybdenum steels will vary with the relative amounts of austenitic and ferritic phases present. When the nickel runs 12-14% and the chromium about 17%, the formation of ferrite is suppressed, and the alloy is austenitic throughout. Generally, these steels are not as pliable and ductile as the corresponding alloys without molybdenum. As in the Cr-Ni-Fe stainless steels, the austenite of alloys with molybdenum is not a stable phase in the true sense of phase equilibrium but undergoes

change with temperature. Short time heating (as in welding) may or may not cause carbide precipitation, depending on composition, thickness, and other factors.

The addition of columbium to molybdenum-bearing alloys is receiving attention. The use of silicon imparts resistance to high temperature (1650° F.) oxidation. Manganese is being substituted for nickel in those countries where the latter metal is scarce. The inevitable small changes in temperature or in concentration of corrosive agent will not change the alloy from "passive" to "active."

The tests in common use for checking on the processing of the metal and methods of construction are: (1) boiling in concentrated nitric acid for 48 hours, calculating as weight loss, and reporting as inches of penetration in unit of time; (2) boiling in solution of copper sulphate and sulphuric acid under reflux condenser to detect precipitated carbides, often in the welded material; (3) immersing in 10% nitric and 4% hydrofluoric acids at 140° F. or boiling, to evaluate the properties of the welded metal; (4) immersing in ferric chloride solutions (at times modified by the addition of sodium chloride) to judge resistance to pinhole corrosion; and (5) salt spraying for general corrosion resistance.

*Digest:* Permanency of the microstructural stability of stainless steel is only relative, and can be enhanced by addition of other metals and by careful use.

J. H. SHRADER.

## The Pouring Lip and Cap As Sanitary Factors in Bottled Milk

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Doctor Harvey D. Thatcher of Potsdam, New York, in 1884 introduced the glass milk bottle. The disc cap was introduced in 1889. Both the bottle and disc are used now with little modifications since they were first put into commercial use.

It is necessary to standardize technical procedures in order to do sound scientific work. The methods, technic, etc. must be of such a nature that each step can be duplicated and repeated in any laboratory. This is a well-known and universally recognized method in scientific work. When we began our studies it was necessary to first determine the area of the glass bottle which should be covered by the closure in order to prevent contamination of the pouring lip. We constructed a simple apparatus for pouring a series of six bottles of milk at the same time under identical conditions. This allowed us to compare the behavior of fresh, fluid milk poured out of bottles with six different types of finishes or dressings.

### LIP CONTAMINATION

It became apparent very early in our work that we were dealing with a problem in physics. We used fresh fluid Grade A pasteurized milk in all of our studies. The cohesive forces of such a standard fluid could therefore be considered as a constant. The adhesive forces of the glass surface over which the milk was poured was the principal variant when the pouring technic was uniform for all bottles under all conditions. We soon observed that a smooth glass surface exerted considerable adhesive pull so that a film of milk spread over this surface,

thereby increasing the size of the pouring lip.

The results of this study have been reported (Arnold, 1938)\*. The use of a standard pouring apparatus allows one to determine the behavior of milk poured from glass bottles of different designs. The area to be covered by a closure for various types of bottles can readily be determined by this method.

The experiments and discussion presented in this paper are an extension of the above mentioned article, and deal to a great extent with the principle to be considered in the sanitary closure of glass milk bottles.

The following experiment was carried out to determine the influence of the dripping of the milk down the side of the bottle upon contaminating subsequent samples poured from the same bottle. Quart bottles with common sense finishes were sterilized and filled with fresh fluid Grade A pasteurized milk. The external surface of the bottle 1½ inches below the top rim were seeded with a twenty-four hour old culture of *B. prodigiosus* for a distance of two inches around the circumference of the bottle. The culture was placed on this wide zone by means of a sterile cotton swab. One hour was allowed to elapse to allow the culture fluid to evaporate. Hand pours were done in the following manner. One glass of milk was poured and discarded. Two successive pours were made into sterile glasses. Five cc. of milk were removed from each glass and divided

\*Arnold, Lloyd: A Glass Milk Bottle with Narrow Pouring Lip and Minimum Drip. *J. Milk Technol.* 1, 6, 5-14 (Sept., 1938).

between two agar pour plates. One hundred and eighty bottles were poured in this manner. Thirty-eight of the second and third pours were contaminated with *B. prodigiosus*. Twenty-one percent indicated the drip returned to the top of the bottle and was transferred to the glass of milk during pouring.

We could not repeat this experiment with bottles types 14, 21 and 31 (Arnold, 1938) because these dressings do not allow dripping of the milk.

