Editorials

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

THE ATLANTIC CITY MEETING

The Program Committee, consisting of the three Junior Officers of the Association, is organizing a program that will interest everyone whose profession is dairy and food sanitation. The most qualified speakers have been engaged to discuss such interesting subjects as permanent piping, the Ring test, Q fever, administration of sanitation programs, interstate shipments, detergent sanitizers, bacteriology, milk and cream dispensers, milk ordinances, food plant personnel training, extraneous material, and others. Excellent color moving pictures showing various food processing operations have been scheduled. A special feature of the meeting this year will be get-together breakfasts. The various committees of the Association will meet in a breakfast session; representatives of the Affiliated Association officers at another; a luncheon meeting for the membership at large.

The officers of the various committees are requested to submit to the Program Committee promptly the titles of the subjects of study for the past year so that they can be included in the printing of the general program. The Committee will welcome suggestions for speakers and subjects but these must be submitted promptly.

The members of the Committee are:

K. G. Weckel, Chairman, University of Wisconsin, Madison, Wisconsin.
C. S. Leete, New York State Dept. of Health, Albany, N. Y.
H. L. Thomasson, Indiana State Board of Health, Indianapolis, Indiana.

The officers and members of the International Association of Ice Cream Manufacturers join President McKenzie in inviting the members of the International Association of Milk and Food Sanitarians to attend the International Ice Cream Convention in Atlantic City on October 18, 19, and 20, 1950.

The convention opens with a joint session with the Milk Industry Foundation on the morning of October 18th, and will run through to the final General Session on Friday afternoon, October 20th.

The Milk and Food Sanitarians are invited to attend the sessions as guests of the International Association. Those desiring to attend should register at the Traymore Hotel.
NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS

On June 1, 2, and 3, more than one thousand representatives from health organizations, sanitary livestock boards, agriculture and industry from twenty-six odd states met in St. Louis to work out a practical procedure for receiving milk from distant producing areas. The conference was chaired by Mr. J. L. Rowland, Director, Bureau of Food and Drugs, Missouri Division of Health.

It was the outgrowth of a series of efforts, dating back to the 1946 Conference of State and Territorial Health Officers, to pass the Public Health Service to develop a program for certification of interstate milk shippers, and to arrange for an interstate conference on milk shipments to be held again next year on June 4, 5, and 6, 1951, at the Hotel Statler, St. Louis, Mo.

Regulations. The 1939 edition of the U.S. Public Health Service Milk Ordinance and Code will be used as the basic regulation, and that compliance therewith will be measured by the U.S.P.H.S. milk sanitation rating method as outlined in the U.S. Public Health Service Bulletin, No. 670, "Methods of Milk Sanitation Rating."

Supervision. The receiving state were urged to recognize inspection of milk by full-time local and state health and agricultural department personnel. Supervision shall be measured by the enforcement of rating procedure outlined in Public Health Service Reports, Region No. 5, 1940, "Methods of Rating Milk Sheds."

Certification. Receiving states were urged to accept ratings made on milk by certified rating officials of either the U.S. Public Health Service or the State Health Department. Certification should include the rating procedure outlined in Public Health Service Reports, Region No. 5, 1940, "Methods of Milk Sanitation Rating."

Channel for Requesting and Reporting Information. An individual in receiving state desiring information on a milk supply should make the request to the public health officer in his own state who will transmit the request to the Regional Office of the Public Health Service for the state.

The state health department in the shipping state will request the results of the survey to the regional office of the Public Health Service. The state health officer of the shipping state will immediately notify the local health officer and/or the individual requesting the survey. Industry in a shipping state desiring a survey should likewise make the request to the regulatory officials in his own state.

To expedite the requesting and reporting process, the immediate future, requests and reports can be sent to the Regional Office of the Public Health Service. The latter shall consider the certified list of milk shippers and circulate all state agencies monthly who in turn are urged to avail their local health officers and/or industry.

Role of the Public Health Service. The state regulatory authorities should carry the work load involved in the interstate milk program, with the assistance of the U.S. Public Health Service. The latter shall be prepared to extend to state regulatory authorities and educational institutions such assistance in the training of field representatives of the state and local government units or of industry, field personnel, and local personnel.

The Public Health Service should also train or assist in training laboratory personnel of state, local, or industrial laboratories as required in the operating of the interstate milk shipment plan. The Public Health Service should also train or assist in training laboratory personnel of state, local, or industrial laboratories as required in the operating of the interstate milk shipment plan. The Public Health Service should also train or assist in training laboratory personnel of state, local, or industrial laboratories as required in the operating of the interstate milk shipment plan. The Public Health Service should also train or assist in training laboratory personnel of state, local, or industrial laboratories as required in the operating of the interstate milk shipment plan. The Public Health Service should also train or assist in training laboratory personnel of state, local, or industrial laboratories as required in the operating of the interstate milk shipment plan.
It should furnish state regulatory agencies periodically with interpretations and regulations based on questions submitted by such agencies and also that state authorities relay such interpretations to local enforcement agencies and/or industry.

Statement of Industry. Representatives of industry suggested that copies of the proceedings and recommendations of this meeting be forwarded to the respective trade organizations. Local and national trade organizations should be invited to participate in any permanent organization.

Getting into Operation.

Receiving States:
1. Local health departments and industry should anticipate in advance the amount of milk needed and the season in which it will be needed.
2. Requests for surveys should be in early.
3. There should be established an intra-state reporting system in order that all local areas could be kept currently informed.
4. A record system should reveal the following: certification information, and sources of incoming milk.
5. Furnish council and supervisory service to local health departments.

Producing States:
1. Establish sound supervisory system in the local health departments and in plants that are not under the health department.
2. Establish survey and certification system.
3. Set up an efficient record system along with the supervisory system.
4. Provide a laboratory certification program.
5. Review and check state regulations, and revise and bring them up to date.

Receiving and Producing States:
1. Standardize procedures and personnel.
2. Work out a rapid requesting and reporting system. (An ideal goal is to have survey reports out within ten days after the completion of the work.)
3. Establish closer liaison with local health officials and industry.
4. Provide an effective educational service.

U.S. Public Health Service:
1. Regional office provide a rapid system of sending on requests and reporting information to the states.
2. Assist states in the standardization of procedures and personnel.
3. Spot check state agencies to maintain uniformity in operation.
4. Assist states, if possible, with the work load.
5. Assist states with organization and administration problems.

BOTTLE-WASHING STUDIES UNDER PLANT CONDITIONS

C. N. STARK, R. F. HOLLAND, J. C. WHITE, AND M. J. GURDIAN
Laboratory of Bacteriology and Department of Dairy Industry, Cornell University, Ithaca, N. Y.

It little profits a milk dealer to enter an involved quality control program, pasteurize milk in the best of equipment, and handle it with extreme care if the milk is placed in containers which are not essentially sterile. On the other hand, any operator with an eye to economy will not want to use in his bottle washer caustic solutions which are stronger than necessary nor want to burden his washer with temperatures higher than are necessary to do an adequate job. For these reasons, it was believed that a thorough investigation should be made of the relationship between temperature, time of exposure, and caustic concentration in a soaker-type milk bottle washer. The recommendations for caustic temperature relationships which are in general use in the dairy industry throughout the United States are based on the work of Levine and co-workers, who investigated the washing of carbonated beverage bottles.

Laboratory tests were made on milk bottles and it is known that cleaning and "sterilizing" the milk bottle presents a radically different problem from the carbonated beverage bottle. The beverage bottle will contain residues of sugar, acids, and perhaps some extraneous matter; the milk bottle contains, in addition to these materials, a complex film of fat, protein, and minerals. This complex film provides a medium which is ideal for the growth of bacteria. The elimination of these factors from the bottle, together with the milk solids, presents the practical and important problem for the dairy plant. It is a problem comparable in importance with the pasteurization process itself. The plant operator must produce a bottle which is clean and spotless in order to hold the consumer's confidence, and which has a bacterial count low enough to be safe and to meet public health standards.

The question, what constitutes optimum conditions for operating a bottle washing machine, is frequently answered in different ways. The manufacturer of the equipment will have his recommendations, but the representatives of the health department are the ultimate authorities and, in any event, must be satisfied. Recommendations of all equipment manufacturers and all health departments are not necessarily the same. Arnold and Levine state "For any given temperature and concentration (of alkali) it would be necessary to expose the bottle 18.1 times as long to meet the New York City requirements as would be required to comply with the Chicago law." New York City requires a minimum soaking time of 7 minutes at the temperature of 150° F. with 2 percent of sodium hydroxide in the soaker tank; Chicago specifies a minimum of 5 minutes at 120° F. with 1.6 percent sodium hydroxide. There is little information available to show what standards are necessary to produce a clean, safe milk bottle.

The Association of Bottlers of Carbonated Beverages has recommendations, based on the work of Levine and associates, stipulating that "Unclean bottles shall be exposed to a percent alkali of which not less than 60 percent is sodium hydroxide for a period of not less than 5 minutes at a temperature of not less than 130° F., or an equivalent cleansing and sterilizing process." It has not been proved that...
the standards for cleaning and "sterilizing" beverage bottles are necessarily difficult to establish for milk bottles.

In an attempt to determine the adaptability of these recommendations to milk bottle washing, a project for the study of the problems under practical commercial conditions was organized at Cornell University. The aims of the project were: (1) to determine the germicidal and detergent efficiency of different concentrations of certain caustic solutions at different temperatures; (2) to observe the importance of exceptionally high-temperature operation to cleaning and "sterilizing" efficiency; and (3) to observe the physical cleanliness of the bottles under these various conditions.

**Experimental Methods**

All the experiments of this project were carried out at the Cornell University dairy plant at Ithaca, New York. This plant bottles quarts, pints, and half-pints. Most of the quarts are sold to household trade at the retail store in the plant, while most of the half-pints are consumed by the students in dormitories and cafeterias on the campus. The dirty bottles returned from the routes are stored indoors until they are washed and refilled. Quart bottles normally are rinsed by the consumer before being returned, but the pint and half-pint bottles rarely receive any rinse. They usually contain milk and very commonly cigarette ashes and other extraneous materials.

The plant is equipped with a Cherry-Boiler Model C eight-wide soaker washer. This bottle washer had been in operation at the plant for only a few months when this project was under way. The ABCB standards for washing bottles were used as a guide for setting up the temperature-caustic concentration relationships up to 150°F. Above this temperature a caustic concentration of 0.9 percent was maintained as a practical minimum. These caustic-temperature combinations are indicated when the ABCB standards are converted to the equivalent of a 3-minute soaking period. Table 1 gives these relationships.

| Temperature | Causticy
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<tr>
<td>120°F</td>
<td>4.6</td>
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<tr>
<td>130°F</td>
<td>4.2</td>
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<tr>
<td>140°F</td>
<td>3.9</td>
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<tr>
<td>150°F</td>
<td>3.6</td>
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<tr>
<td>160°F</td>
<td>3.3</td>
</tr>
<tr>
<td>170°F</td>
<td>3.0</td>
</tr>
<tr>
<td>180°F</td>
<td>2.7</td>
</tr>
</tbody>
</table>

The washer feed water hardness varied between 5 and 7 grains per gallon during the experiment.

To assure accurate control of the soaking temperature at all times, a thermostatically controlled steam valve was installed on the washer. An abundance of high-pressure steam was available at all times.

Two separate recording thermometers made continuous daily records of the temperature of the soak solution and of the tank providing the water for the so-called first rinse or pressure wash. The washer was operated at a speed corresponding to an immersion period of 3 minutes and 15 seconds and was maintained throughout the experiment. This relatively high speed was selected to produce the shortest soaking time that would be encountered normally in commercial operation, thus placing the greatest possible load on the temperature and alkalinity combinations used in the tests.

For each given temperature, the tests were divided into three 5-day periods, in a few cases the periods were 10 days. During the third period tetrasodium pyrophosphate was added in the same proportion as tetrasodium phosphate, while maintaining as a base the same concentration of sodium hydroxide that was used during the second period. A 32-pound sodium hydroxide charge was required to produce a solution having 1 percent caustic alkalinity in this machine. The indicated concentrations of these alkalies were maintained by stored additions to the soak tank during the day's run. The required amount of sodium hydroxide was added and other materials, when in use, were added in direct proportion.

**Results**

Sodium hydroxide soak solution. In table 2, recording the findings on tests made for 38 days using only sodium hydroxide in the soak tank, certain observations appear to be of interest. More than 55 percent of the bottles tested failed to show any colonies of bacteria. The average colony count per bottle never exceeded 15. The highest colony count of bacteria for any one bottle was 170; only once did the bacterial colony count exceed 100. Since the accepted standard requires less than 238 colonies per half-pint bottle, all of the alkalies and acid-fast organisms to strong visual cleanliness. Twelve quart bottles, collected at random daily, were used for making observations on visual cleanliness.

