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A Nopco Subsidiary
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MILK SANITATION

and

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AS a manufacturer of equipment used in every branch of the Dairy Industry, and recognizing the vital part which correctly designed machinery plays in the processing of milk and milk products, Cherry-Burrell engineers give particular attention to the sanitary details of every piece of equipment bearing the Cherry-Burrell nameplate.

Cherry-Burrell engineers recognize the problems confronting the Milk Sanitarian and design machinery that makes the work of the Inspector easier and control of sanitation more certain. Our entire organization is committed to that policy. We always welcome constructive suggestions from those who are responsible for milk sanitation.

CHERRY-BURRELL CORPORATION

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INTERNATIONAL ASSOCIATION OF MILK SANITARIANS

Secretary's Office: State Department of Health, State Office Building, Albany, N. Y.

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LOCAL COMMITTEE ON ARRANGEMENTS FOR THE 1938 MEETING
(To be appointed when place of meeting selected).
THE International Association of Milk Sanitarians, now in its twenty-seventh year, is continuing the good work which has characterized its program for so long. The spirit of the men who founded it and who helped to develop it through the years still rides on to inspire us.

At the last annual meeting of the International Association of Milk Sanitarians, the Journal of Milk Technology was created and made its official organ to be issued in lieu of the annual report which has been published for the past twenty-five years. The Executive Committee was authorized to formulate the necessary publication policies, appoint the editors, and proceed with the publication of the Journal. Already the Association has received new applications for membership as the result of this development.

The Journal itself has received many subscriptions from individuals, institutions, and companies, and requests from numerous libraries in this and foreign countries. An association of state and municipal milk sanitarians has indicated their interest in making the Journal their official organ, and negotiations are now in progress to this end.

Let every one of us try to secure a new member—one who is truly interested in the sanitary production of milk and its products. We are not interested in mere numbers. We are looking for real quality, not just quantity. A united effort will overcome all obstacles. So we face this new year with high hopes and with faith in you.

A. R. Tolland, President.
EDITORIAL SECTION

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.

THE PHOSPHATASE TEST

For a long time public health control officers have felt the need for some dependable test that would indicate when a given milk supply had been properly pasteurized. Various chemical methods have been proposed. At best they gave results of only approximate value and none were supported by a sufficient volume of careful laboratory research and field application to convince the scientific world of their utility. Within the last several years, the phosphatase test has been developed by Graham and Kay (1), based on the property of the heat-sensitivity of the enzyme phosphatase to liberate phenol from sodium phenyl phosphate and then the determination of the phenol quantitatively by a delicate color reaction. The enzyme is less rapidly destroyed by heat than the most pathogenic organism likely to be present in milk (2). These authors developed a field test to detect gross pasteurization errors and a more refined laboratory test which they stated would show the addition of as little as 0.25 percent of raw milk to a properly pasteurized milk, or reveal a pasteurizing error of 1½°F. underheat. Kay and Neave found (3) that the method is applicable also to homogenized milk, cream, butter and cheese.

Gilcreas and Davis reported (4) the results of studies on about 100 samples of commercially pasteurized milk. The method correctly measured the heat treatment in 97 percent of the samples, and showed that the addition of as little as 0.1 percent of raw milk and a variation of 5 minutes in the heating time were significantly determinable. Scharer has made substantial refinements in the technique. The method has been adopted by the Association of Official Agricultural Chemists, and the details will appear in the forthcoming number of their journal.

Other workers are finding that there seem to be factors which affect the test. Smith reports (5) anomalous results, including positive tests after repasteurization. Koppejan (6) finds the reaction accelerated by such salts as NaCl, KCl, Na₂HPO₄ and others. Contardi and his associates (7) state that ascorbic acid destroys phosphatase activity. Herschdorfer shows (8) that the rate of cooling exerts a marked effect. Storrs and Burgwald found (9) that the limits of sensitivity for detecting the addition of raw milk were 0.2 percent, 0.4 percent, and 1 to 2 per-
cent, respectively, in three series of tests. Although they state that their figures show no relation between the phosphatase activity and the butterfat content, these conclusions are not convincing.

Folley and Kay state (10) that the phosphatase enzyme is synthesized in the mammary gland. It would be interesting, possibly significant, to know the effect of its pathologic condition on the enzyme. Tauber (11) reports that the phosphatase content of the blood is known to increase in various diseases—in humans, to be sure, but possibly somewhat significant in other animals. Enzymic concentration directly affects reaction velocities, but not the final equilibrium (Bodansky). Inasmuch as reaction velocities are functions of time, temperature, and concentration, each should be known for the phosphatase reaction.

The effects of known enzymic poisons like copper, and possibly aluminum, iron, and nickel, the changes in milk composition and microbic flora with season and feed, and the influences of the pH value and fat content should be studied. Their effects must be evaluated before we shall be sure that we are measuring what we think we are to the degree of delicacy claimed.

We recall how much work has been expended to establish the limitations of the methylene blue test and even the Babcock. The data already presented on the phosphatase test makes the method look decidedly hopeful. The workers in this field have made a noteworthy contribution to the armamentarium of the milk control officers. We look forward with great interest to the supporting data of controlled research to establish the limits of the significance and the dependability of this test of so much promise.

(1) J. Dairy Research 6, 191 (1935).
(2) Canadian Public Health J. 27, 551 (1936).
(3) Dairy Ind. 2, 5 (1937).
(5) Canadian Dairy and Ice Cream J. 9, 26 (1937).
(9) Ibid. 1, 96 (1936) from Chem. Abs. 30, 7600 (1936).
(10) J. Milk Technology, this issue.
Sanitary Aspects of Paper Milk Containers*

M. J. PRUCHA

University of Illinois, Urbana, Illinois

The bottling of fluid milk in paper containers is not a new idea. In a book by Kenelm Winslow, entitled "Production and Handling of Clae Milk," published in 1909, the following statement appears on page 140: "The latest departure in the way of a milk bottle is the single service milk container of pulp wood invented and made by G. W. Maxwell of 1201 Falsom Street, San Francisco, California. It is now in actual use by dairymen in Los Angeles, California."

While the paper milk container was invented some thirty years ago, very little attention was paid to it by the fluid milk industry or by the milk sanitarians. It is only within the last few years that the paper milk container has forced its way into the fluid milk industry and to the attention of the milk sanitarians.

The paper milk container brings with it new problems, economic, practical, and sanitary.

As a result of demand for information concerning the paper milk container, a study on the subject was undertaken by the Dairy Department, University of Illinois. This paper is a progress report on some sanitary problems connected with the use of paper containers for fluid milk.

There are on the market at present at least five different types of paper milk containers. The study reported here was carried on specially with the Pure-Pak milk container. In the case of this container, the paper (after it is made in the paper mill) is packed in large bundles and is shipped to the carton manufacturing company. Here the container is cut out, printed, and sealed along its long edge. It is packed and shipped to the dairy plant in a collapsed condition. In the milk plant the containers are fed into the Pure-Pak machine, made by the Ex-Cell-O Corporation of Detroit, Michigan. The container enters the machine at one end in its collapsed condition and comes out at the other end filled with milk and sealed. As it passes through the machine the container is formed, its bottom is sealed, it is dipped in hot paraffin, passed through a cooling chamber, filled with milk, and finally it is sealed and dated.

A paper mill requires a great deal of water so every mill is located by some abundant body of water—it may be lake, river or well. The spruce tree trunks are first washed and cleaned of bits of bark. As they pass from the washing machine to the cutter they are sorted, as only sound and free-from-bark-and-knots logs are acceptable. The logs are cut into $\frac{3}{8}$ inch chips. The chips are sorted as they pass on their way to the cooker. The cooking solution is made from sulphur and lime. The wood

*Read before the Twenty-sixth Annual Meeting of the International Association of Milk Sanitarians, Louisville, Ky., October 11-13, 1937.
chips are cooked in large steel cylinders, lined with brick, for about 15 to 20 hours at a temperature of 120° C. or above.

The digested wood pulp is dumped into large vats where it is washed with fresh filtered water. From here the pulp passes over screens and continues to be washed and cleaned of bits of knots. When in desired condition the pulp passes into large white-tile lined tanks where it is bleached. The bleached pulp passes into another series of tiled tanks where it is again washed, to remove the excess of chlorine and bleaching residue.

At this stage an emulsion of partly saponified rosin and paper makers' alum $\text{Al}_2\text{(SO}_4\text{)}_3 \cdot 18\text{H}_2\text{O}$ is added. The pulp then is pumped to a Jordan engine and then it passes to the paper machine vat. From here the pulp is picked out by revolving cylinders covered with fine mesh wire cloth. Layers of the pulp adhering to the cylinder are then carried on and pass over a large number—about 30—of large steel cylinders. These cylinders are heated with steam at about 15 pounds guage pressure. As the pulp—paper—comes off the last cylinder it is dry and hot, the water being evaporated from it; or rather it "boiled off" as the pulp passed over the hot cylinders. The paper is cut in suitable sheets and packed.

In the sulphite process of preparing pulp, the treatment is such that pulp is made practically sterile in three places. First in the steam digesting cylinders, the pulp comes out absolutely sterile. During the washing process it becomes contaminated by the water and equipment. In the bleaching vat it is again sterilized. It again becomes contaminated by the water as it is washed and diluted. The size also adds a few bacteria. As the pulp enters the paper machine and passes over the steam rollers it again is at least partially sterilized.

The bacteriological examination showed the pulp to be sterile in the bleaching vats. After it was washed, diluted, and treated with size, it gave a bacterial plate count of 400. As the diluted pulp entered the wire screen the count was 40. Two by two inch strips of paper, taken as they left the hot rollers, placed in 100 ml. of sterile water, and after shaking, plated, gave no colonies on 1 ml. plates. In other words, the paper was practically sterile on its surface.

Milk sanitarians should inspect paper mills and check on the pulp. In the first place, the water used for diluting the pulp usually comes from polluted streams or lakes. Some paper mills have proper sanitary control over the water supply while other paper mills have not.

In the suggested standards for paper as printed in The Milk Sanitarian, Volume 6, pages 11-13, it is suggested that paper milk containers be made from virgin chemical or mechanical pulp and that prior to moisture-proofing the paper shall not have a count exceeding 500 colonies per gram of the disintegrated paper. Such a standard is extremely liberal. A quart paper container when dry weighs about 42 grams. A container made from such paper would harbor 21,000 in its walls.

When paper is made by the sulphite process and is bleached and is made in a continuous operation—that is, not stored in moist condition for some days—the bacter-
ial population of the paper is extremely low. A number of containers were beaten in a small glass churn until reduced to pulp. About 1500 ml. of sterile water were used for one container. Ten plates were poured, one ml. in each. Five plates had no colonies, two plates had one colony each, and three plates had two colonies each. Such counts mean practically nothing, except that the paper was practically sterile. In another test, twenty quart containers were broken up and jammed into a three-gallon milk can containing six liters of sterile water. After soaking for 20 minutes and shaking vigorously, one ml. of the water was plated in each plate. After two and one-half hours, of soaking another set of plates was made. Again the plates gave counts of one or two colonies or none. The calculations from such counts gave 2.3 bacteria per square inch of surface.

Where paper is made for milk containers some medical examination of the worker handling and packing the paper should be required. Some mills have such standards. Occasional inspection of the premises and of the bundles of the paper are desirable. The Chicago Carton Company, where the containers are cut and printed, was found in an excellent sanitary condition. If paper containers become extensively used, such an establishment should be visited and inspected by milk sanitarians.

So far in our study, most of the experiments have been carried on with the Pure-Pak paper container. As stated already, the containers are shipped to the milk plant from the carton company and are paraffined by the machine at the milk plant. The machine is set as to

\[ \text{time but the temperature of the paraffin can be set as desired.} \]

The bacteriological condition of the paraffined containers was determined at first by the standard methods recommendations. The containers were passed through the machine and were sealed, but no milk was put into them. At the laboratory they were opened, one hundred ml. of sterile water were poured in and after a thorough shaking, two plates—each containing one ml. of the rinse water—were plated. Most of these empty bottles were collected during two months, in which time the machine was used for bottling milk. Each day the first six bottles passing through the machine and also the last six after the milk was bottled, were taken for the test. Space does not permit the giving of the counts of individual containers, of which several hundred were examined. Most of the plates had one, sometimes two, and more often no colonies. The results forced us to the conclusion that the containers were practically sterile and that the standard method is no good for this purpose.

The following method was adopted after this. About 25-30 ml. of nutrient agar were poured directly into the container. After some agitation to bring all the surface in contact with the agar, the containers were incubated. To count the colonies the container was cut open and the slab of agar was placed on the counting glass, and the colonies were counted.

One hundred and ten bottles paraffined at 170° F. had all together 100 colonies; ninety-three containers paraffined at 180° F. had 49 colonies; ninety-eight containers paraffined at 185° F. had
29 colonies; and ninety-five contain-
ters paraffined at 190° F. had
53 colonies. Part of the time the
standard agar plus one per cent
lactose was used as nutrient agar
and most of the time the tryptone
dextrose agar was used. The con-
clusion drawn from these tests is
that when sulphite-process bleach-
ed pulp is used for the paper, it is
practically sterile, and when the
containers are paraffined they also
are practically sterile. It made no
difference whether they were par-
affined at 170, 180 or 190 degrees F.

Another group of 1,000 bottles
from a different source was exam-
ined. The results of that examina-
tion were very similar. In this
group of containers 45 percent of
them developed no colonies, 38 per-
cent of them had spreaders—that
is, spore producers—and 25 per-
cent of the containers had paper
defects. Of those with paper de-
fects, 71 percent harbored spread-
ers. In Table I are given the colony

Table I

Twelve Worst Containers Among 1000 Examined

<table>
<thead>
<tr>
<th>Number</th>
<th>Total Count</th>
<th>Spreader Defects in Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>309</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>126</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>118</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>111</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>110</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>99</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>67</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>64</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>63</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>54</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>49</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Present — absent

counts of the twelve worst con-
tainers and the presence of spread-
ers and paper defects is indicated.
The paper defects manifested
themselves as brownish patches in
the paper under the paraffin and
appeared to be caused by some hy-
drolytic change in the pulp.

Taken as a whole, as far as can
be determined, the paper milk con-
tainers after they are paraffined
are practically free from bacteria.

The paraffining of the containers
is done primarily for the purpose
of making the paper impervious to
the milk. Since paraffining must be
done at relatively high tempera-
ture the process also involves a
bactericidal treatment.