#### CAP CLOSURES

There seems to be considerable confusion regarding the closing of milk bottles. There is little unbiased scientific information available. The disc or plug cap is used to close the bottle. These discs do not properly protect the milk against contamination and adulteration during the average handling procedures in dairy practice. The pouring lip has no protection when a disc cap alone is used. Various other devices are used to augment or replace the disc cap. These are composed of paper, metal, cellophane, and other materials. Bottle finishes have been designed to receive certain types of caps.

There are a few basic factors that should be considered in connection with the closures of milk bottles. The disc or plug cap has been used for many years and its form, consistency, and construction have been standardized. This cap serves to hold the milk in the bottle. In order to facilitate easy removal of the disc from the bottle, wire holders which perforate the disc and paper tabs which are a part of the disc are extensively used. The primary purpose of the disc cap is to make the bottle liquid tight. The disc cap can be removed and replaced with ease. This serves a useful purpose in the home if the bottle is not emptied immediately after opening. The hood or external closure is for the purpose of protecting the pouring lip and the disc from contamination. The disc cap used at the present time is porous. The material forming the external closure or hood

should be as near sterile as possible; it should be non-absorbent, impermeable, and inert. It would be desirable to have disc caps conform to the same high sanitary standards.

#### SANITARY CAP REQUIREMENTS

We think, after several months of study, that the closure of a glass bottle of milk should meet the following general sanitary standards:

*First*—The closure should cover and protect that portion of the external surface of the bottle in contact with the milk during pouring or what is usually called the pouring lip.

Until a better method has been developed, the Standard Pouring Machine as described in the Journal of Milk Technology, *loc. cit.*, can be used to determine the area designated as the pouring lip.

One practical test for the efficiency of the closure of a bottle of milk is to smear the glass just below the closure with a *B. prodigiosus* broth culture. Remove the closure and pour the milk. Several glasses of milk should be poured in succession, and 10 cc. of milk from each glass put into sterile test tubes and incubated for forty-eight hours. The red color developing indicates the milk contacted the zone below the closure.

*Second*—The closure of the bottle should be so constructed and formed that it cannot be removed and replaced by hand without detection.

An absolute tamper-proof closure would be a burglar-proof closure. This is obviously impractical. A common-sense interpretation must be placed upon this rule.

*Third*—A closure of a bottle should be accomplished by means of a mechanical automatic machine in the dairy.

Hand operations increase the hazards of contamination. Hand capping devices do not yield uniform results due to variations in pressure, time, etc. of the hand power used in this operation. Milk bottle closures applied by hand are not tamper-proof. The mechanical application in the dairy of the closure insures

uniformity of operation and safety of the fluid milk contents of the bottle.

*Fourth*—The material forming the closure of the bottle should have smooth, inert, and non-absorbent surfaces.

Porous materials allow milk to permeate and to be absorbed. Porosity cannot be controlled. Chemical substances and bacterial contamination can be transmitted to milk when it is in contact with permeable and porous surfaces. The safe public health procedure is to use only non-absorbent materials as closures for bottles of milk.

*Fifth*—The closure shall maintain its form and seal under variations in temperature ordinarily encountered in dairy practices.

Milk should be protected against contamination due to defective closures produced by hot or cold weather conditions. Extremes in weather are unusual, but average temperature changes should not expose the milk to contamination.

*Sixth*—The closure should not be broken or punctured by the use of ice in maintaining the proper temperature of bottled milk.

It is necessary to keep milk at a low temperature. Several methods are now employed. Direct icing of closed bottles of milk is common practice. Contamination of the milk would occur if the closure were punctured or loosened by such a refrigerating method.

*Seventh*—The material forming the closure of the bottle should be of such a sanitary quality that no bacteria, chemicals, extractives, etc., can be transferred to the milk.

The purpose of this closure is to protect the milk against contamination and adulteration. Such a closure should be free of viable bacteria, non-absorbent, impermeable and inert.

*Eighth*—It would be advantageous and desirable in the interest of protecting the

health of the public if in addition to the above suggested standards, the following could be considered:

- a) It would be desirable to have the disc cap as impermeable, inert, and sterile as the hood or external closure.
- b) It would also be desirable to retain a protective covering over the opening of the bottle of milk after a portion of the contents have been removed.
- c) It would be desirable that the closure, when removed, leave the smooth glass surface of the bottle clean with no adhering substances.

#### PROJECTED STUDIES

Fifteen different closures for milk bottles have been studied. This work is still in progress. The manufacturers of closures for milk bottles are resourceful and competent; they are developing and perfecting new closures. One of the purposes of this study was to clarify this field and indicate desirable future trends in the sanitary closing of milk bottles.