Specific gravity. The specific gravity of the solutions was measured at the beginning and at the end of each of the three periods by means of two hydrometers, the scales of which covered the range of the solutions. All of these measurements were made at 25°C.

Surface tension. The surface tension of the solutions was determined with the same frequency as the specific gravities. Cenco-du Nouy precision tensiometer No. 10402, having a 4-cm. platinum ring, was used. The apparatus was properly calibrated and leveled; the platinum ring was washed with distilled water and dried with an alcohol flame before each determination.

For surface tension measurements the temperature of the solution was adjusted to 20°C in a water bath.

**Visual cleanliness.** Twelve quart bottles, collected at random daily, were used for making observations on visual cleanliness.
concentrations of alkalis, it would be expected that many such organisms would survive this treatment. It should be mentioned that the medium and incubation time used in these studies and less alkali resistant organisms of surviving acid-fast organisms. Doubtless, all of the less heat-resistant and less alkali resistant organisms in these bottles were killed by any of these treatments. The observed bacterial colonies were formed by the more resistant types. Since the lowest concentrations of sodium hydroxide employed gave a pH of around 13.0, the alkali killing effect on many less resistant bacteria would be high in all the alkali concentrations tested. Because these bottles failed to rinse well, the use of only sodium hydroxide in the soaker tank is not recommended. Because of excessive bottle breakage at temperatures of 170°F, and above, these temperatures are not recommended for commercial operation. This is especially true under winter conditions when bottles may be extremely cold and rinse water temperatures near freezing. A limited number of runs made at 180°F and 190°F gave results of the same order as at 160°F and 170°F.

**Sodium hydroxide and trisodium phosphate soak solution.** If the addition of trisodium phosphate to the sodium hydroxide solution in the soaker tank of a milk bottle washing machine is an advantage, these tests should indicate it, since the trisodium phosphate was added to the same concentration of sodium hydroxide as used in the previously reported 42 tests. It is true that the amounts of trisodium phosphate added were small but they do not contribute to the quantities incorporated in bottle washing mixtures sold to the dairy trade.

In table 3, reporting the results of 29 days tests using sodium hydroxide and trisodium phosphate in the soaker tank, 23 percent of the bottles tested showed no colonies of bacteria by the time bottles were 30 minutes old. Only one bottle exceeded the permissible standard limit, having a count of 350. The bacterial counts were more variable and some were higher. The results indicate that the addition of these amounts of trisodium phosphate adds nothing to the bottle-cleaning properties of the soak solution and may be detrimental.

**Sodium hydroxide, trisodium phosphate, and tetrasodium pyrophosphate soak solution.** Tetrasodium pyrophosphate was added to the same solutions of sodium hydroxide and trisodium phosphate used in previously reported tests. The observations made during running of the 25 tests using these materials are reported in Table 4.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td><strong>The Bacterial Content of Half-Pint Milk Bottles Soaked for 3.25 Minutes in Sodium Hydroxide Solution</strong></td>
</tr>
<tr>
<td><strong>Caustic Soda</strong></td>
</tr>
<tr>
<td>%</td>
</tr>
<tr>
<td>4.2</td>
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<tr>
<td>2.9</td>
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<tr>
<td>1.9</td>
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<td>1.3</td>
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<td>0.9</td>
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**TABLE 3**

| **The Bacterial Content of Half-Pint Milk Bottles Soaked for 3.25 Minutes in a Solution of Sodium Hydroxide and Trisodium Phosphate** |
| **Caustic Soda + Phosphate** | **Soak Temp.** | **No. of days run** | **No. of bottles tested** | **Bottles showing no colonies, any bottle** | **Average no. of colonies, any bottle** |
| % | °F. | | | | |
| 4.2 | 120 | 6 | 54 | 36 | 23 |
| 2.9 | 120 | 5 | 45 | 40 | 7 |
| 1.0 | 120 | 5 | 45 | 45 | 49 |
| 1.3 | 150 | 5 | 45 | 42 | 90 |
| 0.0 | 170 | 5 | 45 | 41 | 49 |

**TABLE 4**

| **The Bacterial Content of Half-Pint Milk Bottles Soaked for 3.25 Minutes in a Solution of Sodium Hydroxide, Trisodium Phosphate, and Tetrasodium Pyrophosphate** |
| **Caustic Soda + Trisodium Phosphate** | **Tetrasodium Pyrophosphate** | **Soak Temp.** | **No. of days run** | **Bottles tested** | **Bottles showing no colonies, any bottle** | **Average no. of colonies, any bottle** |
| % | % | °F. | | | | |
| 4.2 | 0.42 | 120 | 4 | 36 | 31 | 19 |
| 2.9 | 0.28 | 120 | 3 | 27 | 22 | 4 |
| 1.0 | 0.49 | 120 | 6 | 54 | 43 | 7 |
| 1.3 | 0.13 | 150 | 5 | 45 | 42 | 90 |
| 0.0 | 0.09 | 170 | 5 | 45 | 47 | 7 |

In all of the 25 days tests made, 44 percent of the bottles tested failed to show any bacterial colonies. The largest number of bacteria indicated for any one bottle was 125. The majority of the counts per bottle was nearer 25. Less variation in the bacterial counts from day to day was observed. The bottles rinsed satisfactorily at all temperatures and all concentrations of chemicals used. This better rinsing is believed to be a factor in obtaining more uniform and lower bacterial counts. Since an improperly rinsed milk bottle is unsatisfactory, even though it shows a low bacterial count, the findings reported in these studies emphasize the major importance of having tetrasodium pyrophosphate or similar material in the soak solution of the bottle washer. Some other equally good polyphosphate could possibly be used; only tetrasodium was used in these investigations.

Several runs were made using only sodium hydroxide and tetrasodium pyrophosphate in the soaker tank. The results obtained were as good or better than those in which all three alkalis were present, indicating that trisodium phosphate adds little or nothing to the cleaning qualities of the soak solution. **Physical cleanliness of bottles.** In Table 5 it can be seen that tetrasodium pyrophosphate in the soaker tank was necessary to obtain properly rinsed bot-
surface tension is generally held to improve the penetration of the washing solution and to give better rinsing. These readings did not, in any degree, correlate with the rinsing results observed.

The specific gravity would be expected to increase as the soaker solution ages. This does not always occur. Specific gravity measurements in these tests did not measure or correlate with efficiency in washing milk bottles.

Carbonate alkalinity would be assumed to increase as the soaker solution ages. At the higher temperatures and lower causticities the carbonate alkalinity were lower, but no noticeable correlation between efficiency of bottle washing and carbonate alkalinity could be observed.

CONCLUSIONS

1. The concentrations of alkali and the corresponding holding time and temperatures suggested by Levine for washing bottles under commercial plant conditions, when followed, will meet the U. S. Public Health Service standard for a bacteriologically satisfactory milk bottle.

2. To obtain satisfactorily rinsed bottles tetrasodium pyrophosphate was essential. The addition of trisodium phosphate in the amounts used, was not an advantage.

3. The measurements made on surface tension, specific gravity, and carbonate alkalinity did not correlate with the satisfactory rinsing of the bottles or the number of surviving bacteria found. Apparently there are other more important factors which these tests do not measure.

4. Soaker solution temperatures of 170° F. or higher are not needed to obtain a low bacterial count bottle of excellent appearance. Breakage may be high at this temperature and above.

5. The alkalinity, temperature, and time of exposure standards for milk

(Continued on page 205)
The freezing point is not included in this summary table as all samples showed a normal freezing point depression, indicating that none of the samples were adulterated with water. Ten of the samples had refractometer readings indicating added water. Acetic serum reading below 36 indicated added water, while another group of 12 samples showed a normal freezing point depression, indicating that none of the samples were adulterated with water. All samples were chosen because of known low composition, especially abnormally low solids-not-fat content.

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Data have been presented indicating that it is possible to obtain low refractometer readings on unadulterated milk samples having a low composition solids-not-fat.

### Bottle-Washing Studies

(Continued from page 201)

bottle washing maintained by some of the larger cities provide a very large margin of safety.

### References

EFFECT OF ADDED RIBOFLAVIN UPON THE PERMANENCY OF ASCORBIC ACID IN RAW COW MILK

ARTHUR D. HOLMES

Massachusetts Agricultural Experiment Station, Amherst, Massachusetts

It is unfortunate that milk, which contains so many of the essential constituents of the human diet, loses so much of its original reduced ascorbic acid during processing, distribution, and storage. The reduced ascorbic acid content of freshly drawn milk ranges from 20 to 25 mg. of ascorbic acid per liter, but a considerable portion of this is lost during the pasteurization process, during the vicissitudes of distribution, and during storage in the home. Holmes, Tripp, Woelfler, and Satterfield and Holmes, Jones, Wert, and Kuznecov reported a loss of over 18 percent of ascorbic acid during pasteurization of milk in the dark at 143° F for thirty minutes, and Woensner, Weckel and Schulte, Elvehjem, and Mawson and Kon observed a 20 percent loss of ascorbic acid.

A very considerable destruction of reduced ascorbic acid may occur during the commercial distribution of milk, particularly if the milk is allowed to stand for any length of time in the consumer's doorstep unprotected against sunshine or even bright light. Dinnher and Fresenius, Buriana, Hand, Guthrie, and Sharp and others have discussed the effect of sunshine upon the stability of reduced ascorbic acid. Kraus found that pasteurized milk exposed in a colorless bottle at room temperature lost its vitamin C in six hours, and Kownda Watson reported that milk kept in June sunshine for an hour lost all of its reduced ascorbic acid.

Holmes and Jones exposed milk in commercial flint milk bottles to the action of light of varying intensity and found that exposure for only thirty minutes on a rainy day destroyed all the reduced ascorbic acid in the milk.

Even if the pasteurizing and distribution conditions have been ideal and the milk contains a relatively large amount of reduced ascorbic acid when it reaches the consumer, there may be a serious loss of ascorbic acid while the milk is stored in the home refrigerator. Hand studied the loss under such conditions and found that during six day storage at 1° C, the reduced ascorbic acid of commercial milk dropped from 19 mg. to 7 mg. per liter—a loss of over 60 percent. Gunsalus and Hand observed a larger loss, namely, a drop of from 14.9 to 1.7 mg. of reduced ascorbic acid per liter, or an 80 percent loss during six day storage. Contrast these large losses, Holmes and Jones found that mare milk stored in darkness at 10° C for six days lost only about 8 percent of its reduced ascorbic acid. In considering the large loss of reduced ascorbic acid from cow milk during processing, distribution, and storage, and the pronounced difference in the amount and rate of loss of reduced ascorbic acid from cow and mare milk, one notes that the ratio of riboflavin to ascorbic acid is radically different in the milk of the two species of animals. Accordingly, this study was undertaken to investigate whether added riboflavin would have any effect upon the permanency of ascorbic acid in raw cow milk.

EXPERIMENTAL

The milk for this study was obtained weekly from the University dairy department. It was produced by the University herd of seventy cows of different ages and stages of pregnancy and lactation. The milk from the evening and morning milkings was thoroughly mixed in a large stainless steel vat preparatory to pasteurization. The aliquots of the raw milk was withdrawn and taken directly to the laboratory, where it was divided into three identical portions. One portion served as a control. U.S.P. grade riboflavin was added to the other two portions at the rate of 4 mg. and 8 mg. per liter. Similar samples were prepared on seven Monday mornings between the middle of September and the first of March. The samples were assayed at once and at 24-hour intervals during the five-day period from Monday to Friday inclusive. During the 96-hour experimental period, the samples were stored in a home-type electric refrigerator in darkness at 10° C. The length of the storage period doubtless exceeded the period that fluid milk ordinarily is stored in the home, but it was adopted to provide sufficient data for judging the rate of destruction of reduced ascorbic acid in riboflavin-fortified milk during early storage.

Reduced ascorbic acid was determined by the Sharp method, which was modified by using 25 ml. of milk instead of 10 ml. and by using a mixture of 3 percent metaphosphoric acid and 8 percent acetic acid instead of sulfuric acid.