This phase of the study had been
conducted both in the milk plant
and in the laboratory. For this
purpose the containers were im-
pregnated with a bacterial suspen-
sion of a given organism. For the
most part a variety of B. prodi-
giosus was used. In the first part of
this study the containers were dip-
ped in a bacterial suspension usu-
ally having about 200,000,000 plate
count. In dipping the containers
they absorbed on the average about
seven ml. of the suspension. As
soon as the containers were dry
enough they were paraffined. To
test for the presence of the organ-
isms, about 50 ml. of nutrient agar
were poured into each container
and thoroughly shaken, then incu-
bated. Many hundreds of contain-
ers were treated in this manner.
The temperatures of 160, 170, 175,
180, 185 and 190 degrees F. were
tried. Very irregular results were
obtained. While most of the con-
tainers were free from the organ-
ism, invariably there would be a
few containers in which the organ-
isms were present. This was true
of all the temperatures.

In one experiment the suspen-
sion had only 2,000,000 bacteria
per ml. Of the three hundred bot-
tles paraffined at 170°, 180° and
190° F. none had any of the bac-
teria present.
The dipping of the paper containers in a suspension containing so many bacteria was discontinued. Instead of dipping, the containers were inoculated by putting one's hand in the suspension and then rubbing the inside walls of the containers with the hand. In one run six hundred containers were inoculated in this manner and were paraffined at 160°, 170° and 180° F.

Of the two hundred bottles paraffined at 160° F., nine were positive, of those paraffined at 170° F., one was positive, and of those paraffined at 180° F., two were positive.

Another method of inoculating the containers was tried. The operator handling the containers from the cartons in which they were shipped to the paraffining machine infected his hands in the bacterial suspension and then handled the containers. In one test, six hundred containers were paraffined, after being handled in this manner: two hundred at each of 170°, 180° and 190° F. All the containers paraffined at 170° F. and at 180° F. were negative. Of the two hundred containers paraffined at 190° F., one hundred and ninety-nine were negative and one was positive.

In these tests in which inoculated containers were paraffined, and a large number of these were paraffined in each test, invariably one or two containers would be positive, and that was irrespective of the temperature used. Whether the bacteria survived the paraffining or whether in handling such rich suspension an occasional accidental contamination took place, it is difficult to conclude. The heavier the inoculation the more positive cases appeared.

The study of paraffining the paper was also carried on at the laboratory. Here small strips of paper, ½ by 2½ inches, were used. After the strips of paper were paraffined they were dropped in a test tube containing 15 percent milk and 85 percent water. After incubation of several days they were examined for the appearance of color. The results from one of the runs, given in Table II, are typical. In Table III, results are given from

### Table II
Survival of *B. prodigiosus* on Paper Strips ½" x 2½". Strips Dipped in Bacterial Suspension of 20,000,000 Cells Per Ml-

<table>
<thead>
<tr>
<th>Exposed</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>160°F.</td>
<td>+++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>++</td>
</tr>
<tr>
<td>170°F.</td>
<td>+++</td>
<td>+++++</td>
<td>+++++</td>
<td>+++++</td>
<td>+</td>
</tr>
<tr>
<td>180°F.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>190°F.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>200°F.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>212°F.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

++ Bacteria survived.
— None found.

### Table III
Killing of *B. coli* on Paper by Dipping Inoculated Strips of Paper in Hot Water. Each Strip ½" x 2½" Received 5,000,000 Bacteria.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>130°F.</th>
<th>140°F.</th>
<th>145°F.</th>
<th>150°F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>45</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>60</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

one of the runs in which the inoculated strips of paper were exposed to sterile hot water instead of paraffin. The results show that the hot water treatment is more effective than the paraffin treatment.
The difference is very pronounced. Water at 150° F. is more effective than paraffin at 190° F.

Tests were also made to determine how long the bacteria will survive on the impregnated strips of paper. Heavily inoculated strips of paraffined paper were examined daily. No living bacteria were found on the strips after the seventh day. When the unparaffined paper strips were inoculated and examined in the same manner no viable bacteria were present after the sixth day.

The tentative standards suggested in the report from the conference on paper milk containers are good. Perhaps they should be somewhat more exacting. The milk sanitarians should inspect and develop some score card for the mill where the paper is made. Health certificates of the employees handling the paper should also be made. Health certificates of the employees handling the paper should also be required, both in the paper mill, in the carton manufacturing company, and also in the milk plant wherever the milk containers are handled by hands. Inspection of the paper containers at the milk plant is necessary not only as to bacterial condition but as to appearance. The containers that are paraffined in a factory and shipped, ready to be filled, need to be well packed and protected in the shipment and at the milk plant. Also a proper storage room, free from insects and vermin and dust, must be provided at the plant.

Paraffining the containers at 185° F. for 30 seconds, when the containers are protected as above suggested, will result in a practically sterile container, and a container that is safe to use. However, entire dependence on paraffining to insure complete sterility of the container may not be sufficient.
Standardization of Rulings on Equipment and Supplies*
HENRY S. CALVERT†
President, Dairy and Ice Cream Machinery and Supplies Association, New York City

The invitation from your organization to me as President of the Dairy and Ice Cream Machinery and Supplies Association to appear before you at your current annual convention is sincerely appreciated.

The present day duties incumbent upon all of the elements that go to make up the dairy industry are necessarily becoming more and more complex and must require a greater measure of cooperation in order that they may be properly carried on. Too often in discussing matters of this kind we are inclined to overlook our experience as well as the history of events which have transpired.

Let me say at the outset of my remarks that I came here with the distinct idea of asking your organization to join with us in an attempt to set up some forum where there could be an exchange of opinion on the details of construction and operation of dairy equipment so as to avoid the helter skelter, hit and miss method that now prevails. This is primarily what I hope I may be able to get you gentlemen to do.

As a background and reason for this, let me point out what has happened in the dairy industry in the last twenty years. The small, middle and larger dairies in the Eastern area have grown to such a size that equipment has had to be developed to permit their operations to meet the public demand for their product. A great dairying zone has been developed in the South. I can remember being told by Mr. George Villers less than twenty years ago that practically all milk used in the city of New Orleans came from the North. Today it is self-supporting and is an illustration of the growth of the industry.

The same period ago Texas and the Southwest was a pioneer territory for milk. Today what Wisconsin has so finely developed is spreading into the mountain states to the West and the growth and use of milk in the Midwestern states surrounding Missouri has been phenomenal.

Dried milk plants and condensed milk plants abound in Utah, Montana, Idaho and Washington and areas that were never considered milk territory. I need not refer to California which is a center of its own, but I can summarize the picture by saying that the production of milk for home use is practically under way in every state in the Union and this industry which was largely centralized in the Northeast twenty years ago is found in practically every state in the Union.

Along with this spread of the industry has come an increase in volume produced and made pos-

*Road before the Twenty-sixth Annual Meeting of the International Association of Milk Sanitarians, Louisville, Ky., October 11-13, 1937.
†Deceased January 5.
sible by types of equipment which were not available at the beginning of the period under discussion and which permit volume production and handling such as was never conceived of twenty years ago.

You are aware of the fact that one of the greatest Eastern dairy distributors has recently constructed a plant to handle 300,000 gallons of milk per day. You do not believe that with the equipment available twenty or even ten years ago this volume of milk could be handled and made safe and sanitary with equipment of that time.

The design and development of the equipment which makes possible the modern dairy in the metropolitan centers as well as the model dairy in the small towns is made possible by the development of equipment to meet the needs of the industry. It is largely a product of the manufacturers of this equipment, and I need to point to only a few of the outstanding examples to bring the matter clearly before you. In the practical handling of milk there have been three devices developed and made practical to the dairymen which have made large city plants practical and economical. These are the truck tank, the storage tank and the car tank. Their application applies not only to the milk, but also to the ice cream industry. In the ice cream industry we have had such outstanding developments as the continuous freezer, a new type of refrigeration, and a new type of packaging equipment, the value of which is without dispute.

In the modern pasteurizing plant there have been developed methods of heating and cooling as well as automatic pasteurizers so that capacities are permissible up to 50,000 pounds per hour and possibly beyond. Running parallel to these developments have been the design and building of model bottle washing machines and also model bottling machines which are necessary to permit the uniform development of size and capacity noted above. My intention here is only to point out some of the outstanding things that have been produced by the machinery and supplies industry to make possible the modern units.

It stands to reason that in the development of this equipment the technical men in the Association of Milk Sanitarians as well as the engineers of the manufacturing companies have all had their share.

This has not been done without a large measure of dispute and complications at this point and that.

In connection with the development of the lines of equipment in which I as a manufacturer am particularly interested, it is to be noted and is hereby stated to be the fact that I have yet to find a milk inspector, or sanitarian if you please, who has not been willing to cooperate to the limit of his official capacity in discussing any proposed progress of this character. The powers conveyed under the laws of the states of this country as well as local laws give to the milk inspector a measure of power which is probably unusual as compared with the powers given any other groups of local officers anywhere and the remarkable thing to me is the fact that these powers are used properly and with the full idea of the support of the industry and care of public health.

The statement was recently made at one of the milk publicity organizations of the United States that the daily turnout of milk in
quart bottles was 30,000,000 quarts. All of the milk used in filling these 30,000,000 quarts passes under the inspection of your organization in one or another of its forms. Necessarily it passes through various types of equipment devised and built by the members of our organization.

It is, therefore, of the greatest importance that two organizations whose functions are as important to the public welfare as are those of these two organizations should have a common meeting ground to discuss their problems.

In order to prepare itself for a wider use of its facilities the Dairy and Ice Cream Machinery and Supplies Association recently enlarged the membership of its "Technical Committee." The purpose of this enlargement was to give membership to more men representing more types of equipment than had been possible under the previous set-up. Membership has been selected with the idea of securing from member companies the assistance and attendance of its technical men. This is the working organization of our Association and the one through which we would like to establish contact with your organization. I shall discuss the purpose of these proposed contacts a little later in this paper.

Considerable publicity has been given to the statement that our Association has endorsed in toto the proposed regulations of the United States Public Health Service. This statement is not true. Our organization has volunteered and agreed to the support of such regulations of the U. S. Public Health Ordinance and Code as cover machinery, supplies, and equipment only. We are not concerned with the regulation of milk supply or any of the other features of the U. S. Public Health Ordinance that may be adapted to any other body. We, therefore, are in a position to discuss with agents of your organization or any other organization which is equally representative the problem of sanitation and sanitary constructions.

Speaking entirely as an individual, it seems to me unfortunate that with the type of men you have in the membership of your organization it has not been possible to get them to sit down together and attempt to do this work in a formal way. Our Association realizes that any arrangements or discussions we have at this time are tentative and that the plan must be gradually worked out. Some of the most brilliant men with whom I have the pleasure of acquaintance in the dairy industry are in your group and they assert their abilities and capacities within the limits of the organizations to which they are now attached, but I feel sure that the usefulness of their experience and ideas could be extended widely through the proper type of organization.

I want to point out in closing one of two things that we are missing at the present time. So far as machinery men are concerned they have no central point of learning what changes take place in a given territory and are frequently embarrassed by this fact.

All of us are God made, but none of us are perfect. The result is that we get regulations in one territory which are not acceptable to the authorities in another territory, and properly so in some instances. It would seem possible to have quarterly, or even monthly
meetings for a time at which disputable changes in the codes and in the types of construction could be discussed. If a committee of your organization and a similar committee of our organization arranged for this, I believe it would be the beginning of a much better step in the improvement and development of sanitary features and dairy equipment.

The above is a general statement or ideas which I have in mind to advance at this particular time. Unfortunately, due to a condition of my health I am not able to attend your meeting and argue, if anybody cares to argue, the validity of the points I am trying to make. However, the paper will be presented by Mr. Roberts Everett, Executive Vice-President of our Association, who is probably much more familiar with the details of what we are asking you to accept than I might be, and I bespeak for him your kindest consideration and acceptance.

Abstract of Report of Joint Committee on Milk Supply
(Presented before the Public Health Engineering Section and Conference of State Sanitary Engineers, Sixty-sixth Annual Meeting, American Public Health Association, New York, October 5-8, 1937)

Very little progress can be made to regulate the sanitary production and handling of milk until the public can be educated to understand the importance of these measures. It is desirable to organize an advisory committee of representatives of the producers, industry, educational groups and related departments to advise with the control officials.

State officers should establish a state-wide program of uniform control, using the milk program of the U. S. Public Health Service or those of the several states as guides.

State and local milk sanitarians should have the following qualifications:

(a) Education: college degree in dairying, dairy technology, sanitary engineering, medicine, or veterinary medicine.

(b) Experience: state or chief local inspector—2 years of practical experience in milk work. Local supervisory work—1 year of practical experience in milk control.

(c) Personality: ability to sell the milk program and instruct farmers and dealers, and preferably should have training and experience in pasteurizing plant work.

It is highly desirable in the interest of developing better qualified personnel that college courses be instituted specially designed for milk sanitarians.

Regional and state training schools were urged for the training of personnel in milk sanitation control, leading to a more uniform and effective milk control. Every state department should employ at least one milk sanitarian to assist local health departments in their practical problems, to train inspectors, to give technical advice, and to rate supervisory performance. There should also be at least one highly trained sanitary engineer capable of assisting local health departments in the more technical matters of plant design and operation.

All plans for new construction and alterations of milk plants and equipment should first be approved by the state officials.
Suggestions for Sanitation of Ice Cream and Ice Cream Plants*

J. H. FRANDSEN

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Ice cream is generally conceded our most popular dessert and, when made from good, wholesome dairy products, should rightly be considered one of the finest foods. It was first known as "cream ice" or "butter ice," and its manufacture, to the surprise of most of us who think of it largely as an American dessert of recent origin, really dates back to the fifteenth century.

The real growth of the ice cream industry began with the discovery of artificial refrigeration and with the general acceptance of ice cream as a really worthwhile food. According to the latest available figures (Hibben), more than 242,906,000 gallons are manufactured annually in more than five thousand different places in the United States.

The need for sanitation in ice cream making should be apparent to both the manufacturer and the inspector. Ice cream, because of its ideal food content in available form, can readily become a carrier of disease-producing bacteria or of bacteria which cause flavor defects in the cream. To guard against such conditions, city and state regulations prescribe definite bacterial standards.

According to data available, ice cream has on some occasions been responsible for outbreaks of typhoid fever, scarlet fever, diphtheria, digestive disturbances such as diarrhea, enteritis, and what is generally known as ptomain poisoning. It is generally felt that ice cream ranks second to milk as a cause of epidemics.

In Bulletin 56, United States Public Health Service, bacterial counts are given from 263 ice cream samples collected between October 1906 and July 1907. The average number of bacteria per ml. of ice cream was 26 million. During August and September of 1936, according to Prucha and Tracy, 480 samples from 166 different ice cream makers were tested. The average bacterial count was 1 million per ml. It should be noted that some of these samples had less than 10 thousand bacteria per ml. The increased interest in low count ice cream has already resulted in many states having specific laws requiring bacterial counts of not over 100 thousand per cc.