The absence of standards to determine the area of glass to be covered by a closure, and confusion as to the fundamental purposes of the closure have retarded progress. There has been a tendency to alter the finish of the glass bottle so as to be able to close it with a cap as economically as possible, disregarding several essential sanitary features. It should be kept in mind that there are certain minimum sanitary standards; these cannot and should not be sacrificed for economy of closure. The closure should protect the pouring lip. A smaller closure may be more economical, but the hazard to the health of the consumer offsets any pecuniary advantages. The problem resolves itself into two parts, namely: (1) the development of a finish of glass milk bottles so as to reduce the area to be covered by a closure to the minimum, that is a narrow pouring lip with no drip, and (2) the covering of this area with a sanitary closure which will safeguard the contents.

## Summary of the Report of the Chief of the Bureau of Dairy Industry, 1938\*

### INTRODUCTION

Nearly 40 percent of the whole milk produced in the United States is made into butter and cheese. Of the nearly 46 billion pounds of skim milk available for processing annually, about 38 billion is used as livestock feed, less than 7 billion is used for human food, and about 1 billion for making about 35 million pounds of casein.

The Bureau has worked out methods for packaging skim milk powder in small consumer-size units for home cooking.

A plant is now in active production of lactic acid by fermenting the milk sugar of the whey from casein manufacture. Recently the Bureau has found it possible to make a resin from lactic acid. This promises to be useful in making varnishes and lacquers that are resistant to alcohol and water, and that adhere to metals. Experiments are now under way to convert lactic acid into acrylic acid or acrylic esters, used in making plastic glass.

The Bureau's new process in the utilization of casein whey recovers the milk sugar somewhat more cheaply than formerly by making available the soluble albumin (useful in infant foods) and a residue rich in flavins (used by feed manufacturers). A new process enables the smaller cheese factories to concentrate and preserve whey for sale to feed manufacturers and confectioners.

The production of butterfat in the Bureau's experimental herd, over the past twenty years has increased by 52 pounds per cow per year (678 to 730 pounds) in the Holstein-Friesian herd, and by 40 pounds (634 to 674) in the Jersey herd, by the use of sires of known transmitting ability.

\* Report of the Chief of the Bureau of Dairy Industry, 1938. U. S. Department of Agriculture.

### DAIRY HERD IMPROVEMENT

The number of associations increased 11.5 percent during the year 1937, reaching a total of 1,106, and these also showed an increase in association members. During 1937, the average butterfat production of cows on test in dairy herd improvement associations was 320 pounds whereas that of all cows in the United States was estimated to be only 170 pounds. In the last ten years, the average yearly butterfat production of associations has increased about 30 pounds per cow. Copies of proved sire records for 1068 sires are available for local agents and cow testers.

The earlier program of the herd improvement associations has been expanded from providing feed and production records on individual cows to include a progressive breeding program:

### BREEDING, FEEDING, AND MANAGEMENT INVESTIGATIONS

Projects have been continued to develop pure strains for high level of production through the continuous use of unrelated proved sires.

On the average, 29.2 and 29.0 percent of the total milk yield was obtained from the left and right rear quarters, respectively, and 20.2 and 21.6 percent from the respective left and right front quarters. Specimens of udders have been classified and mounted on panels for display.

### DIVISION OF NUTRITION AND PHYSIOLOGY

Carotene. It has been determined that cows in the experimental herd required 86 milligrams or more of carotene daily to produce strong and normal calves. Deficiencies can be supplied by cod-liver oil and equally well by feeding 5 pounds of carrots daily when not milking and 20 pounds when milking. The addition of

bonemeal to a low-calcium timothy hay ration has not shown any beneficial effect on growth. Methods are being studied to determine the carotene in cattle feeds. Whereas roughages were formerly regarded as necessary for their indigestible material, they now are recognized as also valuable for their contents of minerals and vitamins.

Discrepancies between the spectrophotometric and biological determination of the vitamin A potency of butter have led to studies which reveal the presence of several pigments in butter.

### DIVISION OF DAIRY RESEARCH LABORATORIES

Oxidized flavor. Extensive data show that the oxidized or metallic flavor of milk is not due to enzymic action but to an oxidation which occurs only when certain conditions in the milk are favorable. The most important factor to cause this is an inorganic catalyzer, particularly salts of copper. No particular feed is involved except that the difficulty is much more likely to occur in milk from cows on dry feed.

Mastitis. An investigation into certain phases of mastitis makes it possible to trace the development of the infection. In some cows, before any significant evidence of bacterial invasion occurs, there are indications of an inflammatory condition of the udder, particularly an increase in leucocytes and chlorides in the milk. In other cows, typical mastitis streptococci are present for considerable time before other significant changes occur in the milk, or before a definite infection of the udder takes place. More or less coincident with a chlorides content of over 0.12 percent, there occurs a marked increase in the numbers of leucocytes and infecting organisms, and a marked loss in the rennet-coagulating property of the milk. As the disease progresses to its peak, the pH value of the milk is markedly changed and there is further loss of rennet coagulability. Following the peak of the disease, the numbers of infecting organisms and the

percentage of chlorides are reduced, but there is a continued abnormality in pH value and in number of leucocytes until a marked trend toward recovery is evident.