RESULTS AND DISCUSSION

The results of the ascorbic acid assays of the three series of seventeen samples of milk are summarized in Table 1. The raw milk as it arrived at the laboratory contained from 15.4 to 19.9 mg. per liter of reduced ascorbic acid and averaged 17.9 mg. per liter. Since the major portion of the milk under consideration was produced during the winter months by stall-fed cows, it was assumed to be representative of freshly drawn, high-quality commercial winter milk. Both the control and the riboflavin-fortified samples lost reduced ascorbic acid continuously during the storage period. The daily losses of reduced ascorbic acid from the controls during the different 24-hour periods were 20 percent, 15 percent, 18 percent, and 13 percent, or a total of 64 percent during the 96 hours of storage in darkness at 10° C. The corresponding losses for the raw milk fortified with U.S.P. riboflavin at the rate of 4 mg. per liter were 18 percent, 16 percent, 17 percent, and 13 percent, or a total of 64 percent. Similar results were obtained for the raw milk enriched with riboflavin at the rate of 8 mg. per liter; namely, the daily losses of reduced ascorbic acid were 18 percent, 15 percent, 18 percent, and 15 percent respectively, or a total loss of 66 percent. Thus, even though the losses during the different daily intervals were not identical for the three series of samples, the total losses for the raw milk, for the raw milk fortified with riboflavin at the rate of 4 mg. per liter, and for that enriched with riboflavin at the rate of

TABLE 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ascorbic Acid mg./l</th>
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<tr>
<td>Monday</td>
<td>Tuesday</td>
</tr>
<tr>
<td>17.9</td>
<td>14.3</td>
</tr>
<tr>
<td>17.9</td>
<td>14.6</td>
</tr>
<tr>
<td>17.9</td>
<td>14.6</td>
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8 mg. per liter, were essentially identical.

Holmes obtained similar results in a study of the addition of riboflavin to pasteurized cow milk, namely, the addition of riboflavin to pasteurized milk did not increase the amount or the rate of destruction of reduced ascorbic acid. In fact, the total losses during storage of the pasteurized milk and the pasteurized milk fortified with 4 mg. and with 8 mg. of riboflavin per liter were 77 percent, 73 percent, and 69 percent respectively.

These data show that enrichment of cow milk with riboflavin caused little if any change in the amount or rate of destruction of reduced ascorbic acid during 96 hours of storage in glass containers in darkness at 10°C.

SUMMARY

Samples of pooled raw herd milk and the raw milk fortified with riboflavin at the rate of 4 mg. and 8 mg. per liter were prepared weekly during the fall and winter months and assayed for reduced ascorbic acid. Since the three series of samples lost reduced ascorbic acid at essentially the same rate and the total amount of reduced ascorbic acid lost was the same for the three series, it is apparent that the added riboflavin did not influence the loss of reduced ascorbic acid from raw milk stored in darkness under the conditions employed in this study.

REFERENCES

9. Holmes, A. D., and Jones, C. P. The Effect of Sunlight upon the Ascorbic Acid and Riboflavin Content of Milk. Ibid. 29, 201-209 (1945).

RECENT ADVANCES IN THE MICROBIOLOGICAL METHODS FOR THE DETERMINATION OF VITAMINS AND AMINO ACIDS

NORMAN W. DESROSIERS

Assistant Research Professor, Department of Food Technology, University of Massachusetts, Amherst, Massachusetts

INTRODUCTION

The use of microorganisms in the assay of biologically active materials is the result of many brilliant investigations. After extensive studies on the growth of yeast and other microorganisms, Pasteur concluded that a medium containing the water soluble fraction of yeast, ammonium tartrate, and sugar was sufficient for the growth of yeast and for fermentation. Liebig, however, failed to obtain yeast growth with this medium. Both men were careful workers and the discrepancy appeared to be important.

Wildi and others in alcoholic fermentation, began experiments of his own on the nutrition of yeasts. He concentrated a substance which he called "bios," necessary for the growth of yeasts, and postulated that the bios of inoculum had been comparatively large and sufficient "bios" had been included in the inoculum to bring about growth and fermentation in an otherwise "bios"-deficient medium. Some of the active principles of Wildi's bios have been isolated and identified as thiamine, niacin and pantothenic acid.

These discoveries stimulated exhaustive investigations of the nutritional requirements of a number of yeasts, molds, and bacteria. These investigations revealed that the simple substances supplied carbon, nitrogen, and other necessary elements, the metabolic processes of microorganisms require a number of specific chemical substances for rapid growth and metabolism.

Adequate nutritive media for numerous organisms can now be formulated using pure chemical substances. If the medium is deficient in any one of the growth factors it will fail to support normal metabolism and growth. There is, consequently, a direct relationship between the amount of a substance that is present in the media and the amount of growth of the organism.

The discovery that riboflavin played an essential role in the nutrition of yeasts was one of the first indications of the essential role the vitamins play in the nutrition of microorganisms. Thiamine was shown to be essential to the growth of yeasts provided all the fragments for its synthesis were absent; and nicotine acid was found to be essential for the growth of Staphylococcus aureus.

Today the growth factors essential for, or stimulating to, the growth of a large number of microorganisms are known. Most organisms require pantotenic acid, biotin, and nicotinic acid, while they display great variations in their requirements for thiamine, riboflavin, pyridoxine and folic acid (Anon. 1946).

Many vitamins have been shown to be essential portions of enzyme systems. Along with the recent discovery of the multitude of enzymatic processes, both degradative and synthetic, in the metabolism of all organisms, there is a recognition of the important role which vitamins play in these enzyme systems.
Certain amino acids are also essential to the nutrition of both microorganisms and animals. Just as in the case of the vitamins, the essential amino acids can be assayed, with great accuracy, by their effect on the growth of microorganisms in appropriately deficient media. The essential nature of certain amino acids in human and animal nutrition, as well as the desirability of supplying products rich in these essential amino acids, makes methods for their assay highly significant.

**Microbiological Assays for Vitamins**

Microbiological assay methods have been divided into four classes according to the method of observing growth of the microorganism. They are:

1. **Yeast fermentation and measurement of gas production (thiamine assay)**
2. **Lactic fermentation and measurement of acidity produced (riboflavin, biotin, pantothenic acid assays)**
3. **Bacterial growth and the measurement of resultant turbidity (folic acid assay)**
4. **Mold growth and measurement by weight of mycelia produced (pyridoxine assay)**

A considerable number of papers have appeared during the past few years directed toward improving the specificity and range of applicability of the microbiological assays for the vitamins. Some are based on changes in the technical details of earlier methods, in preliminary hydrolytic procedures, in the composition of the media, or in conditions of inoculation. Others involve the use of microorganisms hitherto not used for the specific vitamins in question (Oser, 1949).

**Thiamine**

Although most workers prefer the thiochrome method of Hennesey (1941) for the estimation of thiamine, improvements in microbiological methods have been described based upon macrofermentation with yeast, on the growth of *Lactobacillus fermentum* 36, and of the fungus *Phycomyces blakesleeanus*.

The Shultz, Atkin, and Frey (1937) yeast method of assay depends upon the production of gas during an alcoholic fermentation. As little as 1 gamma (0.000001 g.) of thiamine may be detected. The sulfite cleavage has been reported by Shultz, Atkin, and Frey (1942) to be incomplete in some substrates, and must be determined for each type of substrate assayed.

Sarett and Cheldelin (1944) reported a method for thiamine determination based on the growth of *Lactobacillus fermentum*. The growth response is measured turbidimetrically. Good recovery has been reported by this assay method.

Schopfer and Jung (1937) and Cotton (1947) reported a fungus (*Phycomyces blakesleeanus*) growth method for determining thiamine. Because of the cost of the apparatus required for the thiochrome procedure, this fungus growth method may be of interest. The equation to run this assay is very insensitive. The mycelia mats produced are dried and weighed and the thiamine content is read from a standard curve, simultaneously produced. A two-week incubation period is recommended. The results agree closely with the thiochrome method and within 3-8 percent of the rat curative tests.

**Riboflavin**

In the case of riboflavin, Oser (1949) reports that many analysts seem to prefer modifications of the fluorometric method, although the official microbiological procedure has many adherents because of its unquestionably great specificity. The Snell and Strong (1939) method utilizes *Lactobacillus casei*. The acid production is measured by titration with NaOH or by pH value measurements. Standard curves are prepared by plotting the pH value or milliliters of base against concentrations of the standard, and the concentration in the aliquot is interpolated from the curve.

Kornberg, Langdon, and Cheldelin (1948) reported a method of assay using *Lactobacillus mesenteroides* 10 as the test organism. The results are reported to be more sensitive, as the organism responds to 1/50th the riboflavin required by *Lactobacillus casei*.

**Nicotinic Acid**

Improvements in the media for the Snell and Wright (1941) *Lactobacillus arabinosus* 17-5 assay for nicotinic acid have been reported. *Acetobacter suboxydans* and *Proteus HX19* have also been used for the assay of nicotinic acid. A yeast (*Torula rennioris* 2512) assay has been adapted to the differentiation of nicotinic acid (or its amide) trigonelline and N'-methylcotinamide by varying the conditions of hydrolysis (Williams, 1946). The millifiers of base required for titration of the acid produced or the pH value may be used for the calibration of standard curves.

Karram and Dalles (1944) reported that nicotinic acid was essential for the growth of *Acetobacter suboxydans* and described a method for the determination of the vitamin.

The use of *Proteus HX19* in the determination of nicotinic acid has resulted in a tenfold increase in sensitivity over other methods, is faster, and the results are of the same precision as the other methods for determination (Grossowicz and Sherstinsky, 1947).

**Pyridoxine**

Melnick, Hochberg, Himes and Oser (1945) reported that the microbiological assays of the *B₆* content of materials with *Saccharomyces cerevisiae* underlines the *B₆* content because biologically active pyridoxine derivatives are less stimulatory for this organism. These compounds however show comparable activity for *Saccharomyces cerevisiae* and for the rat (Hopkins and Pennington, 1947).

The method of hydrolysis in the estimation of the vitamin in natural materials is of importance as well as the choice of the organism. Oser (1949) reported that 0.055 N H₂SO₄ was more effective in extracting total *B₆* than 2 N H₂SO₄. This has been confirmed by other workers, and autoclaving at 20 pound steam pressure for 3 hours in 0.55 N H₂SO₄ is generally recommended. Oser also reported that the most reliable method for determining the total *B₆* in natural products is the microbiological method with *Saccharomyces cerevisiae*. In pure pharmaceutical preparations in which pyridoxine is the only member of the *B₆* complex present, *Saccharomyces cerevisiae* is satisfactory.

The animal assay for *B₆* continues to play an important role due to incompleteness of extraction. Pyridoxine, pyridoxal, and pyridoxamine when injected all have equal potency. When included in the diet, pyridoxine is more active (Sarma, Snell, and Elvehjem, 1946). Pyridoxine may be determined by titration method. A pyridoxine-less mutant No. 299 of *Neurospora sitophila* is employed, and the weight of mycelia, after drying, is used for calibration. Standard curves are produced by growing the mold on known levels of pyridoxine (Stokes, Guiness, Dwyer, and Coswell, 1943).

*Saccharomyces cerevisiae* and *Streptococcus faecalis* as the organism have been proposed for pyridoxal and pyridoxamine assays. Sodium citrate, substituted for sodium acetate, and sterile cystine are added to improve the medium (Rabinowitz and Snell, 1947).

The determination of pantothenic acid by the method of Strong, Feeney, and Earle (1941) using *Lactobacillus casei* has been modified. Ives and Strong (1946) reported that the release of the vitamin by the use of an enzyme preparation Mylase P, and the use of *Lactobacillus arabinosus* 17-5 as
the test organism have advantages. Hoag, Sackett, and Cheldelin (1945) observed that Lactobacillus arabinosus was not as sensitive as Lactobacillus casei to the interfering effects of fats and starches. A greater response and more rapid growth of Lactobacillus arabinosus with the vitamin has been reported. Results may be read by turbidity in 14 hours or by acid production in 24 hours.

**Biotin**

Lactobacillus casei has been generally used for determining biotin. Neurospora crassa, a choline-less mutant, has also been employed in estimating biotin as well as choline in milk products (Hodson, 1945). Because biotin is the first vitamin for which this method has been described, it is interesting to refer to the agar plate method recently proposed in this country (Hoag and Partridge, 1948). In the method of Genghof, Partridge, and Carpenter (1948), filter paper discs are inoculated with doses of standard and unknown and placed on agar plates seeded with test organisms. The diameter of observed growth has been found to be a linear function of the logarithm of the concentration over a range of 1–1000 per milliliter. This method is claimed to be as sensitive as those using liquid media, and may have as wide an application in other assays for growth promoters as it has in evaluating the zone of inhibition of antibiotics.

**Folic Acid**

This vitamin may be determined by the method of turbidity after 16 hours’ growth of Streptococcus lactis R. Microbiological estimations of folic acid have been subject to recent collaborative studies (Oser, 1949) and satisfactory results have been reported using either Streptococcus faecalis or Lactobacillus casei as the test organism. A modified medium has been recommended which is applicable to either organism. Dehydrated bacteriological media are available commercially for assay of several vitamins, and recently a dehydrated medium has been described for the folic acid assay (Capps, Hobbs, and Fox, 1948). For liberation of bound folic acid prior to microbiological assay, enzymatic digestion with extracts of hog kidney or chicken pancreas are used, but this procedure is not applicable to plant extracts.