Numerous investigators have definitely demonstrated that freezing is not a safeguard against contamination of ice cream with infectious disease germs. C. W. Mitchell found that B. typhosus survived in ice cream for from 12 to 39 days. Similarly, the organisms which cause septic sore throat and others which cause digestive disturbances are known to survive the

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*Read before the Twenty-sixth Annual Meeting of the International Association of Milk Sanitarians, Louisville, Ky., October 11-13, 1937.
freezing process. Paratyphoid and undulant fever organisms remain viable in ice cream for as much as 36 months, and certain strains of tubercle bacilli for more than 30 months.

**Sources of Infection**

Prucha reports the following sources of infection that should be watched and controlled by the ice cream maker:
1. The equipment that comes in contact with pasteurized ice cream mix and with ice cream.
2. The plant operator
3. The ingredients that are added to the mix after it is pasteurized.
4. The retail dispensing of ice cream.

Obviously, the production of ice cream should be surrounded with adequate safeguards to avoid any contamination with pathogenic organisms. The problem involves adequate pasteurization and guarding against re-contamination.

Since the mix must be heated in order to dissolve certain ingredients, and also to homogenize to best advantage, the logical safeguard is to pasteurize the mix at a temperature of from 150° F. to 155° F. for 30 minutes. Care should be taken to insure that every particle of mix is subjected to this treatment.

Since any one of the ingredients of ice cream may be responsible for contamination, it is well to arrange for a check of the bacterial content of all products used in the ice cream mix.

**Skim milk powder** — Use only standard or extra grades.

**Gelatin**—Bacteriologists have reported bacterial counts of 10 to 108 million per gram. Only the better qualities should be permitted.

**Sugar**—Although this product is seldom a source of bacterial contamination, some investigators have, however, found high bacterial content in sugar. Therefore, it would seem best to make occasional counts of sugar as a safeguard.

**Flavoring and colors**—These are significant from a bacterial standpoint. Newman and Reynolds report counts of as high as 16,000,000 per cc.

**Egg and nuts**—These are a source of infection and should be watched. Investigators have found a variation of 620 to 45 million bacteria per gram.

**Milk and cream**—These should be of the same good quality as that used for fluid consumption.

A good rule is to have bacterial counts made of all products used in ice cream, particularly if the count of the ice cream is running high.

The problem of re-contamination is very complex because the ice cream is exposed to so much equipment, some of which is difficult to sterilize, and to human contact.

**Bacteriological Condition of Dye (Color) Solutions**

(According to Prucha, University of Illinois)

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. coll &amp; Mold per gram</td>
<td></td>
</tr>
<tr>
<td>1. Caramel color</td>
<td>0</td>
</tr>
<tr>
<td>2. Caramel color</td>
<td>0</td>
</tr>
<tr>
<td>3. Caramel color</td>
<td>0</td>
</tr>
<tr>
<td>4. Caramel color</td>
<td>0</td>
</tr>
<tr>
<td>5. Blue color</td>
<td>0</td>
</tr>
<tr>
<td>6. Blue color</td>
<td>+</td>
</tr>
<tr>
<td>7. Blue color</td>
<td>0</td>
</tr>
<tr>
<td>8. Green color</td>
<td>+</td>
</tr>
<tr>
<td>9. Green color</td>
<td>0</td>
</tr>
<tr>
<td>10. Green color</td>
<td>+</td>
</tr>
<tr>
<td>11. New York color</td>
<td>+</td>
</tr>
<tr>
<td>12. Yellow color</td>
<td>+</td>
</tr>
<tr>
<td>13. Red color</td>
<td>0</td>
</tr>
<tr>
<td>14. Orange color</td>
<td>0</td>
</tr>
</tbody>
</table>

**Equipment**

Equipment should be sterilized by steaming, scalding, or use of
suitable chemical solutions. Human contact should be eliminated as far as possible, and where it can not be avoided the health of the worker should be supervised and he should be compelled to practice hygienic habits.

_Treatment of equipment_ — The cooler presents the same problem as in milk cooling. Other equipment—aging cans, vats, tanks, freezers, packaging machines, and ice cream cans—all are sources of contamination unless they are sterilized properly. A. C. Fay mentions a case where the mix showed a count of 26,000 and the frozen product count was 625,000; undoubtedly the greater part of this increase was due to re-contamination.

Methods for sterilizing the freezer are substantially as follows: Run cold water through for a first rinse; follow this by warm water. Disassemble the freezer. Brush each part in warm water containing washing powder. Reassemble and steam; if desired, follow by chlorine rinse as an additional safeguard, using 100 parts per million. If necessary, the chlorine solution rinse may be used just before the mix is put into the freezer.

_Homogenization_ is apt to increase bacterial count in two ways:
1. It breaks up the groups or clumps of bacterial cells and thus facilitates their growth.
2. Bacteria are harbored in the machine. This must be controlled by efficient washing and sterilizing.

_Homogenizer_—From a sanitary point of view this machine presents a problem, because it can not be taken apart anywhere nearly as completely as other dairy equipment.

The cleaning procedure for this piece of equipment is about as follows:

1. Check the quality of all ingredients used.
2. See that the mix is pasteurized at a temperature of not less than 155° F. for 30 minutes.
3. See that there is as prompt freezing as possible of the mix after processing.
4. See that there are facilities for the careful sterilizing and cleaning of all ice cream equipment.
5. See that a competent person checks the quality and bacterial content of all fruits, syrups, flavors, and other ingredients used in the ice cream mix.
6. If cause of high count has not so far been located, arrange for line run tests to determine the source of contamination.

**The Human Equation**

It is well to bear in mind that the human equation is as important in checking on ice cream as for any other dairy food. In this connection the suggestions recently
made by F. W. Fabian may be timely and worthy of consideration.

"It has long been a custom to require that employees have health examinations once a year. This sounds good but hasn't always produced results. Recently New York City discontinued issuing medical certificates to food handlers. They say that this does not mean that they have become lax, but rather that they have become stricter in their enforcement. Now they require that each employer see to it that he maintains such standards that no one will be hired or retained as a food handler who will spread disease. They place the responsibility on both the employer and employee, and hold each jointly responsible for eliminating infected food handlers. Briefly, their experience in the food and dairy industry indicates:

1. That every employee should be required to have a complete medical examination by a competent physician and submit the necessary samples of blood, feces, and urine for laboratory examination, together with any other tests necessary, at the time he is employed and semi-annually thereafter. If the Board of Health doesn't require such an examination, the employer should, for his own protection.

2. That, supplementing the above examination and as an added protection, all employees coming in direct contact with food, milk or other dairy products should be examined regularly by a nurse, foreman, or some other competent person for evidences of contagious diseases. By means of education and intelligent application, many cases of potential or actual disease may be found in this way.

3. Whether this system or any other is used in trying to find persons suffering from contagious disease, they must not be penalized by being discharged or temporarily laid off without due compensation.

4. Only people who are inherently clean should be employed in the food or dairy industry. All new employees should be watched carefully until they have been so classified."

While these specific suggestions apply directly to ice cream, the milk sanitarian will of course realize that the general rules of sanitation also should apply to the inspection of ice cream quite as much as to any other food product that comes under his supervision.
The Phosphatase Test*

ARNOLD B. STORRS AND L. H. BURGWALD

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The importance of proper pasteurization of milk cannot be under-estimated, and a laboratory test for accurately determining the efficiency of the pasteurizing process used would be of utmost value.

Within recent years a number of tests have been developed for determining whether or not milk has been properly pasteurized. These tests have dealt with the inactivation of enzymes in milk due to pasteurization, and according to the investigators have shown much merit.

The first test developed was one dealing with the enzyme amylase and was reported by Rothenfusser in 1930. In 1932 Gould confirmed Rothenfusser's findings and suggested a modification. In 1934 Leahy reported another modification of the test and acclaimed it of great value in detecting improperly pasteurized milk and also pasteurized milk containing as little as one to two per cent added raw milk. Gilcreas and Davis reported in 1936 that they found the amylase test could not be relied on to detect accurately the degree of treatment.

Richardson and Hankinson in 1936 concluded after carefully controlled investigation that there were both alpha and beta amylase in milk in variable amounts and that alpha amylase is inactivated at 55°C for 30 minutes, but beta amylase maintains its original activity even after a period of 30 minutes at 65°C.

In 1935 Kay and Graham reported on the phosphatase test for detecting improperly pasteurized milk. They were able to detect a deficiency in pasteurization temperature of as little as 1.5°F.; a holding time of 20 minutes instead of 30 minutes, and of an admixture of as little as 0.25 per cent raw milk to properly pasteurized milk.

In 1936 Gilcreas and Davis reported the phosphatase test to be of much greater value in detecting improperly pasteurized milk than the amylase test. Using Kay and Graham's procedure modified only in the method of reading by preparing color standards containing known amounts of phenol instead of a tintometer and using a twenty-four hour incubation period, they easily detected variations in heating temperature. Variations of five minutes or more in heating time, and the addition of as little as 0.1 per cent raw milk to properly pasteurized gave results indicative of incomplete pasteurization. They were able to correctly determine the treatment to which milk was subjected in 97 per cent of samples submitted.

*Read before the Twenty-sixth Annual Meeting of the International Association of Milk Sanitarians, Louisville, Ky., October 11-13, 1937.
TABLE I
Preparation of Phenol Standards
(Gilcreas and Davis4)

<table>
<thead>
<tr>
<th>Phenol mg./0.5 ml.</th>
<th>Color Solution</th>
<th>ml.</th>
<th>ml.</th>
<th>ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>red§</td>
<td>0.106</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>red§</td>
<td>0.140</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>red§</td>
<td>0.180</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>red§</td>
<td>0.218</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>red§</td>
<td>0.238</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>red§</td>
<td>0.326</td>
<td>4.40</td>
<td></td>
</tr>
<tr>
<td>0.09</td>
<td>red§</td>
<td>0.360</td>
<td>5.70</td>
<td></td>
</tr>
<tr>
<td>0.12</td>
<td>red§</td>
<td>0.396</td>
<td>7.10</td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>red§</td>
<td>0.426</td>
<td>7.60</td>
<td></td>
</tr>
</tbody>
</table>

The amounts of color solutions given above are diluted to 10 ml. with distilled water.

†Gray color solution. Cobalt chloride (CoCl₂·6H₂O) 31.9 grams; copper sulphate (CuSO₄·5H₂O) 75.0 grams; concentrated hydrochloric acid, 32 ml.; concentrated sulphuric acid, 45 ml.; dissolved in distilled water and diluted to 500 ml.

‡Red color solution. Cobalt chloride (CoCl₂·6H₂O) 476 grams, dissolved in distilled water and filtered. 100 ml. concentrated hydrochloric acid added and diluted to one liter.

§Blue color solution. Copper sulphate crystals (CuSO₄·5H₂O) 300 grams and 20 ml. of concentrated sulphuric acid, dissolved in distilled water and diluted to one liter.

Note: Gilcreas and Davis⁴ concluded that the production of a phenol value equal to 0.037 mg. of phenol per 0.5 ml. of milk is equivalent to 2.3 standard Lovibond blue units.

Results obtained by the authors with this test were far from satisfactory. The procedure used was that suggested by Leahy⁵. One of the major objections to the test is the rapid fading of the color, making it impossible to measure its intensity, thereby preventing the detection of minor variations in the pasteurizing treatment. It was found impossible to detect less than from 2.0-4.0 per cent added raw milk to properly pasteurized milk.

PHOSPHATASE TEST
This test proved of immeasurably greater value than did the amylase test, and gives great promise of being a means whereby imperfect pasteurization may be detected.

The test as outlined by Kay and Graham⁶ can be adapted or modified for any pasteurization temperature by simply changing the incubation period. This could readily be established by any health department to fit their ordinance. Kay and Graham⁶ suggested the following incubation periods for different temperatures:

<table>
<thead>
<tr>
<th>Temperature of Pasteurization Degrees F.</th>
<th>Incubation Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>1 hr. 15 min.</td>
</tr>
<tr>
<td>141</td>
<td>1 hr. 30 min.</td>
</tr>
<tr>
<td>142</td>
<td>2 hr. 30 min.</td>
</tr>
<tr>
<td>143</td>
<td>4 hr. 20 min.</td>
</tr>
<tr>
<td>144</td>
<td>7 hr. 45 min.</td>
</tr>
<tr>
<td>145</td>
<td>24 hr. 0 min.</td>
</tr>
</tbody>
</table>

The tests herein reported were for the purpose of aiding in determining the value of the test in detecting improper pasteurization, and to determine whether the standards or limits suggested were satisfactory. To this end the test was applied to milk pasteurized in the laboratory under carefully controlled conditions at temperatures of 140, 142, 143, and 145⁰ F. and at holding periods of various lengths from 20 to 40 minutes.

The tests were also applied to milk pasteurized in the laboratory by the high temperature short time holding method. Temperatures ranging from 154.5 to 163.5⁰ F. for 10, 15, and 20 seconds holding.

Investigation of the possibility of interference of the test due to abnormal or unusual conditions of the raw milk prior to pasteurization was also attempted.
The procedure for the test as outlined by Kay and Graham to-gether with the precautions as outlined by Kay and Neave and Anderson, Herschdorfer and Neave and Scharer were closely followed.

This procedure is:

(a) **Reagents Required:**

1. **Buffer Substrate.** Dissolve 1.09 gms. of disodium phenyl phosphate and 11.54 gms. of "sodium veronal" (sodium diethyl barbiturate) in water saturated with chloroform and make up to one liter. Add a few drops of chloroform to prevent the growth of microorganisms, and keep in the refrigerator;

2. **Folin and Ciocalteu's phenol reagent.** Dissolve 100 gms. of sodium tungstate (Na₂WO₄ . 2H₂O) and 25 gms. of sodium molybdate (Na₂MoO₄ . 2H₂O) in 700 ml. of water in a 1,500 ml. flask connected, preferably by a ground glass joint, with a reflux condenser. Add 50 ml. syrupy (85 per cent) phosphoric acid and 100 ml. of concentrated hydrochloric acid. Reflux the mixture gently for 10 hours. (If an all-glass connection between flask and condenser is not available, use a rubber stopper or cork wrapped in tin foil. Take the greatest care that the solution does not come in contact with the tin foil.) After ten hours, cool, add 150 gms. pure lithium sulphate, 50 ml. water and a few drops (usually 4-6) of liquid bromine. Boil the mixture under the hood without the condenser for 15 minutes to boil off excess of bromine. Cool, dilute to one liter, and filter. The finished reagent should have a golden yellow color with no greenish tint. Any reagent with a greenish tint should be rejected. Keep well protected from dust. Dilute one volume of this stock solution with two volumes of water before use;

3. A 14 per cent solution of anhydrous sodium carbonate. All the chemical substances used should be of analytical reagent quality.