These changes may be produced, almost at will, by increasing the severity of machine milking; that is, by increasing the vacuum and extending the time of operation of the machine. The inference may be drawn that one of the conditions essential to the development of mastitis is an injury to the udder that eventually lowers the resistance until the invasion of the udder tissue by bacteria is possible. Attempts to induce mastitis when the udder has not been injured usually fail. The increase in chlorides in the milk may be taken as evidence of an injury to the udder tissue which permits the infiltration of blood serum into the milk.

Germicidal property of milk. The germicidal property of the milk from different udder quarters affected by mastitis varies with the quarter, and tends to be correlated with the number of leucocytes. This germicidal property increases with the severity of the infection until a general breakdown in resistance occurs.

Casein fiber. Research on the casein-fiber project have been directed to finding substances which will increase the strength and water resistance of the fibers without sacrificing flexibility. Nine applications for patents, have been filed.

Constituents of whey. The alcoholic extraction of the various constituents of whey has been successful on a laboratory scale, and pilot-plant equipment is now being assembled. The process essentially involves the extraction of whey powder or concentrated whey with hot alcohol. The insoluble residue removed by filtration consists of water-soluble protein-rich material, practically free from lactose and exhibiting excellent whipping properties. On standing, 80 percent of the theoretical yield of lactose separates out, giving a high grade product with one crystallization. On evaporation of the alcohol, a third product is obtained which includes the salts, some lactose, and riboflavin 5 to



10 times its solids content of the original whey powder. The riboflavin can be used profitably as a constituent of poultry feeds; the whipping properties of the protein-rich fraction will probably be valuable in certain food products and in confections.

Whey has been particularly useful in dried soups, and also in pea and bean soups. It can be used to advantage with different kinds of candy, such as brittle wheyfers, taffy, caramel, fudge, whipped fudge, and others. It is a substitute for milk solids, sugar, and corn syrup at the same or lower costs, at the same time increasing the nutritive value of the candy, producing a mildly sweet confection, and eliminating the development of a hard casein curd during the cooking.

Ice cream. The relation of temperature to overrun has been reduced to a mathematical formula. Whipping capacity is thus measured as a reproducible property of any mix. These results indicate that the time wasted by the common method of freezing and whipping back, with the refrigerant cut off, may be avoided by adjusting the freezing so that the desired temperature and overrun may be reached simultaneously.

Aging of mix. The effects of aging have been shown to be associated with the heating of the mix, as in pasteurization, and not to be caused mostly by the setting of the gelatin. Homogenization augments the effects of aging. The changes during aging are fundamentally associated with the milk constituents other than fat.

Overrun with butter. The long known difficulty of securing a normal overrun in mixes made with butter can now be secured normally if the butter is first made into cream by homogenizing the butter with skim milk, and then using this cream as normal cream in preparing the mix. In fact, whipping properties better than normal can be secured by making the cream with butter and diluted skim milk. Mixes made with butter in this manner whip much more rapidly than mixes made from normal cream.

Cheese. More uniform production of cheese from day to day is facilitated by a new method of analyzing the curd as it is dipped from the kettle, giving a close approximation to the composition of the green cheese. The water and fat content are important factors in the control of the eye formation, the flavor, and the texture of the Swiss cheese, and this content is subject to a combination of variables during manufacture.

Some Swiss cheese of good quality has been made experimentally from pasteurized milk, but there is a tendency for these cheeses to retain too much water. It is clear that new methods will have to be devised for moisture control in this kind of cheese before uniform results can be obtained.

The deleterious effects of using milk from cows affected with mastitis may be overcome by clarification through the removal of leucocytes and other cellular material from the milk.

The use of the methylene blue reduction test shows that as the reduction time of the kettle milk increases, there is a corresponding decrease in the proportion of undergrade Swiss cheese, and these grades increase sharply if the milk has a reduction time of 3 hours or less. An acidity at dipping above pH 6.51 indicates a failure of the acid-forming bacteria to begin growth properly, and the cheese will almost certainly be undergrade. On the other hand, an acidity at dipping below pH 6.36 shows too great activity of acid-forming bacteria, and the chances are about even that the cheese will be undergrade. The chances of making a No. 1 or special grade cheese is greatly increased if the pH value at dipping is between 6.36 and 6.51.