**B12**

At the present time there is great interest in the development of a microbiological assay for the animal protein, now believed to be identical to B12. Indications are that a strain of Lactobacillus leichmannii will be a suitable test organism for this factor (Snell, Kitay, and McNutt, 1948).

**Amino Acid Assays**

The microbiological methods for the determination of the amino acids are also based upon the limiting effect of an essential nutrient on the growth of the test organism. The amino acid to be determined is left out of the basal medium. Standard curves are prepared, along with the sample material. The amount of acid produced is dependent on the amount of the amino acid present. The amino acid content of the sample is interpolated from the standard curve.

A modified method has been developed during the past few years in revising and improving methods of microbiological assay for amino acids (Oser, 1949). Several systems of assays are based on the use of a single organism for a number of different amino acids. For example, a system of assay has been described by Guinness, Dwyer, and Stokes (1948) for nine essential amino acids using Streptococcus faecalis as the test organism; the tenth, phenylalanine, is determined with Leuconostoc debruchi. More recently a procedure for 13 amino acids has been reported by Boyd, Logan, and Tytell (1948) based on the use of Clostridium perfringens which is claimed to have the advantage of not requiring aseptic conditions. Oser (1949) reported that the present tendency is towards methods utilizing more than one organism for the series of amino acids, each designated to take advantage of the greater accuracy resulting from the specific requirements of the microorganism, special adaptations of media, conditions of incubation, etc. Aside from the conditions affecting bacterial growth the principal technical difficulties are concerned with the preliminary preparation of the protein hydrolysates without the destruction or racemization to inactive forms. The d- and l- forms of the acid has no biological activity, the former being active. The dl forms have 50 percent activity. Methylamine, threonine, cysteine, tryptophan must be enzymatically hydrolyzed as these amino acids are 20–100 percent destroyed by acid hydrolysis.

Attempts have been made to overcome the difficulties of acid hydrolysis of the test organism by modifying the conditions of hydrolysis, using of alkaline hydrolysis, or direct determination on unhydrolyzed material (Oser, 1949).

Analytical data for the amino acid content of proteins and of foods are given in many papers. Henderson, Brickson, and Snell (1948) have described a micromethod for the determination of amino acids which is adaptable to samples as small as 0.2 ml. of sample. Millares and Davis (1949) reported a micromethod for assay requiring only 2 ml. of combined hydrolyzate and medium.

As a matter of interest, the microbiological methods apply not only to the amino acid and vitamin determinations but also to the determination of nucleic acid, which is required by Lactobacillus arabinosus (Bently, Snell, and Phillips, 1947) and potassium which is required by Streptococcus faecalis R. (Barton-Wright, 1945). Several lactic acid bacteria require oleic acid, which suggests the possibility of microbiological assays for fatty acids (Williams, Broquist and Snell, 1947).

**Conclusion**

Recent advances and improvements in the microbiological methods for the determination of the vitamins and amino acids have been reviewed. The majority of the changes have been directed toward improving the specificity and range of applicability of the assay methods.

**References**

20. Oser, B. L. Recent Developments in Food Analysis. Ibid. 21, 216-227 (1949).

CLEANING AND BACTERICIDAL VALUES OF DETERGENT SANITIZERS

Turbulent detergent sanitizers, also known as cleaner sanitizers, consist essentially of a quaternary ammonium compound and a nonionic wetting agent, with or without various combinations of polyphosphates and alkali cleaners. Their primary purpose has been to improve dairy farm sanitation by making it easier for the dairy farmer to clean and sanitize his equipment properly. Under certain conditions these detergent sanitizers have been found to be highly effective in eliminating or inhibiting the development of thermobacteria and fungi on farm equipment. Studies carried out in this study determined the effectiveness of the compounds in maintaining low bacterial counts with a minimum effort on the dairy farm. The study also generally have been favorable toward the development of detergent sanitizers. 1, 2, 3, 4, 5
One study 6 indicated the detergent sanitizer to be comparable to conventional cleaning methods with standard cleaner and hypochlorite germicides. Since the detergent sanitizers are relatively new to the dairy industry and some of their development has been based on uncontrolled studies, a number of details of procedure have been overlooked in published directions. Some examples of these are as follows: Certain directions have failed to specify a rinse with cool or lukewarm water to remove milk solids immediately after use of equipment. There has been great variation in composition of detergent sanitizers on the market and some obviously have been unable to carry out both an effective cleaning and germicidal treatment. Some reports have indicated a pH of 10.0 in use, dilution to be necessary to prevent excessive development of Pseudomonas species. Some directions have specified preparation of detergent sanitizer solution in cold water. Others specify storage of the cup with wet in solution of the waxing or dry. Some directions also specify no rinse just before use of equipment, others, a rinse with water, others, with detergent sanitizer or quaternary. Most of the methods have been designed to make any mention of a periodic disassembling for a thorough removal of accumulated milk solids or other material.

Experimental

In order to obtain information on the above problems, a farm project was initiated consisting of one standard procedure with conventional type of cleaners and hypochlorite germicides and also three variations of a detergent sanitizer procedure. Following a broad survey of a large number of Grade A producers shipping to one plant, 16 farms, all with long-tube milkers of various makes, were selected. Four farms were placed on each of the four procedures. An attempt was made in so far as possible to place two producers with an excellent record for cleaning methods.

The 1950 schedule of public health laboratory courses given by the Communicable Disease Center has been revised as follows:

An additional 1-week course in laboratory diagnosis of enteric bacteria, will be given March 20-24.

An additional 2-week course in laboratory diagnosis of tuberculosis will be given December 4-15.

The previously announced 3-week course in the laboratory diagnosis of tuberculosis will be given August 21-September 7, instead of the dates shown on the course announcement on page 41 of the Bulletin of Public Health Laboratory Courses.

An additional 1-week course in serological diagnosis of ricketsial diseases will be given November 6-10.

Information and application forms should be submitted to the Chief, Laboratory Services, Communicable Disease Center, Public Health Service, Chamble, Georgia.
and two with a mediocre or poor quality record on each procedure. Sanitation equipment was inspected at least twice during the two to three-week preliminary period before starting on the experimental procedures and every two weeks for the remainder of the experiment. Inspections were carried out in order to obtain first hand information on condition. Additional observations were made on the various processes and pasteurized plate counts on most of the producers for two years prior to the experiment were provided by the plant.

Evaluation of effectiveness of various procedures was based on inspection of farm equipment, raw plate counts, plate counts on milk samples pasteurized in the laboratory, and microscopic examination of milk samples collected from the wash vats at the milk plant. Other data included temperature of incoming milk, atmospheric temperature and relative humidity, and farm water hardness tests. All milk cans for farms on the experiment were hand-cleaned at the beginning of the trials and cans were checked at each sampling to insure that they were not influencing results. Cans also were sanitized with hypochlorite solution just before use to further eliminate them as a factor influencing experimental results.

The cleaner and germicidal compounds furnished the producers for the experiment were as follows: The standard cleaner was a balanced, alkaline washing compound. The standard acid cleaner was a balanced, organic-acid containing a wetting agent. The hypochlorite was a sodium hypochlorite solution. The detergent sanitizer powder consisted chiefly of anorganic acid, nonionic wetting agent, and quaternary compound. The liquid detergent sanitizer consisted chiefly of an organic acid, nonionic wetting agent, and quaternary compound.

Following are the detailed procedures that were carefully outlined and turned over to the respective producers.

**Standard Alternate Method**

(1) Immediately after milking, draw one pailful of lukewarm water (100°F) through the unit raising and lowering the teat cup assembly for scrubbing action. (2) Rinse milk pail and all milking utensils thorough with this solution. (3) Rack to dry with no further treatment. (4) Just before milking, draw one pailful of lukewarm water through the unit. (5) Thoroughly rinse all milk cans before use with one pailful of 200 ppm strength hypochlorite solution.

**Original Cleanup Procedure Used at Beginning of Experimental Period**

In order to insure clean equipment as the farms went onto the experimental procedures, they were instructed either to equip machines with new rubber parts, or to subject rubber parts to the following detergent cleanup: (1) Place all rubber parts in a stone crock containing a solution prepared by adding two ounces of organic acid detergent per gallon of warm water. Soak all rubber parts in this solution from the morning milking to the night milking. (2) Before the night milking, take all parts thus soaked and brush them vigorously inside and out. After this has been done, roll out the milk tubes several times, or until the rod comes clean. (3) When all parts are completely clean and free of milstone, wash them thoroughly in a balanced alkaline dairy cleaner solution. Rinse all parts thoroughly with hot water. These parts are now ready to be reassembled.

**Detergent Sanitizer**

The metal parts of the milker, and other utensils where necessary, also were thoroughly cleaned with the acid detergent. The above procedure for rubber parts and the cleaning of metal parts with the detergent was specified at certain times during the experiment. The time of such acid treatment is shown in the figures presenting results over the entire period.

**Purpose of Various Procedures**

The standard procedure was included in order to provide a standard control for detergent sanitizer method. Previous studies had shown this method to yield satisfactory results when properly carried out. The second procedure, the standard detergent sanitizer method, represented a relatively simple standard detergent sanitizer procedure.

The third, the detergent sanitizer alternate method, applied the alternate alkaline-acid principle to detergent sanitizers in order to prevent build-up of alkaline residues on equipment. The fourth method represented the simplest possible modification of the detergent sanitizer procedure, was far superior to some instructions for use of detergent sanitizers on the market and it was employed to determine effectiveness of the so-called flush-wash procedure under the most severe conditions of use. Field observations in the past have indicated the flush-wash procedure for milking machines to be far more common than generally realized.

All producers were called upon to follow the recommended procedure. They also were urged not to expend any additional effort above that needed for an average cleaning job. They were assured of immunity from the routine inspections during the three to four months of the experiment (July 1 to November 1, 1948).
milliliter over the period of the experiment. Thermoculturic counts were correspondingly low with the exception of one producer who gradually lowered his thermoculturic count to a few hundred per milliliter of milk after several weeks on the alternate procedure. The one producer averaging less than 10,000 during the experiment was about to be degraded for high bacterial count when he went onto the experimental procedure. Obviously much of his previous trouble was due merely to a lack of knowledge of the steps required for proper cleaning and germicidal treatment. It became apparent after a few days on the experiment that every one of the producers on this procedure believed that twice a day disassembling and washing was unnecessarily and in spite of our urging soon lapsed into a once a day washing with merely a rinsing after the evening milking. This was true even with those who showed the best records. Such a procedure proved sufficiently adequate to provide low bacterial counts in these trials.

**Standard Detergent Sanitizer Method.** Results with this method are shown in Figure 1. Examination of data of individual producers indicates that there was a gradual increase in raw count of producer 2A. He obviously had not been brushing his teat cups and failed to carry out the complete disassembling and cleaning with acid detergent at the end of the month. When reminded of this fact he immediately carried out the disassembling and cleaned thoroughly with acid cleaner including a soaking of rubber parts. Following this cleanup, which is indicated in the figure, his count dropped to a very low level and remained low for the remainder of the experiment. Producer 2B had been experiencing some difficulty prior to entering the experiment but on this procedure, maintained counts averaging well under 10,000 per milliliter.

The next producer, 2C, also experienced difficulty with high counts prior to entering the experiment; but when he entered the experiment with new rubber teat cups his count dropped to a low level and remained reasonably low. There was some increase in numbers due apparently to accumulation of a practically invisible deposit during the latter part of the first month but this was to be expected because the teat assembly was never taken apart on this procedure during the first month. At the end of the month, this producer was instructed to disassemble the entire machine and subject it to the acid detergent cleaning procedure. His counts were brought even lower and generally remained low for the next six weeks of the experiment. The last producer in this group also had experienced difficulty prior to the experiment. However, his counts came down at the beginning of the experiment and remained low throughout. Frequent examination of his inflations and rubber equipment in general indicated that he persisted in being careless in his washing procedure. There usually was a slight amount of hard deposit in the teat cups that apparently had been carried over by his failure to clean adequately and soak rubber equipment in acid detergent at the beginning of the experiment. Despite his laxity he consistently produced milk with low raw and pasteurized count throughout the test period.