(b) **Special Apparatus Required.**

1. A Lovibond tintometer, or for most purposes a simple comparator with Lovibond Standard cell. (Color standards as prepared by Gilcreas and Davis may be used satisfactorily;

2. Whatman No. 42 filter papers, 9 cm. or acid-washed filter papers of similar quality;

3. An incubator maintained at 98.6-100.4° F.

Other than these, all that is required is a good supply of fairly stout test tubes of constant diameter (5/8 inch) with stoppers, small filter funnels to take the 9 cm. paper comfortably, and a beaker of boiling water.

(c) **The Technique of the Phosphatase Test:**

1. Run 10 ml. of the buffer-substrate solution into each of four test tubes of capacity 20-25 ml. To two tubes (control tubes) add 4.5 ml. of diluted Folin's reagent. To all four tubes add 0.5 ml. of the milk to be tested. Mix well. Add two drops of chloroform, and stopper each of the two tubes in which the milk has not been precipitated, warm to 98.6-100.4° F. and maintain at this temperature (a properly controlled bacteriological incubator will serve) for twenty-four hours;

2. The two tubes containing the control tests should not be incubated but should be carried through the remainder of the test immediately. After the addition of
the milk to the control tubes they should be allowed to stand for three minutes and then filtered. To 10 ml. of the filtrate add 2.0 ml. of the sodium carbonate solution, mix well and place in a boiling water bath for five minutes. Filter. The filtrate is placed in the standard 13 mm. Lovibond cell and the amount of blue color developed determined by comparison with standard glass slides. The controls should develop only a faint blue color. If more than 1.5 standard Lovibond blue units are produced the reagents and apparatus should be examined for traces of free phenol;

(3) At the end of the incubation period the tubes containing the milk under test should be removed from the incubator and cooled immediately in ice water. Then add 4.5 ml. of the diluted Folin's reagent to each tube, allow to stand for three minutes and proceed as given in (2) above to complete the test. If the color developed in the incubated tubes does not exceed 2.3 standard Lovibond units the milk may be said to be properly pasteurized. If more than 2.3 units are developed, then the milk has been improperly pasteurized.

With some experience it will be found possible to eliminate the control test in most instances. They need only be run in cases where there is reason to believe that either the sample may contain free phenol or that the reagents or apparatus may be contaminated.

In the test as described above disodium phenyl phosphate is employed as a substrate. The action of the enzyme phosphatase upon this substrate results in the liberation of free phenol. The amount of phenol thus liberated is then determined colorimetrically by the use of Folin's reagent. The buffer employed, sodium diethyl barbiturate, changes the reaction at which the test takes place to about pH 9.8.

Scharer found that disodium phenyl phosphate often contained considerable free phenol and suggested that the salt should be washed with ethyl ether until the washings gave a negative test for phenol. In the latter portion of the work reported in this paper the disodium phenyl phosphate used was washed three times in ethyl ether, air dried briefly, followed by drying in a dessicator and then stored in a refrigerator.

CARE OF REAGENTS AND APPARATUS

Folin's phenol reagent. This must be prepared according to the directions given previously. The stock solution is stable for at least four months while the diluted reagent undergoes no change over a period of one week.

Buffer substrate. If this solution is kept saturated with chloroform and stored in a refrigerator no hydrolysis appears to occur in at least nine weeks.

Pipettes. Any errors in the measurement of the 0.5 ml. of milk will greatly affect the accuracy of the results; therefore, only pipettes of known accuracy should be used.

Test tubes. Test tubes marked at 10 ml. should be used to receive the first filtrate, the maximum error tolerated being ± 0.1 ml. Tubes should be calibrated before use as some of those offered on the market often show more than this degree of error.

Cleaning of glassware. It has been recommended that all glassware used in the test be washed thoroughly with hot soda solution and rinsed with distilled water. All glassware used in this investiga-
tion was cleaned in a dichromate cleaning solution and, in this instance, tap water was found to be satisfactory as a rinse.

It need hardly be mentioned that care must be taken to keep phenols, disinfectants containing phenols, and soap containing carbolic acid at a safe distance from the test reagents and apparatus used.

**Experimental Methods and Procedure**

**Preparation of Samples**

Pasteurization of samples for experimental purposes was done in the laboratory, over a gas flame and with manual control. Temperature variation did not exceed ± 0.5° F. from the stated temperature in any instance. All samples were cooled in ice water immediately after pasteurization.

At the start of the investigation the samples were pasteurized in test tubes in a water bath. The preheating time was approximately five minutes.

The last portion of the samples pasteurized by the holding method were pasteurized in sealed tubes, prepared as follows: The closed end of a small test tube (3 x 3/8 inches or 4 x 1/2 inches is satisfactory) is placed in the flame of a burner, preferably of the Fisher type, and heated until soft. A bulb is then blown at the end of the tube, taking care not to get the glass too thin. The diameter of this bulb should be approximately 3/4 inch. When the tube has cooled the neck is placed in the burner flame until soft when it is stretched out to about an inch longer than the original length of the tube. The tube is then ready to be filled with milk. After placing about 5 ml. of milk in the tube it is sealed by heating the narrow portion of the neck in the burner flame. When
using these tubes for pasteurizing samples they were totally immersed in the water bath by attaching weights to them. When making up these samples a three-minute preheating time was found to be satisfactory. Within that time the milk contained in the tubes reached the temperature of the water bath in which they were immersed. The desired temperature was maintained by controlling the temperature of the water bath within ±0.5° F.

For preparing samples of milk pasteurized by the short-time high-temperature method, a miniature "flash" pasteurizer was constructed from glass tubing. The details of this unit are shown in the accompanying illustration. A brief description of the equipment follows:

The apparatus was so arranged that the milk flowed through the equipment by gravity, the rate of flow being regulated by an adjustable petcock in the raw milk line. From the raw milk reservoir the milk flowed into a preheater made up of about eleven feet of glass tubing and contained in a water bath. From the preheater the milk passed into a holding chamber into which a thermometer was placed. The entire holding chamber unit was immersed in a separate water bath maintained at the desired temperature. The milk then entered the cooling coils in a bath of ice water. Samples were taken into test tubes at the outlet, after cooling.

The preheating time in this equipment was approximately 40 seconds and the temperature of the water bath for the preheater did not exceed the temperature to which it was desired to heat the milk by more than 5° F.

In order to control the conditions of pasteurization the rate of flow was first adjusted to one ml. of milk per second. The capacity of the holding chamber was adjusted to a known volume. This was accomplished by interchangeable lengths of glass tubing which could be used in the holding chamber unit at will. For example, if the length of the holding period to be used were 15 seconds a section of tubing would be added to the holding chamber unit such that the total capacity of that unit would be 15 ml. Thus, with the rate of flow adjusted to one ml. per second, with a 15 ml. capacity, the length of the holding period would therefore be 15 seconds.

In preparing samples with this equipment three different holding periods were used—10, 15 and 20 seconds.

(a) The Determination of a Proper Standard for Properly Pasteurized Milk

In England where this test was developed the time and temperature requirements for proper pasteurization are 145° F. for 30 minutes. Kay and Graham suggested that the best means of modifying the test to the lower temperature requirements common in this country was to shorten the period of incubation used and retain the same limit for color development, i.e., 2.3 Lovibond units of blue. However, before proceeding further with this investigation it was thought advisable to make certain about this point.

Therefore, a number of samples from various sources were pasteurized in test tubes in the laboratory as described under preparation of samples. All samples were
### TABLE IV
Summary of results of pasteurization of samples at 140° F. for varying holding periods and using different incubation periods for testing.

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>24 hours</th>
<th>5 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holding period in minutes</td>
<td>20 25 30 40</td>
<td>20 25 30 40</td>
<td>20 25 30 40</td>
</tr>
<tr>
<td>Series No.</td>
<td>Lovibond blue units</td>
<td>Lovibond blue units</td>
<td>Lovibond blue units</td>
</tr>
<tr>
<td>30</td>
<td>78.0 7.4 4.3 3.1 6.6 6.5 3.8</td>
<td>8.0 8.0 8.0</td>
<td>8.0 8.0 8.0</td>
</tr>
<tr>
<td>31</td>
<td>&gt;8.0 6.7 5.0 3.3 7.6 6.9 3.8</td>
<td>&gt;8.0 8.0 8.0</td>
<td>&gt;8.0 8.0 8.0</td>
</tr>
<tr>
<td>32</td>
<td>78.0 5.4 3.7 2.9 6.0 4.8 3.3</td>
<td>8.0 8.0 8.0</td>
<td>8.0 8.0 8.0</td>
</tr>
<tr>
<td>33</td>
<td>&gt;8.0 - 4.4 2.8 6.0 - 6.6 3.7 6.0 - 7.0 8.0</td>
<td>&gt;8.0 8.0 8.0</td>
<td>&gt;8.0 8.0 8.0</td>
</tr>
<tr>
<td>34</td>
<td>- 4.4 4.4 3.0 - 6.6 6.6 3.5 - &gt;8.0 8.0 8.0</td>
<td>&gt;8.0 8.0 8.0</td>
<td>&gt;8.0 8.0 8.0</td>
</tr>
<tr>
<td>35</td>
<td>78.0 6.4 4.5 3.0 6.0 7.6 4.6 6.0 7.0 8.0 8.0</td>
<td>7.0 8.0 8.0</td>
<td>7.0 8.0 8.0</td>
</tr>
<tr>
<td>36</td>
<td>6.2 4.8 3.45 2.55 6.0 7.0 5.0 6.0 7.0 8.0 8.0</td>
<td>3.6 8.0 8.0 8.0</td>
<td>8.0 8.0 8.0</td>
</tr>
<tr>
<td>37</td>
<td>78.0 6.2 4.8 2.8 6.0 8.0 7.2 4.4 6.0 8.0 8.0</td>
<td>3.4 8.0 8.0</td>
<td>8.0 8.0 8.0</td>
</tr>
<tr>
<td>38</td>
<td>5.0 6.5 4.6 2.8 6.0 8.0 5.4 6.8 8.0 8.0 8.0</td>
<td>2.9 6.0 8.0 8.0</td>
<td>7.0 8.0 8.0</td>
</tr>
<tr>
<td>39</td>
<td>6.8 5.0 3.2 2.3 6.0 7.6 5.2 2.9 6.0 8.0 8.0</td>
<td>6.8 8.0 8.0</td>
<td>6.8 8.0 8.0</td>
</tr>
<tr>
<td>Average</td>
<td>8.0 5.8 4.25 2.87 6.0 8.0 6.35 3.72 6.0</td>
<td>8.0 8.0 8.0</td>
<td>8.0 8.0 8.0</td>
</tr>
</tbody>
</table>

>8.0 = Color development greater than 8.0 units. Actual color not determined.

### TABLE V
Summary of results of pasteurization of samples at 142° F. for varying holding periods and using different incubation periods for testing.

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>24 hours</th>
<th>5 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holding period in minutes</td>
<td>20 25 30 40</td>
<td>20 25 30 40</td>
</tr>
<tr>
<td>Series No.</td>
<td>Lovibond blue units</td>
<td>Lovibond blue units</td>
</tr>
<tr>
<td>30</td>
<td>3.4</td>
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<td>3.05 2.2</td>
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<td>2.5 2.2</td>
</tr>
<tr>
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<td>2.45 2.2</td>
</tr>
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<td>3.5</td>
<td>2.6 2.3</td>
</tr>
<tr>
<td>35</td>
<td>3.6</td>
<td>2.4 1.8</td>
</tr>
<tr>
<td>36</td>
<td>2.3</td>
<td>2.1 1.7</td>
</tr>
<tr>
<td>37</td>
<td>3.4</td>
<td>2.5 2.1</td>
</tr>
<tr>
<td>38</td>
<td>3.05</td>
<td>2.5 2.1</td>
</tr>
<tr>
<td>39</td>
<td>2.6</td>
<td>1.7 1.65</td>
</tr>
<tr>
<td>Average</td>
<td>3.25</td>
<td>2.41 2.04</td>
</tr>
</tbody>
</table>

>8.0 = Color development greater than 8.0 units. Actual color not determined.
### TABLE VI
Summary of results of pasteurization of samples at 143°F for varying holding periods and using different incubation periods for testing.

<table>
<thead>
<tr>
<th>Holding period in minutes</th>
<th>2½ hours</th>
<th>Incubation periods 5 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Series No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
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<tr>
<td>31</td>
<td>1.55</td>
<td>1.6</td>
<td>1.6</td>
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<td>1.4</td>
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<td>1.7</td>
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<td>1.8</td>
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<td>35</td>
<td>1.4</td>
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<td>1.4</td>
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<td>1.6</td>
<td>1.45</td>
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<td>1.3</td>
<td>1.2</td>
<td>1.25</td>
</tr>
<tr>
<td>39</td>
<td>1.35</td>
<td>1.35</td>
<td>1.4</td>
</tr>
<tr>
<td>Average</td>
<td>1.54</td>
<td>1.5</td>
<td>1.46</td>
</tr>
</tbody>
</table>

### TABLE VII
Summary of results of pasteurization of samples at 145°F for varying holding periods and using different incubation periods for testing.

<table>
<thead>
<tr>
<th>Holding period in minutes</th>
<th>2½ hours</th>
<th>Incubation period 5 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Series No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.3</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>31</td>
<td>2.6</td>
<td>2.2</td>
<td>1.95</td>
</tr>
<tr>
<td>32</td>
<td>2.05</td>
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</tr>
<tr>
<td>33</td>
<td>2.3</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>34</td>
<td>2.4</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>35</td>
<td>2.0</td>
<td>1.55</td>
<td>1.3</td>
</tr>
<tr>
<td>36</td>
<td>2.0</td>
<td>1.6</td>
<td>1.55</td>
</tr>
<tr>
<td>37</td>
<td>2.1</td>
<td>1.75</td>
<td>1.6</td>
</tr>
<tr>
<td>38</td>
<td>2.1</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>39</td>
<td>1.75</td>
<td>1.5</td>
<td>1.55</td>
</tr>
<tr>
<td>Average</td>
<td>2.16</td>
<td>1.85</td>
<td>1.71</td>
</tr>
</tbody>
</table>

Levi bond blue units
heated for 30 minutes at 142° F. Incubation periods of three different lengths were used: 2½ hours, 5 hours and 24 hours. These were then tested in the usual manner with the following results.

Forty-five samples were tested using a 2½ hour incubation period. The minimum blue color developed in any test was 1.4 Lovibond blue units, the maximum was 2.3 units and the average was 1.81 units. Thus it can be seen that the maximum amount of blue color allowable in properly pasteurized milk was developed within 2½ hours in a few instances at least.

Thirty-six samples were tested using a 5 hour incubation period. In this series the minimum color development was equivalent to 1.6 blue units, the maximum 3.4 units and the average 2.22 units. This represented an average increase over the 2½ hour incubation period of 22.7 per cent. Also, 44 per cent of the samples developed more than 2.3 blue units.