With regard to Cheddar cheese, notwithstanding the fact that the manufacture of this kind of cheese has been established for a great many years, there is no criterion on which a standard of milk quality can be based, no dependable rule for the development of acid, no exact limitations on water content, and no established relation between these factors and

the temperature at which the cheese should be ripened to obtain the required flavor.

The manufacture of a Cheddar cheese that at a high temperature exudes only a slight amount of fat has been made possible by first homogenizing the cream, then adding this to the skim milk, pasteurizing to destroy the lipolytic enzymes, and proceeding by the usual process.

There has been an increased interest in canned Cheddar cheese, and three companies are now packing cheese in cans as an established procedure. Two others are making trial packs. The experience of these factories demonstrates that if sufficient care is exercised in the selection of the milk and the inspection of the curd, the loss from poor quality is negligible. One of the companies that has been packing the cheese for its own stores uses exclusively a 5-pound can containing 10 individually wrapped half-pound prints. One used both 2-pound and 12-ounce cans intended for direct sale to the consumer.

#### MARKET MILK INVESTIGATIONS

Mastitis. Studies on mastitis support the following observations: (1) There is a trend toward increased chloride content of the milk as the lactation period advances. (2) Some cows apparently with no infection consistently produce milk with a chloride content higher than that considered normal. (3) Many cows with mild udder infections produce milk with a chloride content and a leucocyte count within the limits usually regarded as normal. (4) An abrupt rise in chloride content and leucocyte count in the milk of a previously normal cow, even though they remain within the so-called normal limits, strongly indicates the beginning of an infection.

The organisms associated with an epizootic of mastitis reported last year were identified as *Pseudomonas aeruginosa*. Previous descriptions of this organism were erroneous in regard to certain of their biochemical reactions.

Bottle losses. A report on ways to reduce bottle losses has been published, to-

gether with a digest of the principal state laws regarding the use and misuse of milk bottles. Protection of owners is accorded by registering the bottles with the proper state official. The bottle laws of a state are enforced in most localities by the bottle exchange.

Single service containers. In New York City over 300,000 units are sold daily in paper containers, principally through the chain stores, and practically all milk handled by chain stores in Philadelphia and Baltimore is in single-service containers. While the paper container itself costs more than glass bottles (which average approximately 30 trips) including the washing of the glass bottle, considerable economies can be effected in the delivery to stores if the entire load of the delivery truck consists of milk in paper because of the smaller space and lower weight required as compared with glass. Although paper containers are not used to any extent for retail delivery of milk, they are used quite extensively for sale of milk through stores and their use for this type of business seems to be increasing.

Bottled cream. A good quality of bottled cream could be produced in a coil-vat pasteurizer, even with the coil revolving at a speed of 60 revolutions per minute, provided the cooling of the cream is done over a separate cooler. When the cream was cooled in a vat, however, a cream plug would form on the bottled cream within 24 hours at room temperature, and within 48 hours at refrigerator temperature. This tendency to form plug was more pronounced as the amount of cooling in the vat increased. When the cream was cooled to only approximately 100° F. in the vat and the remainder of the cooling was done over a separate cooler, no plug resulted; but as the cream was cooled further in the vat, the tendency to form a plug increased.

Agitation of the raw cream at all temperatures between 40° and 100° F. resulted in a tendency to plug formation, but the most critical temperatures seemed to be between 60° and 85°. At these

temperatures, the fat globules tend to form clusters and to churn easily. At lower temperatures the fat is in a more solid state, and at temperatures above 95° the fat is melted. The practical application of these results is that cream should always be below 50° and the milk or cream should be handled quickly and with a minimum of agitation, especially between 55° and 85°.

Curd tension of milk. The following method of making curd-tension determinations has been found to be satisfactory:

The coagulant, N/10 hydrochloric acid containing 0.45 percent of pepsin, is made fresh daily. Ten milliliters of coagulant (at 35° C.) is put into a 200-milliliter tall-form beaker. To the coagulant is added 100 milliliters of milk (at 35°C.). The milk is blown from a 100 milliliter pipette, which has the tip removed so that it drains water in approximately 4.5 seconds. The milk is blown from a pipette held vertically over center of beaker in approximately 3 seconds. The contents of the beaker are not rotated. The beaker containing the milk and coagulant is immediately placed in a water bath at 35° and kept there for 10 minutes.

In measuring the curd tension, any instrument may be used which has the American Curd-o-meter-type knife or a knife having the same design and amount of cutting surface. The instrument, however, must give an automatic movement of the knife or receptacle at a constant speed. The speed of the knife used in this work is approximately 1 inch in 7 seconds. The curd tension (in grams) is recorded as the steady value after the knife has started through the curd.

Samples are run in duplicate. If the curd tension of the duplicate samples varies more than 10 percent, the sample is retested. If the variation is 10 percent or less, the average of the two readings is reported as the curd tension for that particular sample.