Producers on the standard detergent sanitizer method demonstrated rather definitely that it could be employed successfully during the period of the year when most of them experienced difficulty with both high raw and pasteurized counts. Thermoculturic counts remained low in the case of every producer on this procedure. Only one producer of the group was equipped with a mechanical cooler. The others used surface coolers with well water as the cooling agent. Temperatures of milk at the plant for these producers usually ranged from about 60° to 64° F. This procedure proved easy to follow and probably was carried on more conscientiously than that of the conventional standard alternate method. Every farmer on the project reported considerable difficulty with milkstone before these experiments began, yet there was no evidence at any time of milkstone accumulation on equipment while the detergent sanitizer was employed. This contrast was quite striking and was one of the most frequent comments of the producers. There was some accumulation of brown deposit on the outside surfaces of teat cups, milk tubes, and on wash vats. This deposit could be readily removed by vigorous washing in warm water and was not considered objectionable.
tionable. One producer experienced considerable difficulty with his air hose slipping off the metal nozzles when the detergent sanitizer was used. This was reported in some other instances but only with one make of milking machine.

**Detergent Sanitizer Alternate Method.** Results with this method are shown in Figure 2. Results of two of the producers had to be discounted. One had defective equipment that contributed to high counts and there was also evidence that he did not follow instructions properly. When this was corrected late in the experiment, his counts dropped to a low level and remained low. The other producer failed to follow instructions, apparently because he did not have confidence in the residual film of detergent sanitizer and insisted on rinsing it off after washing. The results of the other two producers should be more representative of what might be expected with this method. Results of another farm on this method are shown in Figure 3.

The raw counts of producer 3A are interesting in that they indicate satisfactory results with this method for about the first three weeks and then a gradual increase in numbers due to accumulation of a soft deposit of milk solids in the inflations. The thermodynamic bacteria were definitely inhibited by the quaternary during this period. A complete disassembling and cleaning with organic acid detergent removed the accumulation and the count then remained low for the remainder of the experiment. Apparently under the conditions encountered in the experiment the alternate use of acid detergent sanitizer solution in the daily cleaning procedure was not entirely effective in removing accumulated deposit or in preventing such accumulation.

Counts of producer 3B are interesting for another reason and emphasize the necessity for frequent and careful inspection of each producer's equipment and operation in an experiment of this type. This producer started out with high counts and continued so on the experimental procedure. Inspection indicated that his milking machine, which was an old type, could not prevent escape of milk to the vacuum line. When this was pointed out to him, he immediately purchased a new machine with the results shown in Figure 3. His counts, both raw and pasteurized, remained low throughout the experiment. Both this producer and 3A reported that the acid compound appeared especially effective in removing milk solids from metal equipment.

Results of another trial with the alternate detergent sanitizer procedure are shown in Figure 3. In this case, the experiment was begun with test cups that were clean but were slightly cracked and checked from use. The result as indicated by bacterial analyses was a gradual increase in raw count due, undoubtedly, to accumulation of milk solids in the porous rubber. On complete disassembling and cleaning with organic acid detergent, the deposit was removed. However, it immediately began to accumulate again on the same procedure. All during this period the thermodynamic count remained low.

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**Detergent Sanitizer Short Method.** Results of this group, Figure 4, were the most interesting of the lot. It was expected that counts might run excessively high since the detergent sanitizer had to perform the operation of a water rinse, a cleaning, and a germicidal treatment in one single flushing operation. Contrary to expectations most of the farms on this procedure produced a high quality milk and it was definitely established that they were following instructions relative to the details of the procedure.

Producer 4A did not replace his rubber inflations and milk tube at the beginning of the experiment, but instead carried out the disinfecting with a solution of acid detergent as outlined. Consequently the lack of a water rinse apparently placed too great a demand on the cleaner and there was a gradual accumulation of deposit in the slightly porous rubber. Examination at the end of the experiment indicated that the rubber was saturated with fat. The result was a gradual increase in bacterial count of the milk followed by a sharp drop after complete disassembling and acid detergent cleaning at the end of the first month. The accumulation again occurred with subsequent
DISCUSSION

This study has emphasized a number of factors not previously reported affecting application of detergent sanitizers. One of the most striking observations brought out was the cleaning ability of the detergent sanitizer powder preparation used. In spite of the fact that water hardness on these farms usually ranged from 3 to 15 grains per gallon, almost all of the farms reported considerable milkstone accumulation on equipment with conventional cleaners previously used. Other studies carried out since this experiment was completed have emphasized the importance of the farm water supply in affecting success of detergent sanitizers. A detergent sanitizer preparation of high cleaning ability appears to be essential to cope with varying water conditions from standpoint of both prevention of milkstone deposits and bactericidal effectiveness of the preparation. It is believed on the basis of the studies reported here that superior cleaning and sequestering ability of the alkaline detergent sanitizer employed in the experiment was in a large measure responsible for the low bacterial counts obtained with it. Examination of milking machine infestations on all procedures, noting them open at the end of the experiment emphasized this fact. Those on the standard detergent sanitizer procedure were exceptionally clean and free of deposit. The results suggest that the detergent action may be as important as the bactericidal action in compounding a detergent sanitizer. The effect of detergent sanitizers on thermic bacteria also was marked. The explanation for this lies in the bactericidal or sequestering activity of the quaternary against the gram-negative Pseudomonas aeruginosa and thermic streptococci. In these studies as well as others, factors other than detergent action may be important in cleaning with an acid detergent.

The brown precipitate that developed possibly represented an interaction between the quaternary and milk protein. The fact that it was so constant emphasized the need for a thorough water rinse immediately after use of the equipment. This fact plus the greater accumulation of milk solids, especially fat, where the rinsing and brushing were omitted eliminates the short method as a practical means of cleaning with a detergent sanitizer.

SUMMARY

1. Results are reported on a farm project designed to compare a standard conventional cleaner and hypochlorite sanitizer with a detergent sanitizer procedure. Under the farm water conditions encountered (water hardness ranging from 3 to 15 grains per gallon), both the standard conventional cleaner and hypochlorite sanitizer yielded satisfactory results when reasonable care was taken to follow instructions for their use.

2. Bacterial counts and observations on some previous condition of farm utensils and milking machines indicated that the minimum steps necessary in a detergent sanitizer procedure included a
thorough rinse in cold or lukewarm water immediately after use, thorough brushing with a warm detergent sanitizer solution, and a periodic disassembling and thorough soaking and cleaning in a balanced organic acid detergent in order to remove accumulated solids.

3. Observations indicated that the cleaning ability of the detergent sanitizer was of paramount importance. Effective cleaning was provided by a detergent sanitizer powder preparation consisting essentially of nonionic wetting agent, polyphosphate, and quaternary ammonium compound.

4. The detergent sanitizer compounds exhibited a remarkable specificity against thermotolerant bacteria. No accumulation of fluorescent Pseudomonas species occurred with procedures but could be eliminated largely by thorough rinsing with cold or lukewarm water to remove milk solids immediately after use, and by thorough brushing subsequently in the warm washing solution.

REFERENCES


CONTESTATION FROM DYE SOLUTION IN THE METHYLENE BLUE AND RESAZURIN REDUCTION TESTS

C. K. JOHNS

Division of Bacteriology and Dairy Research, Canada Department of Agriculture, Ottawa

Recently some concern has been voiced over the possibility that the dye solutions used in the methylene blue and resazurin reduction tests might contain sufficient numbers of bacteria to affect the results of these tests. That this may occur when solutions are prepared with unsterile glassware was reported by Thomas (2), and in 1946 the British Ministry of Agriculture (3) prescribed that resazurin solution should not show 37°C counts of more than 10 per ml. At the suggestion of Dr. A. H. Robertson, Chairman of the Committee on Standard Methods for the Examination of Dairy Products of the American Public Health Association, several series of tests were carried out in these laboratories in which dye solutions were prepared according to official directions (1) and compared with similar solutions prepared aseptically. Solutions were analysed at once, then held at room temperature and examined at intervals up to 14 days. Plates were prepared in triplicate and incubated at 37°C for 48 hours.

The results suggest that if solutions are prepared as directed, contamination from this source will be negligible. While one freshly prepared resazurin solution gave a count of 21 per ml, subsequent counts were less than 1 at 4 days, 3 at 7 days and 5 at 14 days. The solutions prepared aseptically showed less than 1 colony per ml. Counts of similar magnitude were obtained from methylene blue solutions.

REFERENCES


Wisconsin Winter Course in Dairy Manufacturing

The winter course in dairy manufacturing at the University of Wisconsin is scheduled to begin with registration on September 20, 1950; instruction ends December 16. During the first semester (September 20 to November 15), the courses will be dairy arithmetic, bacteriology, cattle diseases, mechanics, sanitation, marketing, and milk composition and tests. In the second semester (November 16 to December 15), students may elect two courses: ice cream making or buttermaking, and market milk or cheesemaking. Reservations must be made before August 15, 1950, by writing to Professor H. C. Jackson, Department of Dairy Industry, University of Wisconsin, Madison 6, Wisconsin.

Vermont Annual Conference

Twenty-ninth Annual Conference for Dairy Plant Operators and Milk Distributors is scheduled for October 25 and 26, 1950, by the Department of Animal and Dairy Husbandry of the University of Vermont and State Agricultural College, Burlington. The program will be built around milk quality, the newer dairy techniques and a round table discussion of milk plant problems.
LABORATORY DETECTION OF FOOD POISONING ATTRIBUTABLE TO DAIRY PRODUCTS

R. W. Newman
Dairy Bacteriologist
California Dairy Service Laboratory
Sacramento, Cal.

Gastro-intestinal disturbances in human beings are the characteristic symptoms of food poisoning. Food poisoning often is incorrectly referred to as "toxic" poisoning. No "toxins" ever have been found in food or anywhere else. It is a word which was used to describe a condition before its cause was discovered.

Food poisoning is a term loosely applied to symptoms which may follow ingestion of various foods contaminated by the organisms or by toxins of the following bacterial genera or species: Clostridium botulinum, Staphylococcus aureus, and Salmonella. In addition, certain species of Proteus, Streptococcus, the coli-aerogenes group, and some of the paracolonics occasionally have been implicated.

CAUSES OF FOOD POISONING

True food poisoning, however, is caused only by Clostridium botulinum and by some of the staphylococci. That is, only these two have been shown to produce a true toxin or poison. Ingestion of either of these organisms without toxin does not produce poisoning.

The others (Salmonella, Proteus, Streptococcus, coli-aerogenes, and the paracolonics) apparently elaborate no true toxins, and the symptoms produced by them are the result of a mild infection from the organisms themselves, often masked by gastro-intestinal symptoms simulating true food poisoning.

Botulism. Symptoms of botulism usually occur in 12-36 hours although they may appear at any time within a period of 2-4 days. The toxin usually affects muscle coordination and the nervous system. The patient may or may not show symptoms of nausea, diarrhea, vomiting. Poisoning usually is due to the eating of canned meats, fruits, vegetables, and fish which have been inadequately sterilized. It is rarely found in dairy products although a few, such as home canned cheese and commercially-packed canned milk, have been implicated.

Poisoning is caused by a toxin produced by the growth of the organism in the absence of oxygen and within a relatively narrow temperature and pH range. Mortality: high.

Salmonella. Symptoms usually occur within 12-24 hours after eating implicated food, although occasionally they may appear in 8 hours or as long as 72 hours later. Salmonella cases are characterized by nausea, vomiting, and abdominal cramps followed by diarrhea, sometimes with fever, and are due to an infection resulting from drinking water or eating food contaminated by one or more species, varieties or types of Salmonella. Meat, milk, cheese, and eggs have been implicated.

Mortality: low.

Streptococcus, Proteus, coli-aerogenes group, paracolon organisms. Similar to Salmonella.

Staphylococcus. Symptoms occur within 1-6 hours, usually within 2-3 hours, after eating implicated food. Causes salivation, nausea, vomiting, retching, abdominal cramps, and diarrhea, normally severe. Poisoning may be due to eating meats, fish, custards, salad dressings, creamed foods; creamed soups, sauces, or gravies; pies or cakes with custard or cream fillings or icing; milk, cream, buttermilk, ice cream, butter, cheese, etc., which have been contaminated by organisms from an infected cow or from infected food-handlers. Toxin is rapidly produced by growth of the organisms in unrefrigerated food. Mortality: negligible; patient almost always recovers on the following day.

Although all of these organisms have been implicated in food poisoning traceable to dairy products, by far the most common source of these cases is the Staphylococcus. As a causative agent of food poisoning, each year it is assuming a more prominent place in public health and other reports. Because such poisonings rarely are fatal and because many of them are mild or are unrecognized as food poisoning, there is every reason to believe that many more cases occur than are reported.

Milk and cream continue to play an unfortunately important role as carriers of such poisonings. Since Barber's (1) classic report from the Philippines in 1914, cases have been reported implicating not only raw and pasteurized milk, including Grade A, but also ice cream, cheese, evaporated milk, buttermilk, cream fillings, milk or cream sauces, custards, milk puddings, cream puffs and cakes, cream soups, etc.

Because of their ubiquitous occurrence, it is fortunate that not all strains of Staphylococcus aureus apparently are capable of elaborating gastro-enterotoxins. Just why some strains of S. aureus have this faculty while others do not, is not quite clear. However, enterotoxins on formation in milk, not only will survive ordinary unrefrigerated temperatures but actual boiling.