Twenty-one samples were tested using a 24-hour incubation period. Of these the minimum blue value was 2.6 units, maximum 8.0 units and the average 4.14 units. The increase in blue color over the samples incubated for 2½ hours was 128.7 per cent and was 86.5 per cent higher than those incubated for 5 hours. Since in this series not one sample developed as little as 2.3 Lovibond blue units it is quite obvious that a 24 hour incubation period is far too long for use with milk pasteurized at 142° F. for 30 minutes. This is the minimum requirement for pasteurization in many cities.

These results indicate that for milk pasteurized under these conditions, a period of incubation longer than 2½ hours but not less than 5 hours, could be used without resulting in incorrect findings on some properly pasteurized samples.

(b) Application of the Test to Milk Pasteurized by the Holding Method at Various Temperatures and for Varying Holding Periods.

The milk used in this portion of the work was pasteurized in the laboratory in sealed tubes as described previously. The raw milk was obtained from patrons' milk received at the University Creamery. Ten series of samples were prepared and tested as follows:

Samples of each raw milk were pasteurized at 140° F., 142° F., 143° F. and 145° F., with holding periods of 20, 25, 30 and 40 minutes at each pasteurization temperature. This made a total of sixteen samples in each series and all raw milk used in each series was from the same source. In testing, three incubation periods were employed with each series—2½, 5, and 24 hours. The relative phosphatase content of each raw milk used was determined as well as the butterfat content.

The results of this work are summarized in Tables IV-VII and displayed graphically in Charts I-III.

A study of the data reveals that when a 2½ hour incubation period was used in the test the following results were obtained:

1. Of all samples heated at 140° F., every one gave a reaction indicative of underpasteurization with the exception of one sample which was held at 140° F. for 40 minutes.

2. At a pasteurization temperature of 142° F. nine out of ten samples held for 20 minutes were found to be underpasteurized, while with a twenty-five-
CHART I
Development of color by series of prepared samples with a 2½ hour incubation period

CHART II
Development of color by series of prepared samples with a 5 hour incubation period

CHART III
Development of color by series of prepared samples with a 24 hour incubation period
minute holding period seven out of ten samples were classified as being improperly pasteurized. All samples held for 30 minutes or longer were within the limits of proper pasteurization.

3. Of the samples pasteurized at 143° F. only two which were held for 20 minutes were classified as being improperly pasteurized.

4. All samples pasteurized at 145° F. gave indication of proper pasteurization.

Using a 5 hour incubation period (slightly longer than that recommended by Kay and Graham for milk supposedly pasteurized at 143° F. for 30 minutes) the results were as follows:

1. In no instance was milk pasteurized at 140° F. found to be acceptable.

2. At a pasteurization temperature of 142° F. all samples held for 20 minutes, nine out of ten held for 25 minutes, four out of ten held for 30 minutes and one out of nine held for 40 minutes were classified as being improperly pasteurized.

3. At 143° F. eight out of ten samples held for 20 minutes and two out of ten held for 25 minutes were found to be underpasteurized. All samples held for 30 minutes or longer were classified as being properly pasteurized.

4. All samples pasteurized at 145° F. fell within the limits of proper pasteurization.

If a 24 hour incubation period is used for testing milk supposedly pasteurized at 143° F. for 30 minutes as Gilcreas and Davis have done, the accuracy of the test with respect to detection of underpasteurized milk is greatly increased. However, some instances of a development of blue color greater than the limit of 2.3 Lovibond units with samples pasteurized at 143° F. for 30 minutes did occur in this portion of the work when testing by this method. Although the limit of 2.3 units was not exceeded by more than 0.2 unit in any case, the fact remains that if the heat treatment to which these samples had been subjected were unknown they would be classified as being improperly pasteurized. It is not the purpose of this paper to criticize such a testing procedure but merely to point out the fact that if such methods are employed some cases will in all probability arise wherein properly pasteurized milk will be classified as being underpasteurized.

The results when using a 24-hour incubation period were:

1. All samples pasteurized at 140° F. were found to be underpasteurized.

2. Of those pasteurized at 142° F. all but two which were held at the pasteurizing temperature for 40 minutes were classified as being improperly pasteurized.

3. All samples pasteurized at 143° F. and held for 25 minutes or less were found to be underpasteurized. Of those held for 30 minutes five samples, exactly half, were classified as being improperly pasteurized. All samples held for 40 minutes gave a reaction indicative of proper pasteurization.

4. All samples pasteurized at 145° F. for 20 minutes or longer were classified as being properly pasteurized.

It should be noted that all samples tested in this investigation which were pasteurized at 145° F. for 20 minutes or longer developed a blue color less than the specified standard of 2.3 units. This does not agree with the results of Kay and Graham who reported the ability to detect milk pasteurized at this temperature for 20 minutes or less.

(c) The Relation of the Phosphatase Content to the Butterfat Content of Raw Milk and Their Effect on Results Obtained on the Pasteurized Milk

It was thought when the preceding portion of the work was started
that the phosphatase content of the raw milk used as well as the butterfat content might exert some influence on the results obtained with the pasteurized samples. Therefore, in each series of samples tested a phosphatase determination was run on the raw milk and the butterfat content was also determined, either by testing the milk used or by taking the result of the composite sample test for the period during which the milk was taken. For purposes of comparison the phosphatase content and the butterfat content of the raw milk were compared with a figure which was termed the Average Phenol Value of all of the pasteurized samples within a given series. The Average Phenol Value was arrived at by averaging all of the results obtained from the pasteurized samples within a series; in all cases where the color development exceeded 8.0 Lovibond blue units the color value was assumed to be 8.5 units for the purposes of calculating results. The Average Phenol Value arrived at in this manner has no other significance than as an index of the comparative amount of color development in the pasteurized samples tested in this particular experiment. The results are shown in Table VIII and are arranged in the order of increasing Average Phenol Value.

Unfortunately the number of samples included in these results is rather small. There is a noticeable lack of correlation between the factors considered. Although a more comprehensive examination of these factors might show some definite trend it can be concluded from the data presented that the relative phosphatase content of the raw milk as well as the butterfat content exert little if any influence upon the proportionate development of color in pasteurized samples.

(d) Application of Test to Milk Pasteurized by the Short-Time High-Temperature Method

The milk used in this portion of the work was pasteurized in the laboratory in the "flash" pasteurizing apparatus previously described. The temperature range covered in the experiment was from 154.5 to 163.5° F. and three holding periods—10, 15, and 20 seconds—were studied. Also, the prepared samples were tested using two different incubation periods, 2½ and 24 hours.

The results are expressed graphically in Charts I, II, and III. Several series of samples are repre-

<table>
<thead>
<tr>
<th>Series No.</th>
<th>Average Phenol Value</th>
<th>Phosphatase content raw milk</th>
<th>Butterfat content</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>3.07</td>
<td>330</td>
<td>6.2</td>
</tr>
<tr>
<td>36</td>
<td>3.20</td>
<td>260</td>
<td>4.2</td>
</tr>
<tr>
<td>34</td>
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</tr>
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<td>33</td>
<td>3.39</td>
<td>280</td>
<td>4.9*</td>
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<td>3.50</td>
<td>245</td>
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<td>35</td>
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<tr>
<td>30</td>
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</tr>
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</tr>
<tr>
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<td>4.75</td>
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<tr>
<td>31</td>
<td>4.09</td>
<td>335</td>
<td>4.4*</td>
</tr>
</tbody>
</table>

*Butterfat determined from composite sample test.
CHART I
Amount of blue color produced by samples of milk pasteurized at various temperatures with a 10 second holding period.

CHART II
Amount of blue color produced by samples of milk pasteurized at various temperatures with a 15 second holding period.

CHART III
Amount of blue color produced by samples of milk pasteurized at various temperatures with a 20 second holding period.
presented on each chart, each separate series being indicated by a capital letter. All series bearing the same letter are from the same raw milk.

If 160° F. for a period of 15 seconds is considered as fulfilling the requirements of proper pasteurization, then it will be seen by referring to the charts that if a 2 1/2 hour incubation period is used in the test the results would be interpreted as follows:

1. Milk pasteurized for 10 seconds at 160° F. or higher would be classified as being properly pasteurized.

2. Samples pasteurized within the range of 157 to 160° F. or higher for 15 seconds would be classified as having been properly pasteurized.

3. Milk held for 20 seconds at 158.5° F. or higher would fall within the limits of proper pasteurization.

If a 24 hour incubation period were employed in the test, however, the results would be quite different. They might be tabulated as follows:

1. With a 10 second holding period a pasteurizing temperature of approximately 163° F. or higher would be required in order to produce a properly pasteurized reaction to the test.

2. For milk held for 15 seconds the pasteurizing temperature range necessary in order to give a reaction indicative of proper pasteurization would be from 159.5 to 162° F. or higher.

3. Samples pasteurized for 20 seconds at approximately 169° F. or higher would be considered as being properly pasteurized.

Thus, it can be seen that the adjustment of the phosphatase test to milk pasteurized by the so-called “flash” method is apparently quite delicate. On the basis of the data obtained (insufficient to draw conclusions, however) it appears that the length of the incubation period used for the test can be extended beyond 2 1/2 hours without getting incorrect results on some properly pasteurized samples.

More tests are to be made on this method of pasteurization.

(d) DETECTION OF ADDED RAW MILK TO PROPERLY PASTEURIZED MILK

The possibility of contamination of properly pasteurized milk with raw milk must not be overlooked. In this respect Kay and Graham claimed the ability to detect 0.25 per cent added raw milk, while Gilceas and Davis reported that they were able to detect the presence of as little as 0.1 per cent of added raw milk in properly pasteurized milk.

**TABLE IX**

Detection of Added Raw Milk

Summary of Three Series

<table>
<thead>
<tr>
<th>Percent of raw milk added</th>
<th>Series A</th>
<th>Series B</th>
<th>Series C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1.25</td>
<td>1.35</td>
<td>1.2</td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td>1.4</td>
<td>1.35</td>
</tr>
<tr>
<td>0.2</td>
<td>2.35</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>0.3</td>
<td></td>
<td>1.95</td>
<td>1.5</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td>2.65</td>
<td>1.6</td>
</tr>
<tr>
<td>0.6</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>2.45</td>
<td>2.4</td>
<td>1.55</td>
</tr>
<tr>
<td>0.8</td>
<td>3.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>6.6</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>100.0</td>
<td>Too great to measure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>5.0</td>
<td>4.7</td>
<td>2.75</td>
</tr>
<tr>
<td>1.0</td>
<td>3.5</td>
<td>3.05</td>
<td>2.05</td>
</tr>
</tbody>
</table>

We need to make more tests on this phase before we can draw any conclusion. So far we have made only three series of tests for detecting the presence of added raw milk. In the first series 0.2 per cent added raw milk was detectable. In the second series between 0.3 and 0.4 per cent was detectable and in the third series the smallest amount detectable was between 1.0 and 2.0 per cent. Probably this third sample of milk contained only a very small amount of phosphatase.
In all of these series the milk was pasteurized at 142° F. for 30 minutes and a 2½ hour incubation period was used in testing. No determinations were made of the phosphatase content of the raw milk used.

**Modified Phosphatase Tests**

Quite recently two modifications of the phosphatase test have been suggested by the Department of Health of New York City, one a short field test (4) and the other having a somewhat longer procedure and being better adapted for laboratory use (10). The same reagents are employed in both cases. No color standards are required for the field test but phenol standards are used in interpreting the results of the longer method. Since a very brief investigation of these tests is included in this paper the necessary reagents and methods of procedure are described here.

(1) Reagents

Dibromoquinonechloromide Solution, referred to subsequently as BQC (Eastman Kodak Co. Chemical No. 2304). Dissolve 0.04 gram in 10 ml. of 95 per cent ethyl alcohol. When tightly stoppered and stored in the refrigerator this solution is stable for several days. It was found convenient to keep this solution in an Owens 1/4 ounce green glass pharmaceutical dropper bottle (Design No. 90943). This size dropper bottle delivers 50 drops to the ml. of the alcohol solution of BQC.

Borate buffer solution. Dissolve 1.09 grams of washed and dried disodium phenyl phosphate in 900 ml. of distilled water previously saturated with chloroform. Add 50 ml. of Borate Buffer solution and dilute to a liter with distilled water. Add a few drops of chloroform and store in the refrigerator. The pH of this buffer substrate is about 9.6 and should be checked, using Thymol Blue as an indicator.

Basic lead acetate (prepared by the Horne Method A. C. S. specifications). Boil 280 grams of the dry basic lead acetate in 500 ml. of water for 10 minutes. Cool, allow to settle, filter and dilute to 500 ml.

Phenol standards. Prepare a stock solution of pure phenol in distilled water such that one ml. contains one mg. of phenol. This reagent was standardized by titration with N/10 Bromine in accordance with the method outlined in the U.S.P. Dilute the stock solution to prepare the following phenol solutions:

<table>
<thead>
<tr>
<th>% Gamma of phenol per ml. (1/4 p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

To 5 ml. of each of these phenol solutions, add ½ ml. of the borate buffer solution and two drops (1/25 ml.) of the solution of BQC. Stopper tightly with rubber stoppers. The blue color of the indophenol reaction will develop in a few minutes; the smaller the amount of phenol present, the less rapid the development of color. These color standards, if stored in a refrigerat-
tor when not in use and if kept out of direct sunlight when used, have been found to remain relatively permanent for several months.

It is proposed for convenience to identify these color standards by the number of Gamma of phenol present in each. Thus a phenol standard containing $\frac{1}{2}$ gamma of phenol per ml. would contain 2$\frac{1}{2}$ gamma in 5 ml. or 2$\frac{1}{2}$ units of color. The standard containing 1 gamma of phenol per ml. would contain 5 gamma in 5 ml. or 5 units of color, etc.

Blanks should be run on the reagents daily. If the blank produces any blue color the solution of buffer substrate should be discarded. Other precautions, previously enumerated for the Kay and Graham test, should be observed.

(2) Method for the Field Test

To 10 ml. of the buffer substrate in a test tube add 1 ml. of milk or cream (for this purpose a short 1 ml. throw pipette can be utilized). Cork; shake for about a minute. Place in vest pocket or a water bath at around 100° F. for five to ten minutes.

After this incubation period remove test tube, add 10 drops (1/5 ml.) of BQC. Cork; shake gently by inversion. Wait five minutes, then examine sample. The appearance of any blue indicates that the sample has not been properly pasteurized. Raw milk or cream will be very dark blue. Properly pasteurized milk will be gray or light brown. Properly pasteurized cream will be white or gray.