Although it is impossible to measure digestion accurately in vitro because conditions in the stomach cannot be exactly simulated, it is possible to secure indications of the digestibility. The method used was as follows:

The reagent, consisting of N/10 hydrochloric acid with pepsin as the digestant, was added to the milk to bring the pH value of the mixture of milk and digestant to approximately 3.5. The mixture was held in a water-bath at 37.5° C., and agitated at 15-minute intervals. The amount of digestion was measured by formal titration.

The results obtained indicated that soft-curd milk starts to digest more rapidly than hard-curd milk, but that it is not more completely digested at the end of a reasonable time.

Raw milk, having a curd tension of 75 grams by the Hill method and 46 grams by the hydrochloric acid-pepsin method, was boiled. This lowered the curd-tension to 24 grams and 9.6 grams by the two methods respectively.

The greatest increase in the rate of digestion of soft-curd (boiled) over hard-curd (raw) was during the first 15 minutes. During this time, an average of 76.5 percent more digestion took place in the soft-curd milk than in the hard-curd milk. After the first 15 minutes, the average difference in the amount of digestion which had taken place in the raw and boiled milks decreased, the differences in favor of the latter being 34.8, 25.0, 17.2, 10.3, 8.49, 1.69, and 0.40 percent at 30-minute, 45-minute, 1-, 2-, 3-, 4-, and 5-hour intervals, respectively.

Homogenized milk seems to be more rapidly but not more completely digested than the original milk.

Corrosion of metals. Studies were conducted on the corrosion of 100 and 200 p.p.m. of available chlorine on 13 metals. (Editor.—The pH values are not given.) On the basis of the weight loss, the aluminum alloys showed the greatest corrosion, then the copper alloys, and least were the stainless steels (8-18 and nickel-chromium-iron 80-12-8 ratio).

Dissipation of chlorine. When test strips of metal were immersed in the chlorine solution for 30 minutes, the aluminum alloys reduced the available chlorine of the hypochlorites 10 to 40 percent, but the strengths of the chloramine-T solutions were practically unaffected. The other metals had practically no effect on the available chlorine.

A solution of chloramine-T (with sodium carbonate) decreased in strength from the original value of 312 p.p.m. of available chlorine to 301 p.p.m. after 5 minutes or after washing the udders of 11 cows.

J. H. SHRADER.

## Summary of 1938 Report of Research Committee of The American Butter Institute

### I. *The Problem of Loss in Weight in Print Butter*, V. L. Turgasen, Chairman.

There is a definite loss of moisture in print butter irrespective of methods of churning, working and otherwise preparing butter for individual package form. Further studies will be necessary before definite and comprehensive data can be presented.

### II. *The Study of Methods of Application of pH. Determination in Butter Sera*, C. H. Parsons, Chairman.

The pH measurement of butter sera is becoming of increasing importance as a means of predicting as well as controlling the keeping quality of butter. The two most practical methods used are the colorimetric and the electrometric. Of these two methods, the electrometric is the more reliable.

The electrodes most used in the electrometric measurements are of two types, the glass electrode and the quinhydrone electrode. There is a growing tendency in butter control work to favor the quinhydrone electrode for the following reasons: 1. The Quinhydrone Electrode appears to be a little bit more foolproof and less susceptible to breakage; 2. The Quinhydrone Electrode can be used on butter sera even though it contains some residual fat whereas if the Glass Electrode is used in this same type of sera, it must be thoroughly cleaned with acetone or some other suitable solvent before the next measurement can be made; 3. While the measurement of pH with a Glass Electrode can be made more rapidly, however, unless the precaution of keeping the electrode entirely free of grease film is meticulously observed, inaccurate results may be obtained. The Quinhydrone Electrode is much simpler to clean; 4. There is an apparent need for standardization of procedure in preparing the butter serum samples for pH determinations by the electrometric methods.

In a detailed study of approximately 5,000 individual butter churnings subjected to the keeping quality test at 70° F. for eight days, an average loss in score of one point was ordinarily obtained when the pH of the freshly churned butter was maintained within the pH range 6.6—7.0. An average loss of one-half point in score resulted when the acidity of the butter was maintained at pH of approximately 6.7. The butter samples involved in this study were made in the States of Ohio, Indiana, Illinois, Iowa, Missouri, Nebraska, Kansas and Oklahoma. It was also observed that when the salt content of butter exceeded 2%, there was a tendency for an increasing loss in score, other things being equal.