Normally these organisms may be found on the skins of all animals, including human beings, and food products can become contaminated from either of these sources as well as from utensils or milking machine parts previously so contaminated. They are capable of rapid growth in milk. They are fairly resistant to drying. On certain surfaces and on fabrics, paper, etc., they have been dried and then recovered in viable form several days later and even after a week or more. In the mucous or encapsulated phase of S. aureus, I have repeatedly taken agar plates on which the agar and the colonies had been dried to glass-like hardness for one month, have rubbed a loopful of sterile distilled water over the dried colony, transferred it to a tube of enriched semi-solid agar and had luxuriant visible growth in two to four hours. This, however, is not true of S. aureus in the ordinary smooth phase.

In fluids, such as milk, S. aureus as a rule is killed by a 10-minute exposure to 58°C. (136°F.), and S. albus by a 10-minute exposure to 62°C. (143.6°F.). Pasteurization, therefore, is a bit more than a reasonable safeguard against S. aureus as an organism. However, if S. aureus has succeeded in producing enterotoxin before pasteurization, this toxin will survive pasteurization temperatures and, if sufficient living staphylococci has been recovered, will cause symptoms of food poisoning even though no living staphylococci can be recovered.

Toxin may be boiled for 30 minutes or more with little if any loss of potency. In fact, so heat stable is this enterotoxin that it is common practice actually to boil it prior to cat inoculations in order to eliminate the possible presence of other less stable toxins which also may be present in the preparation.

It is this difference between the relatively low thermal death point of the organism and the high thermal stability of its enterotoxin which explains so many of the puzzling cases reported in the early literature where food poisonings occurred but without the presence of any suspicious organisms. In the light of our present knowledge plus the
Laboratory Determination of Food Poisoning

Symptoms which had been reported, we now can attribute many of these cases to a Staphylococcus enterotoxin. Thus, at last, we have been able to exonerate many a well-mannered and otherwise respectable Streptococcus or coliform.

Staphylococci in Cows

While staphylococci commonly are found on the skin of cows, occasionally they enter the udder and set up an infection which may develop into a staphylococci mastitis. At certain periodic intervals these organisms are liberated into the milk. If the milk is promptly and efficiently cooled and kept cool until it has been pasteurized, no great danger to the consumer results, for ingestion of the organisms themselves will not cause gastro-enterotoxic symptoms. But if the milk is not cooled sufficiently to prevent growth of the organisms and the elaboration of toxin, then poisoning may occur regardless of whether the milk or cream is consumed as raw or as subsequently pasteurized milk. There have been so many instances of this nature, particularly in the warmer areas of this and other states, that the great importance of cooling in the dairy industry can not and must not be ignored.

If one or more cows in the herd are infected with the staphylococci form of mastitis it is, of course, important that these cows should be isolated last, especially if milking machines are used, in order to prevent spreading of infection to the remainder of the herd. In addition, a thorough cleaning and sterilization of these milking machines and other equipment or utensils which have come in contact with staphylococci milk is imperative. Only by observing these precautions can the spread of infection be controlled, and the seeding of other, non-staphylococci, milk be avoided.

The matter of staphylococci mastitis or mammitis too often is considered only from the standpoint of economic loss to the dairymen; in other words, simply as an outbreak of mastitis in the herd. Of equal and perhaps potentially of greater importance is the possible relation of this form of infection to food poisoning. Since the introduction of Chapman's four way Staphylococci Medium some years back, I have been using it over and over and submitting the samples to the laboratory as an aid in determining whether a pathogenic Staphylococcus might be involved. This medium has been found to be very useful for this purpose. In most cases I also have found that on this medium there is no apparent difference in the behavior of mastitis and food poisoning strains of staphylococci, but at times if they were one and the same organism. I do not wish to imply that all food poisoning cases may be traced back to a case of mastitis or that all mastitis strains of staphylococci are potentially capable of causing food poisoning, but do we have some evidence that at least some strains may be responsible for both.

For example: One day a farmer brought in a sample of milk from his cow which was suffering from "gasger." He particularly wanted to know the causative organism before calling in a veterinarian. The leucocyte count was high, but neither microscopic nor cultural methods revealed any suspicious organisms. In conversation with the farmer it came out that the cow would exhibit all the symptoms of mastitis for a period of four or perhaps five days, then become normal for about a week. At the end of this period the symptoms again appeared. I inquired whether there was any way of knowing when the cow was about to show these mastitis symptoms and he replied,"Yes, the night before when gagert becomes feverish and the nervous. It's hard to milk her, and always know she'll come down with gagert the next morning."

I explained to him the saw-tooth chart described by Dr. Litterer: how milk from a cow with Staphylococcus infection will remain free of staphylococci for almost a week then, with explosive suddenness, they will appear by the hundreds or thousands per field in the milk. The peak, in numbers, occurs on the first day, then rapidly declines to zero within the next three or four days and remains negative for about a week. He also kept other explosive discharge of organisms. Parallel with the liberation of staphylococci in the milk there is a corresponding rise in the leucocyte count.

This saw-tooth picture is explained by the sudden breaking of an abscess with liberation of staphylococci into the milk followed by healing, the reforming of the abscess, and then another breaking down with further discharge of organisms.

The farmer was instructed to bring in another sample of milk on the first morning symptoms appeared. This second sample not only was leucocyte positive but contained millions of highly virulent St. aureus per milliliter.

The farmer was asked whether any of his family had been sick lately. He replied, "Yes," that his neighbor had had a birthday dinner to which he and his wife and daughter were invited. Part of the dinner was a sausage which had been purchased for the occasion. However, they had to leave the party early and return home because he and his little girl were not feeling well. Later, they had to leave the party late. He said that they had gotten food poisoning from eating the sausage. However, although everyone else had eaten part of this sausage, the little girl had not. It seems that she had not been feeling well for several weeks, so her father had taken some milk over to the party with them and that all that she had drunk. She drinks some of it, too, to "keep her company." They were the only ones poisoned.

Of course only a single family cow was involved in this incident and, therefore, one might be inclined to dismiss the case as interesting but unimportant. Yet, it must be remembered that only one infected cow in an average dairy herd can eliminate enough organisms of the aureus type to contaminate the entire output from that dairy. There have been many reports of gastro-enteritis traced back to one or two infected cows in the herd. The dairy itself may be observing all of the rules and regulations with regard to cleanliness, methods and cooling; yet, while raw milk, because of these factors, may not be toxic when it leaves the premises, improper refrigeration after delivery to the home or processing plant may cause it to become so.

Staphylococci in Food Handlers

However, it is not always the dairy cow which is at fault. Sometimes outbreaks of gastro-enteritis may be traced to human sources, to food handlers. A few years ago, an inspector rushed into the laboratory with a slice of so-called Boston cream pie. A "Boston" appears to be a single layer of cake plastered over with cherry jam, which in turn is covered over with a meringue. Over this combination is applied the lavish decorative art of white icing in terms of roses, rosettes, and wavy ribbons made of "whipped" cream.

It seems that a pie shop had delivered several of these concoctions to a country store after a 2-hour ride in a closed van on a very hot day. A woman came in for her Saturday shopping at the very moment the pies were being delivered and purchased one of them. After her shopping was over she took her bundles and the pie out to her car and drove back to the farm. There she placed the Boston on a table in the hot kitchen because there was no room for it in the refrigerator. At 8 o'clock that evening, when her husband came in, dinner was served. Part of the pie was served as dessert and the remainder placed in the refrigerator. Two hours later, the father was rolling around and screaming in what seemed like mortal agony, the mother was almost as bad, the younger daughter was very uncomfortable, and the elder daughter was not ill at all. After a night in the hospital, they returned...
strangely enough, no one suffered any symptoms whatever.

Using approved epidemiological methods, everything they had eaten was eliminated except the Boston and two bars of Cuban chocolate. Laboratory examination quickly eliminated the chocolate. The cake itself was negative; so was the cherry jam. However, the whipped cream and, to a lesser extent, the egg meringue were positive for a food poisoning strain of S. aureus.

Two questions immediately arose: Why was not the elder daughter poisoned also? And why was not anyone poisoned when all of them ate the remainder of the pie the next day?

One could understand why these staphylococci had been able to grow and to elaborate enterotoxin during those two hours in the hot delivery van plus a six-hour incubation in the hot kitchen. The only answer I could think of was that the cream itself could not have been contaminated with S. aureus at the time it had been squirted onto the pie for, if it had, the entire pie would have been equally toxic. Instead, there must have been a spot contamination after the pie was eaten that the whipped cream on one edge of the pie and, in doing so, had contaminated it. Then, during the interim between inoculation and eating of the pie, the staphylococci had grown and spread in ever-widening circles away from this original thumb print. As the organisms continued to grow, enterotoxin also was formed so that, later, when the pie was eaten there was a heavy concentration of toxin at the site of the thumb print, less at the periphery, and still less further away from it. It was the father's bad luck to get the original thumb print; the mother got the next slice, the younger daughter the third, and by the time the elder daughter got her slice it was so far removed from the original site that there was insufficient toxin formed at that point to produce symptoms. As the pie was kept in the refrigerator overnight no further growth occurred and no additional toxin was formed. Therefore, no one became ill the next day because they ate the other half of the pie where there was a minimum or absence of toxin.

But where and how had this spot contamination occurred? Obviously, the first step was to visit the bakery. On walking into the pastry room, the first man observed had a severe impetigo on his face which he was continually scratching. He not only was the man who handled the Boston's but was observed to pick them up, one by one, with his thumb pressed against the side of the pie.

**LABORATORY PROCEDURE IN FOOD POISONING**

Solving a food poisoning case is never a simple routine affair. There may be an absence of definite information on symptoms and the time of onset of these symptoms following ingestion of the implicated food. Or, even if this information is supplied, the local physician may call for a Salmonella infection where contamination is toward a Staphylococcus. Of course, the sample itself may be so old and badly contaminated with other organisms that any attempt at isolating the real culprit is like looking for a needle in a haystack. Occasionally, too, will a mixed contamination in which both a Staphylococcus and a Salmonella, or possibly a Shigella, may be involved.

For this reason, it is fairly certain what the organism really is. It is best to begin with a rough screening of the sample which will segregate all likely genera. This involves:

1. Inoculation of tetrahionate broth with 1 gm. (or 1 ml.) of sample per 13 gm. (or 1 liter) of broth, and also a 5 gm. (or 5 ml.) portion of standard sample in 50 ml. of the broth. These are placed in the 37° C. incubator overnight.
2. At the same time, streak 1 gm. (or 1 ml.) of the sample over the surface of freshly poured and hardened plates of bismuth sulfite agar and of SS agar. These plates are placed in the incubator along with the tetrahionate cultures. These plates should serve as a short-cut in the isolation of any Salmonella-Shigella-Proteus-paracolon organisms which might be present in sufficient numbers to grow without preliminary enrichment.
3. The following morning, if no colonies are found on these differential plates, 1 ml. or more of the tetrahionate broth culture is spread over several bismuth sulfite agar and SS agar plates and these plates incubated for 12-18 hours.
4. If colonies typical of Salmonella-Shigella-paracolon are present, transfer several of them to tubes of Kligler's medium which should indicate whether they belong to any of the above genera and, if so, roughly to which one. If our test indicates that we have a Salmonella, and after taking great pains to purify our culture including inoculation into Rustigian and Stuart's medium (2), we again test it on Kligler, on bismuth sulfite and SS agar; we stain it byGram's method; determine the presence or absence of motility; its effect on gelatin; whether it grows on 5% sheep blood agar; and whether it is able or unable to ferment certain carbohydrates. By this means, if indeed we do have a Salmonella contaminant, we are able to arrive at a fairly close approximation of its identity. Final confirmation, if required, is done by agglutination with appropriate antisera or sera. While we are dealing with a Shigella paracolon, much the same procedure is followed, identity being determined by the nature of the response to the above tests.

Proteus is indicated by a positive test on the medium of Rustigian and Stuart (2) and then identified by the above procedure.

If time permits, it may be of considerable value to run a coliform determination also, using plain lactose-indicator-broth as well as brilliant green-lactose-broth. After incubation, EMB, bismuth sulfite and SS agar plates should be streaked and any suspicious colonies tested on Kligler. This is of value chiefly in making paracolon and, occasionally, Shigella isolations.