(3) Method for the Longer Test

By means of a pipette transfer 1 ml. of the milk to be tested to a pyrex test tube; the pipette should be plugged with cotton to prevent contamination with saliva. It was found convenient for rapid work to use a pipette not graduated to the tip. Add 10 ml. of the buffer substrate. Shake well. Incubate for one hour at 37.5° C. After incubation place the tubes in boiling water for five minutes. Cool to room temperature or below. Add 0.1 ml. of basic lead acetate solution. Shake well by placing the thumb over the mouth of the tube. Allow to stand for a minute or two. The proteins will coagulate and separate sharply (note: in some instances it may be necessary to add an additional 0.05 ml. of lead acetate to completely precipitate the protein). Filter; to 5 ml. of the clear filtrate add 0.25 ml. of the borate buffer. Add 2 drops or 0.04 ml. of BQC and shake gently.

The blue color of the indophenol reaction develops within five minutes; the greater the amount of phenol the more rapid the development. The color produced is then compared with the phenol color standards and reported as phosphomonoesterase units, a unit being the amount of enzyme which, under conditions of the test, would produce the color equivalent of 1 gamma of phenol. The phenol standard containing 5 gamma of phenol would be equivalent to 5 units and so on.

<table>
<thead>
<tr>
<th>Temperature °F.</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>138</td>
<td>250-500 units</td>
</tr>
<tr>
<td>140.5</td>
<td>100 units</td>
</tr>
<tr>
<td>142</td>
<td>10-15 units</td>
</tr>
<tr>
<td>143</td>
<td>2$\frac{1}{2}$ units</td>
</tr>
</tbody>
</table>

A comparison was made of these tests with Kay and Graham test. Although the tests made were limited in number, it would appear that the modified laboratory test would check favorably with the
Kay and Graham test and was a considerable time saver.

The short method or field test could readily be used for picking out gross errors or variations in the pasteurizing process. Its simplicity would make it an ideal test for that purpose.

**SUMMARY AND CONCLUSIONS**

The results of the application of the test to milk pasteurized by the holding method at various temperatures and for varying length holding periods may be summarized as follows:

1. Using a 2½ hour incubation period the following samples were classified as being properly pasteurized: (a) all samples pasteurized at 140° F. except one out of ten held for 40 minutes; (b) 90 per cent of the samples held for 20 minutes at 142° F.; (c) 70 per cent of the samples pasteurized at 142° F. for 25 minutes.

2. By employing a 5 hour incubation period the following samples were classified as being improperly pasteurized: (a) all samples heated at 140° F.; (b) all samples heated at 142° F. for 20 minutes, 90 per cent of those held for 25 minutes, 40 per cent of those held for 30 minutes and 11 per cent of those held for 40 minutes; (c) 80 per cent of the samples pasteurized at 143° F. for 20 minutes and 20 per cent of those held for 25 minutes.

3. Using a 24 hour incubation period the following samples were classified as being underpasteurized: (a) all samples heated at 140° F.; (b) all samples pasteurized at 142° F. except two out of nine held for 40 minutes; (c) all samples pasteurized at 143° F. for 25 minutes or less; (d) 50 per cent of those held at 143° for 30 minutes.

In view of these findings it is recommended that a 5 hour incubation period be used for testing milk supposedly pasteurized at 142° F. for 30 minutes and that a 24 hour incubation period should be used where the legal limits of pasteurization are 143° F. for 30 minutes.

These incubation periods are recommended so as to detect all cases of underpasteurization, even though it means that some cases of properly pasteurized milk may give reactions indicative of underpasteurization. Such cases could readily be checked by requiring all pasteurizing plants to save a sample of the raw milk from all vats each day and hold in refrigerator until afternoon of the next day, pasteurization of the raw sample in the laboratory under carefully controlled conditions, and comparing the color developed on these samples with that obtained on the plant pasteurized sample. Under these conditions any incubation period could be used, and no color standards would be required. If the plant pasteurized sample developed a deeper blue color than the laboratory pasteurized sample, it would indicate underpasteurization.

Another factor would be to shorten the incubation time and reduce the phenol value in proportion.

The test was also found to be applicable to milk pasteurized by the high temperature short time holding system. In the few tests made by this method a 2½ hour incubation period was found to be proper for milk pasteurized at 160° F. for 10 seconds. When milk is required to be held at 160° F. for 15 seconds, the 2½ hour incubation period was too short, permitting some underpasteurized milk to be included. A 24 hour incubation period was too long, none of the samples meeting the requirements of 2.3 Lovibond blue units. A 24 hour incubation period was suitable, however, for milk held for
20 seconds at 160° F., all samples meeting the requirements.

There was no relationship between butterfat content in milk samples (mixed herd milk) and phosphatase content. Neither was there any relationship between phosphatase content of raw milk and the blue color developed in the pasteurized samples by this test.

The modifications suggested by the Department of Health, New York City, gave promise of having considerable merit.

REFERENCES
Discussion of the Use of the Phosphatase Test

Paul F. Krueger

Director, Bureau of Dairy Products, Chicago Board of Health, Chicago, Ill.

During the past year the phosphatase test has been applied in Chicago to all samples of milk routinely collected by the Chicago Board of Health. Previous to that time, attempts had been made to use other tests, but each of them were found, at times, to give inaccurate and misleading results. After intensive study, it was found that the phosphatase test could be relied upon to give an accurate report of the conditions surrounding the pasteurization process. The method used for the test was Kay and Graham's original recommendation, the incubation time used being eight hours to correspond to the pasteurization temperature of 144°F. The tintometer was used in recording the color reactions.

It was soon demonstrated to our satisfaction that if milk were handled in accordance with our requirements, which are essentially those of the United States Public Health Service for grade A pasteurized milk and milk products, no sample of milk or cream should react to the phosphatase test; if it does, something is wrong. The particular item to be corrected may not always be easily discovered but eventually it can be found.

Our first use of the test showed a considerably larger number of improperly pasteurized samples than we believed should have been true. With the intensive use of the phosphatase test, it was possible to so reduce the number of samples of pasteurized milk which were shown to be improperly pasteurized that today their occurrence is indeed a rarity.

The cause for the illegal samples first discovered may be briefly summarized as follows:

Of greatest magnitude was our finding that the milk plant operator failed to hold the milk for the full 30-minute period, although the recording thermometer chart apparently showed compliance. The reason for the chart not showing the violation was due, in most instances, to the fact that the operator moved the recorder chart by hand to falsely indicate the proper holding time. In other cases it was found that the outlet valve of the pasteurizer had been opened before the thirty-minute period had elapsed.

Inasmuch as all milk pasteurization equipment in Chicago is equipped with air-space heating devices, most thermometer chart temperature records show very little deviation during the holding time so that fraudulent "pulling" of the chart is difficult to detect.

In order to make it extremely difficult for the operator to make a fraudulent chart, it was required that all recording thermometers be equipped with small pins in the hub of the instrument to which the
charts were attached, so that punctures would be made when the chart was placed on the recorder. If the chart were then tampered with, after being placed on the instrument, either a duplicate set of perforations would occur or the paper would be torn. This correction has been found to be very satisfactory. In order to prevent the outlet valve of the pasteurizer from being opened before the end of the 30-minute holding period, we are at present experimenting with a number of devices which will record outlet valve operation, but as yet cannot be considered practical for use on all installations.

In some cases milk was being improperly pasteurized although the operator of the equipment did not know that any defect existed in the pasteurizing equipment or in its operation. In several instances it was found that the raw milk inlet valve to the vat was not fully closed or was leaking during the holding period, or the pasteurizer outlet valve was not fully closed. In one instance it was found that the outlet valve of a pasteurizer was not closed after the tank was emptied and before the milk inlet valve was opened. In a few cases it was found that pasteurized milk was run through equipment that had previously been used for raw milk and which had not been thoroughly cleaned and sterilized between usages. It was found, in a few installations, that small amounts of milk or cream were pasteurized in a large vat in which the indicating and recording thermometer bulbs did not reach the surface of the liquid. The result was that the air temperature was recorded on the chart and not the temperature of the milk or cream. The use of air-space heating equipment would naturally record a higher temperature than had been reached by the milk or cream.

Only two instances of improperly pasteurized milk were found where automatic equipment was used and both of these defects were shown on the recording thermometer charts. One instance was caused by improper heating of the holder before it was filled with heated milk at the start of the run, and a second instance was due to the fact that the outlet valve of a tubular holder was kept open while being filled with milk at the start of the run.

The phosphatase test may also be applied to cream and most other dairy products with equal accuracy. It may also be used for milk pasteurized by the high-temperature short-time method. The phosphatase test should not be considered as a device that will do away with the necessity for routine milk plant inspection, but should be considered as an extremely invaluable aid to the health officer in insuring the proper pasteurization of all milk and cream.

Dr. Tobey Assumes New Duties

Dr. James A. Tobey, Director of Health Service of The Borden Company for the past eleven years, resigned on October first to become Director of Nutritional Education for the American Institute of Baking, with an office at 9 Rockefeller Plaza in New York City. Dr. Tobey states, however, that he expects to continue his interest in the sanitary, legal, and nutritive aspects of milk and dairy products, since bread and milk is one food combination that offers a virtually perfect diet to the average person.
Milk Plant Equipment of the Future*

C. SIDNEY LEETE

Associate Milk Sanitarian, New York State Department of Health, Albany, N. Y.

For the past few years, it has been thought that milk pasteurizing equipment had reached such a degree of efficiency in construction and operation that it was nearly fool-proof. Such an assumption has been found to be unwarranted.

The phosphatase test, recently developed in England, was studied two years ago by this department. It is a test for determining whether or not milk has been properly pasteurized. It is a quantitative test and will therefore tell the degree of undertreatment of so called pasteurized milk. Undertreatment in this case means (1) short holding, (2) low temperature, (3) mixture of raw with pasteurized milk, or (4) a combination of these items. Exhaustive tests have proven the rest reliable. This test has been used in a routine manner in the control work of the New York State Department of Health throughout the state for the past year. The results have shown that the control officials have not been too critical but rather not critical enough of details.

This brief sketch of what the milk plant equipment of the future will be is based on the findings that have been revealed by the use of the phosphatase test. The greatest cause of underpasteurized or improperly tested milk is not in the construction of the equipment, although this is not ruled out of the picture, but rather in improper operation, to the human element rather than the mechanical.

In the first place, pasteurizing equipment must be made more and more mechanical, thus leaving manual operation less and less a factor to consider.

When one man must operate a battery of pasteurizing vats with manual control of the inlet and outlet valves, it is easy to see why mistakes happen. With four vats there are 4 inlet and 4 outlet valves, each to be opened or closed at a certain definite time. It is easy to open the right valve too soon or even open the wrong valve. When such occurrences take place the product, supposedly properly pasteurized, is open to suspicion. It really is contaminated or underpasteurized milk. Granted that all mechanical devises are not 100% efficient at all times, such devises are better than manual operation. Batteries of batch pasteurizers should have both the inlet and outlet valves operated mechanically.

Single vats should be equipped with a device which accurately records the time of holding. Our present day recorders will show the temperature of pasteurizing

accurately but they do not show the time. To properly interpret the holding time, one must know the rate of filling, the rate of emptying, the amount of milk in the vat, and the location of the thermometer bulb. Obviously each vat would have a different set of dates. If and when an instrument is devised and found practical which will indicate the holding time, such an instrument, in all likelihood will be required as standard equipment.

It has been found that many operators are pushing charts. All that is gained is only a little saving of time at the expense of endangering the entire output of the plant. The operator who stoops to this practice is furthermore endangering the welfare of the whole industry. Operators who have been involved in a milk-borne outbreak do not want to go through the experience again. One way of courting such a disaster is to follow the practice of pushing charts. The phosphatase test has caught up with this practice. Probably soon, all recorders will be required to have a pin attached to a gear disc spindle. The pin will puncture the chart. And only one puncture per chart will be allowed. This may help to stop chart pushing.

It has been thought, up to the present time, that in most modern pasteurizers, agitation during the holding period was unnecessary. But the results of the use of the phosphatase test, both in the field on routine samples and later under controlled tests, indicate that in most cases agitation during holding is necessary. It need not be rapid agitation. Slow agitation will keep the milk from stratifying. Also, it will eliminate cold pockets. An instance out of many that prove that agitation is necessary was a routine sample from a modern plant the first indicated that the milk was underpasteurized. A careful check of the operation of the plant was made. Another test showed an improper reaction. The operation was checked again but this time the agitator was used throughout holding. A properly pasteurized milk resulted. In many plants agitation during holding must be required.

In many states, regulations require foam or air heaters but in New York state, this equipment has not been behived to be essential. However, the phosphatase test has shown that the time is not far distant when New York State will require foam or air heaters. Quantities of foam on pasteurizers will result, at times, in improper pasteurization as determined by the phosphatase test. Foam heaters or air heaters may become shortly standard equipment.

Constant capacity pumps are needed for continuous flow pasteurizers. Experience shows that usually the capacity decreases with use, thus resulting in a longer holding time than required. From the strictly public health viewpoint of safety, this increase of holding time is not serious. But such holding time may effect the flavor of the product. Indirectly this affects consumption and so in a real sense it is a public health problem. In order to overcome this change in capacity, a variable pulley may be used. Such a pulley must be sealed officially at a certain rate and may not be changed except under the supervision of a representative of the health department.

Surveys in this state and in others indicate that the bottle and
can washing operations are not generally being carried out efficiently. Several examples of improper construction and operations indicate that serious consideration should be given to the problem of sterilizing containers.

Recently it was found that bottles at one plant were neither particularly clean or sterile. Investigation revealed that the openings of the water and steam jets were located so that it was impossible for the water and steam to enter several rows of bottles. Equipment such as this must be condemned.

Again in a plant which uses hot water for sterilization it was found that shortly after the washing and sterilizing operation was started, the temperature dropped far below the sterilizing temperature. Along this same line, it has been found that temperatures in many cases, used for sterilizing cans were found too low. To meet this problem, it is necessary that whenever heat is used as a sterilizing agent, recording thermometers with proper charts should be installed.

It is well known that milk cans and other metal containers wear out and rust. Usually the first indication of rust is along a soldered joint. At the present time seamless, welded cans are available. One complaint entered against such equipment is that rust appears along the weld, probably because the metal which is to be welded is drawn to a feather edge. Such thin construction at the weld apparently is susceptible to rust. If and when satisfactory seamless cans are available at a reasonable price, it is probable that serious consideration will be given to making such equipment mandatory. The use of such cans would undoubtedly be of real value in dairy practice.

The above items are those which are receiving the serious consideration of milk control officials. They may be required because modern methods of testing the safety of milk indicate that they are closely related to the quality of milk and cream as delivered to the consumer. The progressive dealers will probably demand them and the manufacturer will supply this demand even before they are legally required. Experience shows that most dealers and manufacturers demand the best in equipment and operation.