### III. *The Study of Methods for Determining the Keeping Quality of Butter*, C. H. Parsons, Chairman.

About 800,000 tests have been made on butter which has been scored for quality after incubating seven days at 70° F., and about 200,000 tests have been made after holding butter fourteen days at a temperature of 60° F. The results indicate the following:

1. Butter which has been carefully judged for its quality by scoring, held fourteen days at a temperature of 60° and then carefully scored again, tends to give fairly reliable information as to what can be expected of this butter when handled under average merchandising conditions. Properly manufactured butter will show very little loss in score (not over one point) in the above test. If this butter has a tendency to deteriorate in merchandising channels, it will show about the same sort of deterioration in the above outlined test.

2. Butter held seven days at a temperature of 70° tends to indicate practically all of the flavor defects which it may contain with the exception of limburger or surface taint. Such defects may or may not manifest themselves

in this test. Butter which is inclined to develop limburger taint while it may not show this defect as a limburger taint in this test, usually will show a peculiar type of cheesiness. We recommend that instead of holding butter seven days at 70° F., the keeping quality test be modified to ten days at 65°-68° F. if limburger or surface taints are to be included.

3. The keeping quality test embodying holding butter at a temperature of 70° F. for seven days has come into rather extensive use by the industry. Collectively, the number of keeping quality tests which have been made under the supervision of the members of the Research Committee has been estimated at approximately two million. It has become an indispensable test to those responsible for the quality maintenance of creamery butter.

4. The indications are that butter held fourteen days at 60° F. will compare favorably with butter held ten days at 65-68° F., and the results of either of these tests will simulate average commercial conditions under which this butter is held during its period of merchandising.

5. While there is the practical objection to keeping quality tests that the merchandise involved is often consumed or in trade channels before the results are known, generally the experience has been that defects are the results of cumulative quality control imperfections or failures. Therefore, close and serious attention to the results of keeping quality tests will enable the creamery using the same to anticipate trouble in many instances before the defect reaches a stage of development whereby it becomes noticeable to the trade.

#### IV. *The Handling and Checking of Standard Solutions for Members of the Institute.* Laboratory Management Committee.

The Institute Laboratory is prepared to supply standard solutions to members of the Institute upon request.

#### V. *The Study of Comparative Methods for Determining Numbers of Molds and Yeast in Salted and Unsalted Butters.* R. V. Hussong, Chairman.

Substantial changes in the methods of buttermaking have given rise to defects which otherwise might not as readily develop. The most troublesome have been the so-called "limburger" and "surface" taints, and these are not adequately controlled by evaluating the number of molds and yeast in salted and unsalted butters. Research work is under way in the laboratories of members of the Research Committee as well as several Agricultural Colleges to develop a method which will anticipate such taints due to microbiological contaminations or infections.

#### VI. *The Study of Special Chemical Methods for Evaluation of Quality in Creamery Butter.* R. V. Hussong, Chairman.

In the past year considerable attention has been given the application of the phosphatase test to market milk supplies for attesting the thoroughness of its pasteurization. While in the case of creamery butter, the phosphatase test has only limited application from the public health standpoint, it is invaluable in checking the thoroughness of pasteurization of the cream used provided the butter samples are tested when fresh and have not been subjected to any adverse temperature conditions. Cream must be flash pasteurized at a temperature not below 185° F. to give a negative reaction in the pasteurized cream and its butter. Further work on the application of this test to creamery butter is contemplated.

The chemical identification of weedy taints in butter has been attempted and the results of such studies will be given in the joint report of the Research Committee of the American Butter Institute and the Cream Quality Committee of the American Dairy Science Association.

M. E. PARKER, *Chairman.*  
V. L. TURGASEN, *Secretary.*

# JOURNAL OF MILK TECHNOLOGY

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## Association News

## NEW YORK STATE ASSOCIATION OF DAIRY AND MILK INSPECTORS

The executive committee of this Association met recently and determined that the next annual meeting will be held in Syracuse, N. Y., on September 27, 28 and 29, 1939.

President Clyde L. Kern appointed the following committee to cooperate with a committee of the New York State Association of Milk Distributors and the state Department of Health to study and attempt to find a solution of the problem of sales of questionable raw milk on the outskirts of cities in which the sale of such milk is prohibited.

Wilbur H. Rothery, Auburn, N. Y.  
John C. Fitzgerald, Troy, N. Y.  
J. J. Regan, D.V.M., Utica, N. Y.

W. D. TIEDEMAN,  
Secretary-Treasurer.

## METROPOLITAN DAIRY TECHNOLOGY SOCIETY

This society meets on January 17, 1939, at the McGraw-Hill Bldg., 330 W. 42d St., New York City. After an informal dinner at 6:30, followed by a short business session, the address of the evening follows at 8 P. M.

At the December meeting, Mr. George W. Putnam gave an illustrated address on high-short milk pasteurization. It drew a large attendance.