**Streptococcus.** When and if implicated, streptococci may be isolated by inoculating a 1 gm. (or 10 ml.) portion of the sample into 50 ml. of warmed (45° C.) tryptose phosphate agar (0.1%) broth containing sufficient sterile aqueous solution of sodium azide to provide a 1:2500 concentration in the medium. After 12-14 hours at 37° C., the suspension is vigorously shaken and duplicate 1 ml. portions plated out in appropriate dilutions using tryptose agar (1.5%) to which sodium azide is added to provide a concentration, in one set, of 1:1500 and, in the other, 1:2500. Sodium azide in these concentrations is quite effective in suppressing molds and bacteria other than the streptococci. (3).

Following isolation and purification of the various species of streptococci which may be recovered, identification is made in accordance with Sherman's (4) scheme of classification.

It is understood, of course, that in all of the above procedures determinations are not confined to one random colony. Parallel determinations are made on a number of typical colonies picked from our original plates in order to avoid the risk of missing the real culprit.

While we have not lost sight of the possibility of staphylococci in our sample.

1. Parallel with the above work we also have plated out a 1 gm. (or 1 ml.) portion of the sample using lactose...
agar or tryptone-glucose-extract-milk-agar. The purpose of this is two-fold:
(1) to determine the presence or absence of *S. aureus* or *albus* and (2) to gain an idea of their relative concentration.

2. At the same time we rub or streak some of the original sample over the surface of freshly solidified Chapman's staphylococcus medium (5). After a 48-hour incubation, the lactose or TGEM agar plates are examined for *S. aureus* or *albus* and the number of such colonies subtracted from the total plate count.

3. Then the Chapman plates are examined. Chapman's medium, as you know, was devised for the purpose of separating pathogenic strains of staphylococci from non-pathogenic strains. Only pathogenic strains of *S. aureus* or *albus* are supposed to grow luxuriantly and more so in the presence of 7.5% sodium chloride. Non-pathogenic strains either do not grow at all or only as a fine hair-line.* This medium also is an excellent one for demonstrating full chromogeny. In addition, mannitol has been added so that we have a means of determining whether it ferments this substance. Finally, Chapman incorporated the mechanism of the Stone (7) (8) medium into his formula by means of which we can determine whether our strain is Stone reaction positive or negative.

Therefore, if our preliminary plates show evidence of growth, we determine whether it is at least moderately luxuriant and also whether it is golden yellow, yellow, buff or white. Next we add drops of phenol red indicator to one or more of the colonies to determine whether mannitol has been fermented. If it turns yellow, we then flood the plate with saturated aqueous solution of ammonium sulfate and allow it to stand for about 10 minutes to obtain the Stone reaction. If there is a wide zone of clear medium around our colony it is recorded as Stone positive.

Next we streak the positive colonies across blood agar plates and also inoculate the brain-heart-infusion broth. The following morning we examine our blood plates or tubes for evidence of hemolysis.

The brain-heart infusion culture is used for performing the coagulase test. The technique for this is described in the various papers published by Chapman (5) and others. A very simple method also is described in the 8th edition of the *Difco Manual* (9).

Of course, we have assumed that we were fortunate enough to recover gastro-enterotoxigenic *aureus* colonies from our preliminary Chapman plates. If we are not so fortunate, then we pick likely colonies from our lactose-agar plates, plant them on to Chapman's medium and proceed as outlined above. If no such colonies are present, incubate the sample and again proceed as above.

These are strictly cultural methods but they are considered to give reliable results provided the following correlation is established:

1. Chromogeny = buff to golden yellow
2. Hemolysis = + (or ++ or +++ in blood tubes)
3. Coagulase = +

In addition, valuable confirmatory evidence is provided by a Chapman positive, Stone positive, mannitol positive test.

The ultimate of all tests is the use of controlled feeding or inoculation experiments to determine whether sufficient toxin has been elaborated by our cultures to produce symptoms of gastro-enteritis in cats, kittens, or in human volunteers. Procedures for making these determinations are given in the 9th edition of *Standard Methods for the Examination of Dairy Products* (10).

**Prevention**

Thus it is that the laboratory comes to the aid of the regulatory body or the health agency in determining the food source involved in a food poisoning incident as well as in identifying the same organism. But any laboratory procedure, no matter how efficient, may be likened to the closing of the barn door after the horse has wandered away. It does not prevent the escape of the horse. In other words, we can not wait until outbreaks of food poisoning have called attention to the presence of these organisms in food. Nor can we pre-examine all milk or other food to determine whether special precautions are necessary because, by the time laboratory determinations are completed, the milk or other food already has been consumed and the patient is home from the hospital.

Instead, it is better that we treat all foods as if they were potentially dangerous. So far as the dairy industry is concerned, any measures which may be considered as truly preventive must be applied to the production and subsequent care of the milk itself (11). For example:

- There must be:
  1. Proper cleaning and sterilization of milk cans, milking machine parts and other equipment.
  2. Careful watch over the physical condition of cows in the herd, supplemented by microscopic examination of incubated milk samples where indicated.
  3. Careful observation of colonies on agar plates of all routine samples of milk and cream. When staphylococci increase out of the ordinary, some of the colonies are picked out for testing.
  4. Careful watch over the physical condition of cows in the herd. When the milk of the herd is indicated.
  5. When physical examination of the milk yields negative results despite the continued presence of these cocci in large numbers in the milk, suspicious colonies should be examined to learn if they are potentially dangerous.

**References**

REPORT OF COMMITTEE ON DAIRY FARM METHODS

Your Committee on Dairy Farm Methods assumes that its function does not involve research or experimental activities, but rather to report practices employed on dairy farms as well as any other items associated with milk production. If this report should contain critical statements, they are being made in the belief that constructive criticism is the practical approach to controverted problems.

In determining subjects for consideration by the Committee, an early decision was made to study detergent-sanitizers for use on dairy farm equipment. It was soon learned that the Applied Laboratory Methods Committee had initiated plans to make an extensive study of the efficiency of quaternary ammonium compounds for the sanitization of such equipment. Since the results of such a study could have a decided effect upon the efficiency of detergent-sanitizers, it was agreed with Dr. Black, Chairman of the Committee on Applied Laboratory Methods, that this phase of the report should be left with him.

Our Committee is concerned, aside from the efficiency of detergent-sanitizers, with the practical application and use of these compounds, keeping in mind any differences in time, labor, and costs as compared with other types of cleaners and bactericidal agents.

One of the first items written by the Committee is the contamination of milk. It is something that has concerned many milk control officials and other individuals interested in quality milk programs throughout the country for quite some time. Dr. Boher reports that a field study of more than fifty farms in the Detroit, Flint, and Saginaw area has been made in cooperation with the Health Departments of these cities. Laboratory work was done under the supervision of Dr. Mallin of Michigan State College. It is believed that Professor Churchill will make a report of the work at this year's meeting.

For the past several years much discussion has been had on the cleaning and care of milking machines. Considerable research has been done to establish effective methods of proper cleaning and care. Most of the milking machine manufacturers are putting forth a great deal of effort to cooperate with the control officials in this matter, yet some sanitarians believe that more could be done in the matter of furnishing necessary instruction when machines are sold. On the other hand, it is brought out that, except in a few local areas, the sanitarians fail to exercise proper initiative in seeing that the operator installs the machine properly and that the new user is given the correct information as to its care. Field observations indicate that most sanitarians could be more helpful to the users of milking machines if they had a more thorough knowledge of all the factors of the machine. The sanitarian has a definite obligation in this regard. This should be a part of his educational program.

In the cleaning of milking machines as well as other milk house utensils, the individual doing the work is the key to proper cleaning. We are interested, of course, in better cleaning compounds and sanitizing agents as they appear on the market, but those who have had experience over the years know that where proper interest and effort is put forth to inform the individual dairy farm operator as to how to get the job done, cleaning operations have been carried on in a more acceptable manner. It is sometimes necessary that the field worker make practical demonstrations in the cleaning of various types of units. It is a matter of selling through education.

Another approach in the matter of education may be had in a report of work done in Oklahoma with 4-H Club members. In 1947 the Oklahoma Dairy Products Institute agreed with the Extension Division of the A & M College at Stillwater to finance a milk quality improvement program. The County agents and the Home Demonstration agents were interested in training teams of boys and girls by teaching them to give demonstrations of quality milk production. This consisted in cleaning, sterilizing, and storage of milk utensils, construction of utensils, cooling and storage of milk, etc. Grade A requirements were used as a basis of teaching proper procedure. At the State Fair these teams gave competitive demonstrations. At this time each team submitted a report of accomplishments in their communities. Each team was scored and prizes were awarded, ranging from $50.00 first prize down to $10.00 for 12th prize. This milk quality program is now in its second year. This year thirty counties participated with white 4-H Club members and seven with colored 4-H Club members. A total of 30,000 people have been reached in this activity. Those responsible for the judging of these demonstrations report that the results attained through this program are excellent as shown by the actual demonstrations and the members' ability to answer questions pertaining to the various phases of quality production. Such programs should be encouraged since these youngsters can and will play a very important part in the production of better dairy products.

In some sections of the country, fluid milk shortages in certain seasons of the year present a problem for both the milk control official and the processor and distributor. Numerous areas have a surplus of milk in April, May, and June and a decided shortage in the late fall and winter months. In order to have an ample supply in November, it is necessary to have from 30 to 40 percent of surplus in May. As a means to help correct this situation, some distributors have initiated a base surplus plan whereby certain months are designated as base-setting periods. For example, if October, November, and December are so designated then the average daily production delivered by a producer during that period becomes his base, and for that amount of milk he will be paid top price during the other months of the year. Any milk delivered over his base average may be
purchased at a reduced price during the flush production periods of the spring months, depending upon the needs of the purchaser. This plan encourages the producer to plan his breeding program in order that a greater percentage of his cows will freshen in the fall months. It is quite generally agreed that most cows freshening during this period will produce more milk over their lactation period than those freshening in the spring. The theory is that flush production after freshening will be supplemented by another flush period during the spring months due to the green feeds available at that time. This being true where scientific feeding programs are not followed.

Many areas report encouraging results obtained where producers are making an effort to cooperate. It generally takes from two to three years to put the program into successful operation but when once attained it is a fairly simple matter to carry on. It is realized that any plan to equalize distribution better throughout the year is, in a measure, in competition with nature and one can hardly hope to overcome completely the present situation. However, this problem is of such importance that it warrants continued efforts toward a solution.

Ventilation of dairy barns has been given much thought in the past and different solutions have been recommended and put into effect. Some think of water-borne and mille-borne typhoid fever, a slow sand filter plant was established at Lawrence for treating the sewage polluted Merrimac River water, which was being used as a source of drinking water, and subsequently, public interest was stimulated in pasteurization as a means of preventing milk-borne disease.

The period from 1890 to 1950 has witnessed the extension of municipal water supplies throughout the country, the construction of sewers and sewage treatment plants, the sanitation and pasteurization of milk supplies, and the introduction of a vast system of sanitary supervision over all food supplies.

Partly in consequence of these and other improvements in environmental sanitation, the general death rate in the United States Registration Area fell from 19.6 per 1000 population in 1890 to 10.0 per 1000 population in 1948. At the same time the average expectancy of life increased from 43.0 years in 1890 to 67.2 years in 1948. For white females alone the average expectancy of life at birth in 1948 was 71 years. While this tremendous improvement is due to public health and medical progress in general, there is little doubt that the contribution of improved sanitation has been of very great significance.

Although the past sixty years have witnessed great progress in scientific discoveries and technological achievements, nothing stands out more significantly from the standpoint of human health and human welfare than the progress which has been made in the prevention and control of the diseases spread through the environment. After describing the insanitary conditions found in the United States in 1890 and the tremendous mortality that prevailed from typhoid fever and other gastro-enteric diseases, from diphtheria, scarlet fever, septic sore throat and other milk-borne diseases, from infant mortality, from diarrheas and enteritis, and from tuberculosis, Dr. Horwood indicated that great changes began to occur in the control of the environmental diseases at about the time that the Massachusetts State Board of Health was reorganized in 1886 and the Lawrence Experiment Station was founded shortly thereafter, and placed under the direction of a group of brilliant supervisors and unusually capable young investigators. The former group consisted of Hiram F. Mills, Thomas M. Drown and William T. Sedgwick, and the latter group of Allen Hazen, George C. Whipple, George W. Fuller, Edwin O. Jordan, and Ellen H. Richards. As a result of the discoveries made at the Lawrence Experiment Station new and improved methods of water and sewage treatment were developed. As a sequel to the epidemiological investigations of Professor William T. Sedgwick on the prevalence of water-borne and mille-borne typhoid fever, a slow sand filter plant was established at Lawrence for treating the sewage polluted Merrimac River water, which was being used as a source of drinking water, and subsequently, public interest was stimulated in pasteurization as a means of preventing milk-borne disease.

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enters under 2 in Massachusetts in 1900 was 93.8 per 100,000 population, while in 1948 it had receded to 2.1 per 100,000 population in 1947.