Association Member in Headlines

Said to be the first to see measles "inclusion bodies", Dr. Jean Broadhurst, described by Time as "64, tall, stately, silver-haired professor of bacteriology at Columbia University", found herself very much in the public eye in November, as a result of a paper published in the Journal of Infectious Diseases. Measles has long been considered to be caused by a filterable virus. By the use of a stain called nigrosin, Dr. Broadhurst "brought to sight", apparently for the first time, inclusion bodies believed to be characteristic. It is believed that this may provide a means of diagnosis of measles in the early stage when it is otherwise practically indistinguishable from a severe cold. Dr. Broadhurst was elected to Associate membership in the International Association of Milk Sanitarians in 1936.
Proposed Standards for Paper Milk Containers

J. R. Sanborn

New York Agricultural Experiment Station,
Geneva, New York

Sanitary Requirements of Food Container Board

There is no reason why a paper container made from clean, sound, pulp wood according to methods consistent with food standards should not be hygienically suitable for perishable foods. Investigation has demonstrated the feasibility and effectiveness of this means of packaging milk.

Commercial mill practices in manufacturing pulp and paper and the condition of finished products are often not sufficiently sanitary to meet with the approval of food sanitarians and public health officials. The paper maker needs a sanitary technique comparable to the procedures employed for edible products. The intimate associations of cellulose material with food and household uses admit of no compromise with cleanliness and microbiological control.

Fear of increased operative costs and hesitancy to make changes from long established practices intensify the opposition to improved sanitary methods on the part of some pulp and paper mills. On the other hand, several producers of food wrappers and container board already exercise effective sanitary control which enables them to manufacture a clean, high quality product practically free from microorganisms. By insuring purity of process water, cleanliness of mill systems and operations, and adequate protection of the finished product, these plants are setting a standard of quality which indicates the suitability and safety of properly made paper for the packaging of perishable and easily contaminated food products such as milk.

Consistently high standards of quality in paper mill operations and possible approval by public health officials will depend on constant adherence to a program of microbiological control. The industry has suggested that sanitary inspections be made of plants manufacturing milk container board. Apparently the only way this can be done is by federal supervision or voluntary action on the part of pulp and paper mills or conversion plants. Many purchasers and consumers of paper food containers are sensitive to the bacterial counts of contained foods and to the presence of undesirable organisms. With all the cleanliness and care used in preparing and processing foods, it is obviously inconsistent to package these foods in unsuitable and contaminated paper.

Read before the International Association of Milk Sanitarians, Louisville, Kentucky, October 11-13, 1937.
SANITARY QUALITY OF PAPER MILK CONTAINERS

An institute has been established at the New York State Agricultural Experiment Station for the purpose of securing fundamental information on the sanitary condition of paper for use in contact with such perishable foods as milk and milk products, and establishing standards for this paper and its fabricated forms which will provide health laboratories with cri-

3 Suitable protection and wrapping of finished board
4 Mechanical handling of board and containers at conversion factories and milk plants
5 Protection of board, adhesives, moisture-proofing materials, and finished containers from careless exposure to human contact, contamination, dirt, flushing water, or insects
6 Detailed knowledge and careful selection of all materials composing the container to avoid the possibility of incorporating substances having germicidal

SOME TYPES OF PAPER CONTAINERS NOW ON THE MARKET

or bacteriostatic effects, the use of which is prohibited unless they have been shown to be nontoxic to human beings and without effect on milk.

The bacteriological condition of the original container board usually influences directly the bacterial counts of finished containers. Strictly sanitary methods of handling, conveying, and storing good quality board aid in preserving its sanitary condition. Health laboratories are therefore primarily in-
interested in the hygienic state of the original container board as it is received by conversion factories or milk plants.

**Municipal Regulations and Control**

Critical examination of food wrapper and container board will reveal information relative to the care and sanitary precautions taken by pulp and paper mills in the manufacture of these important grades of paper. Health laboratories may confirm by identification of fibers the nature of the pulp used; calculate the dirt content according to a method of numerical estimation suggested by the Pulp Testing Committee of the Technical Association of the Pulp and Paper Industry; examine slime spots for evidences of slime-forming organisms, growth fragments, or characteristic products of slime production.

Appreciable quantities of dirt and foreign matter may be introduced into new paper and board during their manufacture. Particles of metal, pipe scale, sand, and carbonaceous matter sometimes occur as well as various foreign fibers and organic residues, the presence of which is occasionally associated with unhygienic or careless practices.

Accumulations of microbial growth or slimes in pipe lines, chests and tanks are frequently dislodged and carried along by pulp streams to paper machines where considerable operative difficulties may result. Slimes are caused by bacteria, filamentous fungi, or yeastlike organisms, representative species falling under the genera *Aerobacter, Achromobacter, Flavobacterium, Pseudomonas, Bacillus; Penicillium, Mucor, Aspergillus, Cladosporium, Trichoderma, Oidium, Monilia*. The presence of slime spots in samples of paper suggests inadequate control over the development of microorganisms in pulp and paper mills. Many slime-forming species are unusually aggressive and persistent, controllable only by specific remedial measures and consistent application of programs of slime eradication and prevention. Only those mills which successfully control microorganisms within their systems should be considered by health officials for an approved sanitary status in the manufacture of food wrappers and containers.

It is also possible to grade paper products such as milk container board according to their bacterial content based on the number of colonies developing on standard agar per gram of disintegrated stock. Disintegration of container board may be accomplished aseptically by means of special beaters, shredders, grinders, or agitators. When properly carried out the process is completed in a relatively short time. Ten cc. portions of the pulp suspensions containing ten grams of board to a liter of sterile water, are plated out on standard agar in both 100 mm. and 150 mm. petri dishes. In the case of the regular size plates, 2 cc. amounts of suspension are introduced into each dish, thereby facilitating the counting of colonies from board containing relatively large numbers of organisms. The large size plates, accommodating the entire 10 cc. portion of pulp suspension, furnish the more useful method for the examination of improved milk container stock.

Samples of container board taken at any stage prior to moisture-proofing should not have a
count exceeding 500 colonies per gram of disintegrated board. Certain pulp and paper mills which are able to maintain effective control over the development of microorganisms within their systems, manufacture a product having counts of less than 100 per gram. Ten cc. rinses of paraffined containers made from low count board are often practically free from bacteria. On the other hand, containers produced from board which approaches the maximum count of 500 may yield 100 colonies per container. Average plate counts for all types of paper milk containers are usually less than 50 per container, which is standard for the Baltimore regulation discussed below.

Rinse tests on fabricated containers are made in conformity with the latest standard methods recommended by the American Public Health Association. Paper containers are rinsed with 10 cc. of sterile water all of which is plated out on standard agar, by distributing the rinse among three regular size plates or introducing the entire quantity into a 150 mm. dish. Plates are incubated at 37°C, and counts are made at the end of 48 and 72 hours. It may be considered that present standards for paper containers should be in accord with those suggested by the American Public Health Association for glass bottles. Sanitary conditions in pulp and paper mills, container manufacturing plants and dairies usually enable paper containers to meet a much stricter standard than those ordinarily considered for glass.

Isolated attempts are being made by control officials to regulate the handling and sale of milk and milk products packaged in paper. While these actions are based on information available at the time of formulation, recent findings indicate that the data are usually inadequate. The regulation adopted by the Baltimore City Health Department is deserving of serious study. In accord with this regulation it will be generally agreed that all paper, cardboard or other non-glass containers should be approved by the Commissioner of Health and conform to certain general rules regarding labelling and designation. Item 4 of the more technical portion of the regulation states:

"The paper blanks or non-paraffined containers shall be received by the milk plant in tightly closed packages or cartons and shall be manufactured from the best obtainable white spruce pulp or other material approved by the Commissioner of Health."

In the light of the results of recent investigations, this item may be effectively amended according to the suggestion given below.

"Container board in rolls, sheeted stock or blanks shall be received by conversion or milk plants suitably wrapped and sealed, and shall, until used, be kept unopened, in a clean, dry place."

"Only virgin pulp shall be used in manufacturing milk container board, which is low in dirt count, free from slime spots and has a bacterial count which does not, at any time, exceed 500 colonies per gram of disintegrated board."

Item 5 of the Baltimore regulations makes the following statement:

"The container, if glued, shall be glued with material made from a base of soybean, tapioca, or other product approved by the Commissioner of Health."

The suggested revision of this item would be as follows:

"The gluing of the container shall be accomplished with non-fermentable adhesives of synthetic, thermoplastic varieties or such types having vegetable or casein bases as produce rapidly-drying
films which resist dissolution, decomposition and leaching.'

Item 6 of the Baltimore ruling is as follows:

"The container, if it be one which requires paraffining, shall be paraffined in the milk plant where it is filled with milk or milk products; and shall be mechanically conveyed from the paraffining apparatus to the filling and sealing equipment; and the paraffining, conveying, filling and sealing of all containers shall be so accomplished as to prevent any possible hand or other contamination."

In view of the fact that paraffining may not completely sterilize containers and that premade containers may be as effectively protected from contamination as those that are filled with the milk immediately after paraffining, both types of containers should be permitted. The condition of paper milk containers in actual use at milk plants is the most reliable index to their sanitary quality. Wording such as the following might be substituted.

"Moisture-proofing of containers shall be accomplished by means of fully refined paraffin wax or other suitable materials which are odorless, tasteless, and non-toxic. The operation of paraffining machines shall be supervised by competent mechanics.

"All stock and containers shall be handled mechanically so far as possible and be paraffined, conveyed, filled and sealed so as to prevent contamination from manual contact, dirt, and insects."

This form of amended regulation would seem to provide sufficient restriction and sanitary control without shutting out high quality containers which may reach milk plants already paraffined. It has been demonstrated that it is entirely possible to make paper wrappers and containers which are suitable for milk and other perishable foods. As manufacturers continue to progress in sanitary methods of production and handling, it is probable that standards and specifications will become more stringent.
"Keeping up with the Joneses"

(In recognition of the need for education of the public to enable them to see their advantage in supporting effective milk sanitation control, this popularly written article illustrates a part of the successful program of the New York State Department of Health.—Editor.)

Dr. Mortimer Jones is well-known in New York State as "the health officer of the imaginary village of Utopia and for many years its only physician." He was brought to light through the series of radio health plays called the Health Hunters, in which, for four years, the part of Dr. Jones has been taken by Dr. Paul B. Brooks, an officer of the International Association. Dr. Jones has been described as "a progressive doctor of the old school."

Taking advantage of the reputation which the radio plays had built up for Dr. Jones, the New York State Department of Health a few months ago initiated as a weekly feature a column of comment under the caption "Doctor Jones Says—", of which Dr. Brooks is the unannounced author. As might be expected "Dr. Jones" now and then has something to say about milk, as in the following, which appeared in Health News of September 20:

"Speaking of cows: in one of the state licensing examinations for doctors they asked a question about what bovine mastitis was and one fellow, so I heard, said it was inflammation of the cow's 'rudder'. Some wisecracker said he probably was thinking of a steer. Maybe he came from New York. Certainly there aren't many people up around this way that don't know that the udder is the most important part of the cow, at least if it's milk you're looking for. In fact, Doctor Udall—he's one of the professors over at the Cornell Veterinary College—I heard him say that dairy farmers ought to keep in mind that when they're buying a cow they're 'buying an udder'. What he meant, of course, was that a cow without a good udder was a liability and they ought to take pains to see that the udders were sound and free from mastitis. Yet they don't seem to, always.

"No sir; it's a surprising thing how many people there are—folk that's associated with cows all the time, more or less—that are all mixed up on this mastitis business. One thing they don't seem to be able to get straight is the connection between mastitis and epidemics—scarlet fever and septic sore throat. They know how common mastitis is and when somebody tells 'em some of these epidemics come from cows with mastitis, they say: 'Well; if epidemics come from mastitis why don't we have 'em all the time instead of just once in awhile?' This same question came up at a medical meeting where I was. A couple of health officers were the only ones
there that knew the answer. The thing of it is: the general run of mastitis is caused by a variety of streptococcus that affects cows but not humans—at least doesn't cause scarlet fever and septic sore throat. 'Once in awhile' the cow gets infected with a streptococcus that comes from a human; usually a milker that's had scarlet fever or septic sore throat. That's when we get the epidemics. In other words, when you get an epidemic from a case of mastitis, the bug that causes the mastitis is from a human source.

"One of my patients told me that mastitis caused the dairy farmers so much trouble they were sick of hearing about it: just hearing the word irritated 'em. But, the way it looks to me, it don't irritate the farmer hearing about it any more than it does the cow having it. Getting information is like taking castor oil: it's awful unpleasant but the sooner you get it down the sooner you get rid of the trouble."

Report of the Committee on Milk and Dairy Products

(Presented before the Section on Food and Nutrition, Sixty-sixth Annual Meeting, American Public Health Association, New York, N. Y., October 5-8, 1937)

Although fluid or market milk presents more public health problems than all the other dairy products combined, it is recognized that the control officers cannot ignore the need for supervising the production and handling of ice cream, butter, and cheese without possible hazard to the public health. Outbreaks of disease have been traced to each one of these products.

In spite of the large number of state and municipal standards, laws, and regulations for the handling of milk, they are basically quite similar in their general requirements although very dissimilar in their details. These differences are increased by the interpretation of the enforcement officers. The adoption of the ordinance and code of the U. S. Public Health Service was intended to remedy these difficulties but there is a tendency now to adopt them with modifications to suit local conditions, thereby perpetuating lack of uniformity.

Other efforts to bring about greater uniformity were a conference at the U. S. Bureau of Standards to standardize on the color used for milk bottle closures, and a bill introduced by Senator Cope-land (Senate 2359) to abolish the existing milk control agencies in the U. S. Public Health Service and the U. S. Department of Agriculture, and institute a new Bureau of Regulation in the latter department under a Coordinator.

Vitamin D milk is now being sold to the extent of over half a million quarts daily. There is much difference of opinion as to its desirability, many municipalities favoring it, a few opposing it, and some indifferent. The committee considers that metabolized vitamin D milk (made by feeding irradiated yeast to the producing herd) is an unmodified, natural product, whereas irradiated milk (made by exposing milk to the irradiations of ultraviolet light) and fortified milk (made by adding a vitamin D concentrate to milk) are processed or modified milks and must be labelled accordingly.
Abstracts


This publication reports an extensive investigation of many factors which have been thought to affect the development of oxidized flavor in milk. In the test herd, the authors found that individual cows were usually not consistent in the production of the off-flavor, and that it is inhibited by feeding green feeds such as pasture, green alfalfa and clover. Breed, stage of lactation, chlorine-lactose ratio, and leucocyte count of the milk did not influence its occurrence, confirming the work of Guthrie and Brueckner on breeds. Like numerous other investigators, the authors found that milk is more susceptible to this development when the bacterial count is low, and in confirmation of the work of Tracy, Ramsey, and Ruehe, observed that the incubation of susceptible milk or its inoculation with an active culture tended to prevent the off-flavor.

In order to check Kende's and Chilson's results on the protective effect of reducing substances in the milk, the authors mixed cream from susceptible milk with normal skim milk but found variable results.