The following new officers were elected: Dr. C. H. Kimberly, *president*, succeeding Dr. J. H. Shrader; D. F. Snyder *vice-president*, Dr. O. F. Garrett, *secretary-treasurer*; and Dr. D. Levonwitz, *sergeant-at-arms*.

J. H. SHRADER.

## CENTRAL STATES MILK SANITARIANS

The first annual meeting of the Central States Milk Sanitarians will be held on February 17, at the Knickerbacker Hotel, Chicago, Ill., and will convene at 1:30 P. M. After the disposition of the regular business, the officers for the coming year will be elected. Four or five addresses will be delivered by men prominent in the field of dairy technology. The meeting will close with a banquet at 8 P. M. followed by a dance.

The committee on arrangements for the annual meeting consists of the following members of the Association: Leo Randolph, Chairman, John H. Dorocke, Edward Sullivan, William Hogan, and Edward Jones. The following members constitute the entertainment committee: Peter Larsen, Chairman, Floyd M. Keller, and Edwin S. Roberts.

I should like to ask the members of our Association to make the JOURNAL OF MILK TECHNOLOGY our meeting-place, between the yearly meeting, where each might share the ideas and ideals of the others. Please send me any advices, suggestions, or notices so that I can arrange to have them printed in the Journal.

All members are requested to notify me of any changes in their addresses.

DONALD V. FITZGERALD,  
Secretary-Treasurer.

## Fabian Elevated to Full Professorship

Dr. F. W. Fabian, third vice-president of the International Association of Milk Sanitarians, has been raised in rank at the Michigan State College to that of full professor with the title of Research Professor of Bacteriology. He is being provided with a newly apportioned and equipped laboratory.

## Changes in New Edition of Standard Methods Of Milk Analysis\*

The forthcoming seventh edition of Standard Methods of Milk Analysis (published by the American Public Health Association) will carry the new title "Standard Methods for the Examination of Dairy Products." The ice cream section will be further developed by the work of the Joint Committee on Standard Methods for the Examination of Frozen Desserts as approved by the Laboratory and the Food and Nutrition Sections of the American Public Health Association.

The most important changes in the new edition are as follows:

The bacteriological methods in the first five sections cover samples, agar plate counts, direct microscopic counts, methylene blue reduction technic, and sediment tests. The four following sections describe methods for the isolation and identification of coliform bacteria, hemolytic streptococci, tubercle bacilli, and undulant fever organisms, respectively. Section 10 includes ice cream methods, and Section 11 the butter methods. The text of the bacteriological section has been rewritten, and a subject index prepared.

Beginning July 1, 1939, standard agar medium is to have the following composition:

Bacto-Tryptone .....	5 gr.
Bacto-meat extr. ....	3 gr.
Glucose .....	1 gr.
Skim milk .....	10 cc.
(Where dilutions are greater than 1 : 10)	
Agar .....	15 gr.
Reaction .....	pH 6.6 to 7.0
Preferred reaction .....	7.0

While incubation at 37° C. (not 37.5° C) is to be continued until laboratories doing milk work are more generally equipped with approved incubators, the variation in temperature permitted was changed to 35° — 37° C. rather than 35.5° — 37.5° C. as at present.

Agar plates must be counted under constant illumination. The Quebec Colony Counter is recommended.

The only incubators approved are the water-jacketed and anhydric laboratory types with low temperature heating units operating at temperatures only slightly in excess of 37° C., and incubator rooms of proper construction.

Specifications for glassware are drawn in greater detail than formerly.

More accurate methods are given for rinse tests from glass and paper bottles.

The direct microscopic test is described in greater detail, particularly for the examination of pasteurized milk. All agar plates must be checked frequently enough to detect the presence of excessive numbers of bacteria in milk and cream where they do not grow on the plates. Only in this way can excessive numbers of bacteria be detected.

The methylene blue thiocyanate tablets are accepted as standard.

No reference is made to the resazurin test because further study must be made before the results are accepted as significant.

Reference is made to the modified form of the methylene blue reduction technic now officially approved in England.

The description of the sediment technic has been improved.

Brilliant green lactose bile broth, sodium formate ricinoleate broth, violet red bile agar, and sodium desoxycholate agar are approved for detecting coliform bacteria in dairy products.

Methods for plating on blood agar and the use of Burri agar slants are described for detecting pathogenic, usually hemolytic streptococci. References are given to methods of identifying species of streptococci by cultural and serological procedures. Also tentative methods are included for the identification of tubercle bacilli and *Brucella* organisms in milk and cream.

Directions are given for making yeast and mold counts on butter, and bioassays of vitamin D milk.

It is expected that the new (Seventh) edition will be printed early in 1939.

\* Summarized from *Amer. J. Pub. Health* 28 (12) 1447 (1938).

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