They tested Kende's idea of the role of an enzyme as the causative agent by heating susceptible milk to various temperatures. In agreement with Kende and Chilson, they found that heating milk at 145° F. for 30 minutes (and also 160° F. for 5 minutes) enhanced the off-flavor, but that a temperature of 168° F. destroyed the active factor. This is probably an enzyme.

Kende and later Chilson reported that the presence of vitamin C was an effective reducing substance which prevented the appearance of off-flavor. Anderson et al. had shown that pasteurization at 145° F. for 30 minutes in the presence of oxygen was detrimental to vitamin C. The authors found that this vitamin was not destroyed by the actual heating itself and that at temperatures of 170° and 180° F. there was very little destruction of the vitamin. However, vitamin C is partially destroyed in the subsequent holding period after cooling, but less so in the milk that had been heated to 170° F.

In order to determine whether the destruction of vitamin C in oxidized milk is greater than that in fresh normal milk, the authors determined the ascorbic acid by titration in both kinds of milk, both whole and skim, and noted that the content of vitamin C declined about as much in the normal milk as in that which developed the off-flavor. This indicated that the off-flavor enzyme did not seem to be an appreciable factor in destroying the vitamin C. This vitamin is decreased when off-flavor develops, although the vitamin may be decreased without the occurrence of the off-flavor.
The effect of oxygen on the development of the off-flavor was studied by the removal of the oxygen by bubbling nitrogen through the milk, and also by shaking it in a vacuum. Nitrogen prevented or diminished the development of off-flavor, and the vacuum treatment gave definite decrease. The addition of copper sulphate, and also exposure to sunlight, both independently caused the off-flavor to rise, whether or not free oxygen was present. Moreover, when a milk which had been heated to destroy the enzyme was treated with oxygen, no off-flavor came, thus indicating that something more than oxygen was necessary (as Greenbank and Holm found).

In order to locate the milk fraction which carried this causative factor, susceptible and normal milks were treated with rennet extracts and also papain and pepsin. The wheys were then mixed with cream heated at 170° F. to make a 4 per cent "milk." No off-flavor developed in the supercentrifuged whey devoid of fat but it did come in the whey of the susceptible milk to which the heated cream was added. This indicates that the enzyme-like factor responsible for the off-flavor is carried in the plasma and serum portion of the milk.

Prevention of the development of this oxidized flavor was accomplished by homogenizing the susceptible milk at 145° F., although simply heating at 145° F. for 30 minutes developed this flavor. This work confirmed the findings of Tracy, Ramsey, and Ruehe and also that of Thurston et al. The addition of maleic acid up to 0.04 per cent did not protect it but imparted a noticeable acid taste. Hydroquinone in concentration of 2 ppm, and also 0.2 per cent of oat flour were effective preventives. Carotene mixed with butter oil and emulsified with susceptible skim milk did not prevent the off-flavor development. The addition of 100 mg. ascorbic acid per liter was effective.

Experiments were conducted to ascertain what were the portions of milk that were affected in the development of this off-flavor. Kende has held that the iodine number of the butterfat was materially lowered when this flavor came, whereas Thurston et al., supported somewhat by the work of Chilson, showed that oxidation of the fat globule "membrane" caused the bad taste effects. The authors found that butterfat from susceptible cream, heated at 170° F. to destroy the oxidized-flavor enzyme, showed no change in iodine number nor off-flavor, whereas the susceptible raw cream and the same pasteurized at 145° F. for 30 minutes showed progressively lower iodine numbers. These tests showed that butterfat is affected as the oxidized flavor occurs. The authors extended their experimental series and further found that in the absence of lecithin and the oxidized-flavor enzyme, no off-flavor will develop on exposure to copper, thereby confirming Thurston et al. Therefore, the spontaneous oxidized flavor in milk is due to the oxidation of the phospholipid fraction of the fat globule membrane (lecithin) and the butterfat, and there is a reduction in the iodine number of the butterfat in milk having the oxidized flavor.
Standard Agar Counts as Compared with Counts on Improved Agars at 32° C.—M. W. Yale—
Paper presented before the Laboratory and Food and Nutrition Sections at the 66th Annual Meeting of the American Public Health Association, New York City, October, 1937.

In connection with the collaborative studies to ascertain the relation between the bacteria counts on the standard agar (by the regular official method) and those on the tryptone-glucose-skimmilk-agar at an incubation temperature of 32° C., the author lists 43 reports by 56 laboratories on 23,633 samples of raw and pasteurized milk and cream of different grades.

The reports in general indicated that the colonies were larger on the new tryptone medium than on the standard nutrient agar, and easier to count both because of their increased size and because of the opaqueness of the medium. Counts on the replicate tryptone agar plates at 32° C. were less variable than counts from standard agar plates incubated at 37° C. Types of bacteria predominating in improperly cooled milk and those associated with unclean utensils developed greatly increased counts on the modified medium, particularly when incubated at 32° C. Organisms arising chiefly in normal udders grew about as well on standard agar plates at 37° C. as with modified methods, thereby explaining why some investigators have reported but little difference in the case of Certified Milk.

The percentage of instances in which increases or decreases fall within certain limits shows: (1) that the use of 32° C. incubation had a greater effect on the count than the modified medium and that the combination of the two produced a greater effect than either modification alone; (2) that the increase in the majority of cases is least for raw milk and progressively greater for Grade A pasteurized milk, Grade B pasteurized milk and greatest of all for pasteurized cream; and (3) that wide distribution in the range of decreases and increases shows that adoption of the modified methods will not merely result in a constant percentage increase in count.

A summary of the 43 reports shows that modified methods produce greater spread between counts of truly good quality and truly poor quality products than does the present standard procedure. The numerical increase in counts is so slight in the case of truly high quality products that they will in most cases be able to meet present bacterial count standards. Moreover, adoption of modified methods will result in better correlation between results of milk inspection and bacterial counts than is the case at present.
Journal of Milk Technology

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Application for Membership

I wish to apply for membership in the International Association of Milk Sanitarians.

Name ................................................................. 19

(Print name in full and degree)

Address .............................................................

(Street and City)

Present occupation ................................................

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Application is for:

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Person may qualify for

MEMBER is officially engaged in dairy or milk inspection, or laboratory control, or administration of such function for any country or subdivision thereof; or officially engaged in research or educational work related to dairy or milk inspection for any country or subdivision thereof.

ASSOCIATE MEMBER if interested in the promotion of dairy sanitation.

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Int. Assn. of Milk Sanitarians,
N. Y. State Dept. of Health,
Albany, N. Y.
INTERNATIONAL ASSOCIATION OF MILK SANITARIANS

CONSTITUTION AND BY-LAWS

CONSTITUTION
Adopted October 16, 1911*

NAME
This Association shall be known as the International Association of Milk Sanitarians.

OBJECT
The object of this Association shall be to develop uniform and efficient inspection of dairy farms, milk establishments, milk and milk products, and to place the inspection of the same in the hands of men who have a thorough knowledge of dairy work.

MEMBERSHIP
There shall be two classes of membership in this association: Active and Associate.

The active membership shall be composed of persons who are officially engaged in dairy or milk inspection, or the laboratory control of, or the administration of such function for any country or any subdivision thereof, and of persons who are officially engaged in research or educational work related to dairy or milk inspection for any country or subdivision thereof, provided, however, that all persons who at the time of the adoption of this amendment are members of the Association, shall be active members.

The associate membership shall be composed of any persons not eligible for active membership, who are interested in the promotion of dairy sanitation. Associate members shall not be eligible to vote, serve as officers, hold the chairmanship of any committee, serve on the Resolutions Committee, or serve as majority members of any committee of this Association.

Any properly qualified person may make application for active or associate membership to the Secretary-Treasurer and if application is accepted by the Membership Committee, said applicant may become an active or associate member, as the case may be, upon payment of the annual dues of five dollars ($5.00).

OFFICERS
The officers of this Association shall be a President, three Vice-Presidents, a Secretary-Treasurer, and two Auditors, who shall be elected by a majority ballot at the Annual Meeting of the Association, and shall hold office for one year or until their successors are elected. An Executive Board, which shall direct the affairs of the Association when not in Annual Session, shall consist of the President, the three Vice-Presidents, and the Secretary-Treasurer.

AMENDMENTS
This Constitution may be amended by a two-thirds affirmative vote of those active members of the Association who register their votes with the Secretary. Any member proposing amendments must submit the same in writing to the Secretary-Treasurer at least sixty days before the date of the Annual Meeting, and the Secretary-Treasurer shall at once notify all members that the proposed amendments will be open for discussion at the Annual Meeting immediately succeeding such notification. After discussion at the Annual Meeting such amendments, upon a majority affirmative vote of the members in attendance shall be, within 90 days, submitted to the entire membership of the Association by the Secretary-Treasurer. All members voting on such amendments shall, within 60 days after receipt of such notification, register their vote in writing with the Secretary-Treasurer on blanks furnished by the Association. These ballots shall be opened and recorded by the Executive Committee, and the results shall be reported by the Secretary-Treasurer at the next Annual Meeting; and if the amendments are passed they shall become a part of the Constitution from the date of such report by the Secretary-Treasurer at the Annual Meeting.

BY-LAWS

Adopted October 25, 1913

ORGANIZATION

The Constitution shall be the basis of government of this Association.

ARTICLE 1

MEMBERSHIP

SECTION 1. Any person eligible for membership under the Constitution who shall file an official application, accompanied by the first annual membership dues of five dollars, and whose application for membership shall have the approval of the Membership Committee, may become a member of the Association for one year.

SECTION 2. Any person having once become a member may continue membership in the Association so long as the annual membership dues are paid. Any member who shall fail to pay annual dues within thirty days after having been notified by the Secretary that said dues are due and payable, shall be dropped from membership. Any member so dropped may, within ninety days, be reinstated by the Membership Committee, upon application filed in due form and accompanied by the annual membership dues for that year.

SECTION 3. A member of the Association may be expelled for due cause upon recommendation of the Membership Committee, and a majority vote of the members at any annual meeting. Any member so expelled shall have refunded such pro rata part of his membership dues as may not be covered by his term of membership.

HONORARY MEMBERS

SECTION 4. Members of the Association may elect as honorary members, at any stated meeting, on the recommendation of the Membership Committee, those whose labors have substantially added to the scientific knowledge of milk supply betterment, or those who have been of pronounced practical influence in the improvement of the milk industry. From such members no dues shall be required. They shall have the privilege of attending the meetings of the Association, but they shall not be entitled to vote.

ARTICLE 2

OFFICERS

SECTION 1. The officers of this Association shall be a President, a First, Second, and Third Vice-Presidents, a Secretary-Treasurer, and two Auditors, who shall be chosen by ballot at the annual meeting of the Association, and shall hold office for one year, or until their successors are duly elected.

SECTION 2. The Executive Board shall consist of the President, the three Vice-Presidents, and the Secretary-Treasurer.

SECTION 3. The Membership Committee shall consist of the President, the three Vice-Presidents, and the Secretary-Treasurer.
ARTICLE 3

DUTIES OF OFFICERS

SECTION 1. It shall be the duty of the President to preside at all meetings of the Association. He shall examine and approve all bills previous to their payment, appoint all committees unless otherwise directed by vote of the Association, and perform such other duties as usually devolve upon a presiding officer, or are required of him by the Association.

SECTION 2. The Vice-Presidents, in the order of their selection, shall perform the duties of the President in his absence.

SECTION 3. The Secretary-Treasurer shall record the proceedings of the Association. He shall keep a list of members, and collect all moneys due the Association, giving his receipt therefor. He shall record the amount of each payment, with the name and address of the person so paying. He shall faithfully care for all moneys entrusted to his keeping, paying out the same only with the approval of the President, and taking a receipt therefor. He shall, immediately after his election to office, file with the President of the Association a bond in the sum of five hundred dollars, the expense of which shall be borne by the Association. He shall, at the annual meeting, make a detailed statement of the financial condition of the Association.

It shall also be the duty of the Secretary-Treasurer to assist in making arrangements and preparing a program for the annual meeting, and to compile and prepare for publication all papers, addresses, discussions and other matter worthy of publication, as soon as possible after the annual meeting.

SECTION 4. The full management of the affairs of the Association when the Association is not in session shall be in the hands of the Executive Board, as provided in the Constitution.

SECTION 5. It shall be the duty of the Auditors to examine and audit the accounts of the Secretary-Treasurer and all other financial accounts of the Association, and to make a full report of the condition of the same at the annual meeting.

ARTICLE 4

MEETINGS

SECTION 1. The annual meeting of the Association shall be held at such time and place during the month of October of each year or at such other time as shall be designated by the Executive Board.

SECTION 2. Special meetings of the Association may be called by the Executive Board, of which due notice shall be given to the members by the Secretary.

SECTION 3. Quorum.—Twenty-five per cent of the membership shall constitute a quorum for transaction of business at any annual meeting. Voting by proxy shall not be permitted.

ARTICLE 5

These By-Laws may be altered or amended at any annual meeting of the Association. Any member proposing amendments must seasonably submit the same in writing to the Secretary-Treasurer, who shall then give notice of the proposed amendments by mail to each member of the Association at least thirty days previous to the date of the annual meeting.
Recent research work has shown that "pin-point" bacteria in many cases survive ordinary heat sterilization. In the subsequent pasteurization, they find temperature conditions ideal for rapid multiplication... seriously increasing the bacteria count and often resulting in off-flavors and taste.

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Coming Meetings

Massachusetts Public Health Association, Boston, Mass., Jan. 27.
Southwestern Exposition and Fat Stock Show, Fort Worth, Tex., Mar. 11-20.
Public Health Association of New York City, April.
Dairy Products Association of the Northwest, St. Paul, Minn., April.
Ohio Federation of Public Health Officials, Columbus, Ohio, April.
American Chemical Society, Dallas, Tex., Apr. 18-21.
American Institute of Nutrition, Baltimore, Spring.
Georgia Public Health Association, Atlanta, Ga., May.
Iowa Public Health Association, Des Moines, Ia., May.
Association of Food and Drug Officials of the South Central States, Biloxi, Mass., May.
Pennsylvania Public Health Association, Harrisburg, Pa., May.
South Carolina Public Health Association, Myrtle Beach, S. C., May 23-25.

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THE months ahead are fraught with many difficulties. But, we must continue our forward march in the vital business of milk sanitation.

The Sealtest System of Laboratory Protection will naturally carry on its close cooperative work with Milk Supervisors... in checking and supervising the quality, purity and wholesomeness of those products put forth under the famed red-and-white Sealtest Symbol.

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Bacto-Peptone is the standard peptone for use in the preparation of general purpose culture media for routine use. It is rich in readily available forms of nitrogen and is completely soluble in the usual concentrations. In a one percent solution it has a reaction of pH 7.0.

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