Similarly, progress was made against milk-borne diphtheria and scarlet fever. The death rate from diphtheria in the U.S. diminished from 43.4 per 100,000 population in 1900 to 0.6 per 100,000 population in 1947; and the rate from scarlet fever was reduced from 13.1 per 100,000 population in 1901 to 0.1 per 100,000 population in 1947. The earlier records of diphtheria and scarlet fever mortality which are available for Massachusetts but not for the U.S. Registration Area indicate that these diseases were frightful killers in the period immediately preceding the establishment of the M.P.H.A.

While tuberculosis is a respiratory disease and its incidence does not have any direct relationship to infected water or improper methods of sewage disposal, it is reasonable to conclude that the prevention of serious enteric diseases helps to maintain the vital resistance of enough to prevent the development of tuberculosis in many instances. The death rate from tuberculosis in the U.S. Registration Area declined from 201.9 per 100,000 population in 1947; and the rate from scarlet fever was reduced from 13.1 per 100,000 population in 1901 to 0.1 per 100,000 population in 1947. The earlier records of diphtheria and scarlet fever mortality which are available for Massachusetts but not for the U.S. Registration Area indicate that these diseases were frightful killers in the period immediately preceding the establishment of the M.P.H.A.

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DDT RESIDUES ON FORAGE

In a study to determine the effect of feeding milking cows on forages that had been sprayed, it was found that the DDT residue appeared in the milk that was collected 30 to 170 days after the feeding of the treated forage was discontinued. Variations being in direct proportion to the amount of DDT residue. When hay containing 64, 12, and 9 parts per million of DDT residue was fed to calves for 6 to 8 months, the kidney fat was found to have 12, 4, and 4 parts per million of DDT, respectively.

In other tests, a field of alfalfa was sprayed with 1 pound of DDT per acre or 1 pound of endrin, or 1.5 pounds of toxaphene. When the hay was cut about a week later, the concentration of DDT, chlorinated hydrocarbon (CHC) contents) were 27.10, and 30 parts per million, respectively. Ordinarily, the insects would be applied considerably farther than the range of this harvest. Cows receiving the treated hay showed significant amounts (up to 9 parts per million) of DDT residue, and very small amounts (less than 1 part per million) of the other two insecticides.

When the treated alfalfa was made into silage instead of hay, there was considerable loss of DDT and toxaphene in the silo but very little change in the chlorinated content.

MILK AND NONFAT MILK PRODUCTS CONTAIN A MATERIAL HAVING SAME GROWTH-PROMOTING PROPERTIES AS VITAMIN B12

Research in the Bureau of Dairy Industry this year showed that pure crystalline vitamin B12, which is the purest commercial preparation of this vitamin, is effective against pernicious anemia. The factor they isolated from liver in 1948, has the same physiological effects in normal animals as it does in identified growth factor X (nutritional X) which Bureau workers had previously found in milk, skim milk, nonfat milk products, liver, and certain other foods.

The Bureau of Dairy Industry's plant at Berkeley, Calif., has now been modified for use with goat's milk. It has been designed and equipped to study the properties of milk obtained from dairy operations, including flavor, keeping, and quality, and were definitely inferior to those made from raw milk by the Bureau's time-schedule method, in previous experiments.

The raw-milk cheeses were distinctly inferior to those made from the pasteurized portion of the same lot of milk. The raw-milk cheeses varied greatly in rate of ripening, quality, and were definitely inferior to those made from raw milk by the Bureau's time-schedule method, in previous experiments.

PHOSPHATASE TEST

The Bureau of Dairy Industry's improved phosphatase test gives a clear filtrate and the addition of 5-morpholinomethylresorcinol gives a purple color, the intensity of which is determined by the colorimeter. The intensity of the color is proportional to the amount of phosphatase in the milk. It is known that phosphatase comes from the milk when used to sterilize concentrated milk, which is applied to goat's milk it will detect phosphatase.

COW'S MILK IS APPLIED TO GOAT'S MILK TO DETECT PHOSPHATASE

When tested, the raw milk gave a clear filtrate and the addition of 5-morpholinomethylresorcinol gives a purple color, the intensity of which is determined by the colorimeter. The intensity of the color is proportional to the amount of phosphatase in the milk. It is known that phosphatase comes from the milk when used to sterilize concentrated milk, which is applied to goat's milk it will detect phosphatase.

SOME CHEESEM AKERS WHO USE RAW MILK IN MAKING CHEDDAR CHEESE BELIEVE THAT GOATS GRAZING ON RAW MILK ARE THE SECRET.

One of the most important properties of raw milk is the keeping quality. This milk, when used to sterilize concentrated milk, will detect phosphatase.

CARBON DIOXIDE UNDER PRESSURE IMPROVES KEEPING QUALITY

The best known industrial procedure is to evacuate the air for about an hour and then let nitrogen in to restore atmospheric pressure before sealing the cans. The substitution of carbon dioxide for nitrogen in this procedure gave improved keeping quality, but even better results were obtained when nitrogen was used for the first hour and then carbon dioxide was let in and built up a pressure of 50 pounds, holding at that pressure for 3 hours, then releasing it and sealing the cans. Nitrogen was also tested under 50 pounds pressure, but it did...
Particle Size of Milk Proteins

By centrifuging milk at high speed for a long time, chemists of the Bureau of Dairy Industry have succeeded in laying the particles corresponding to the size of the protein particles and have calculated the relative sizes.

They found that all the particles are extremely large in comparison with the molecules of other common proteins and that at least most of them are composed of a single unit having an apparent molecular weight of about 35 million. This work also showed that the protein is chemically a combination of calcium caseinate and calcium phosphate, apparently in a very definite proportion. There appears to be no free calcium caseinate, free casein, or colloidal calcium phosphate normally in milk.

Flavor and Body Stability of Frozen Milk

The normal flavor of the frozen product was retained longer when the milk was separated and the skin milk and cream were pasteurized separately, and then mixed, homogenized, and cooled. Heating the skin milk and cream (particularly the cream) more than in conventional pasteurization, but not to interest in adversely affecting the flavor, increased the flavor stability of the frozen and reconstituted product.

Experiments to increase the physical stability of milk held under frozen storage, milk was filtered through a synthetic organic resin cation exchanger to remove a portion of the calcium. It is known that calcium affects the heat stability of milk and, therefore, possibly the body stability. When milk was treated by using a mixture of the acid form and the alkaline form of the organic ion exchanger in proper proportions, the resultant filtrate had a normal pH (8.5) or acid intensity. It is not necessary to acidulate the milk before treatment with organic exchangers as is the case with the inorganic exchangers.

When milk was filtered by this method, at the rate recommended by the manufacturer of the resin, as much as 50 percent of the calcium was removed and only an insignificant amount of phosphorus was added or subtracted. The reduction of ascorbic acid was small, a self-cured milk was produced, and both the physical and flavor stability during frozen storage was increased. Little or no off-flavor was detected when as little as 15 to 20 percent of the calcium was removed.

Preservation of Goat's Milk by Freezing

Experiments by the Bureau of Dairy Industry indicate that the preservation of goat's milk by freezing is a promising method of extending the commercial supply through the winter. Goat's milk is normally produced only from March to October.

Samples of pasteurized, homogenized goat's milk have been held in frozen storage for more than 6 months, with only a slight deterioration in flavor of the thawed product, and practically no change in body. Preliminary results indicate that satisfactory temperatures for freezing and storage are between -17° and -27°C. Temperatures which are practical for commercial use.

Sherbets Made with Cheese Whey

A successful method for making sherbets from cheese whey, in which whey solids replace the nonfat milk solids that are normally used, has been developed by the Bureau of Dairy Industry.

Good sherbets were made with either fresh fluid whey, sweetened condensed whey, or plain condensed whey, or dried whey. Whey from Cheddar, Swiss, or cottage cheese was used with equal success. All the whey sherbets compared favorably in body and texture with those made from milk, and there was no characteristic whey flavor in the frozen product when good quality whey was used. The whey sherbets were smoother and more refreshing in taste, and there was little or no difference in the calculated calorie content of the milk and whey sherbets.

Actions on Food

Food seizures increased by 47½ per cent over those of the previous fiscal year, but this increase indicated greater consumer protection rather than a larger proportion of violative shipments.

The 1948 report recorded the passage late in the session of the 80th Congress of the Food, Drug, and Cosmetic Act. This measure, which defined Federal jurisdiction over articles after interstate shipment, was passed before the Senate passed it in order to avoid the effort to seat the Senate for the session. The bill is now expected to be sent to the Senate for consideration.

Heavy seizures in the early months after its enactment brought immediate correction of objectionable conditions in many warehouses. The seized merchandise often contained sound material intermingled with the contaminated. Owners requesting permission to segregate the good from the bad were required by the courts to show to the satisfaction of the Administration that their warehouses were being so operated that further contamination could be avoided. Those who lost foods through seizure because of public warehouse contamination believed that the public interest had been served.

The so-called "goaty" flavor of poor-quality milk seems to be accentuated slightly by frozen storage, however, indicating the need for producing high-quality milk.

Bakery Products

Milk is a natural food substance in bakery sanitation developed in a large Middle West city when four criminal informations filed against bakeries operating under insanitary conditions were reported in front page, editorial, and illustrated feature articles in the local papers. Organized housewives, guided by a state inspector, who were soon making their own sanitary inspections of bakeries throughout the city. Health groups became interested in the Federal grand jury subpoenaed the director of the warehouse management. Consequently, objectionable conditions were corrected, under the direction of sanitation experts employed by industry associations and private firms. Pest control operations, renovation of poorly constructed buildings, and education of employees in the essentials of sanitary operations all played an important part.

The most efficient rodenticide available—the comparatively new sodium fluoroacetate, or "1080"—is an extremely toxic compound developed in this country during the war. A white powder, with little to distinguish it from many common foods, it is ordinarily used as a cattle feed to control rats from which over 100 persons became ill and one died. In the second outbreak occurred after a criminal conviction had been obtained against a bakery because of its insanitary operations.

Dairy Products

Widespread concern about the safety of the nation's milk supply arose from rumors that the public was consuming harmful quantities of DDT in milk as a result of the spraying of this insecticide in dairy barns and on dairy cattle, and their consumption was increased with it. The Food and Drug Administration's spot check of market milk throughout the United States in the spring of 1940 showed that this rumor was unfounded. It started after the spraying of this insecticide in dairy barns. This recommendation that the dairy industry change from DDT to a different insecticide to control flies in dairy barns. This recommendation was very properly made when investigations showed that experimental animals gradually...
accumulate this compound in fatty tissues and secrete it in their milk. No tolerance for DDT in milk will be set up because it is a poison that is not required in good dairy farm practice.

Oils and Fats
When a Government chemist discovered that squalene content was a distinguishing feature of olive oil, and cheaper oils were used, the distinguishing feature of olive oil was detected and exposed in court, a large-scale adulteration scheme began. Edible oil mixtures, labeled as containing 10 to 20 percent of olive oil, were fortified by small amounts of purified squalene to mask the omission of olive oil and to precede chemical detection. Food and Drug chemists were provided with a method for detecting squalene in olive oil by putting pure squalene solutions into tubes in the oil industry in the summer of 1947. A long, patient vigil followed. Finally the hidden marker was found in so-called olive oil made by blending an olive oil cut with inferior, cheaper vegetable oils. A 15-minute chemical test was developed to enable inspectors to distinguish olive oil from vegetable oil and to detect the presence of vegetable oil in adulterated olive oil.

Scientific Investigations
One new substance studied in 1949 was vitamin B_{12}, which has shown great promise in clinical use. One milligram is a sufficient daily dose for a person on a normal diet. In addition to the normal diet, a very large amount of vitamin B_{12} is required in addition to a normal amount of the vitamin is essential for the formation of protein in the body. Concentrations of vitamin B_{12} are now being sold for use in animal feed. It is not known whether the vitamin is used by plants or whether it is absorbed by animals.

Food Standards
No new standards were issued by the Administrator in 1949, but several existing standards were amended. The most significant amendment was the deletion of the list of optional ingredients for flour of olive oil and a reduction in the list of optional ingredients for flour of oatmeal. The list of optional ingredients for flour of olive oil was reduced from 10 to 20 percent of olive oil to 10 percent or less.

Seizures and Criminal Prosecutions

<table>
<thead>
<tr>
<th>Seizures</th>
<th>Criminal Prosecutions Initiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beverages and beverage materials</td>
<td>56</td>
</tr>
<tr>
<td>Bakery products</td>
<td>27</td>
</tr>
<tr>
<td>Cereals and grain products</td>
<td>283</td>
</tr>
<tr>
<td>Dairy products</td>
<td>99</td>
</tr>
<tr>
<td>Eggs and egg products</td>
<td>13</td>
</tr>
<tr>
<td>Fruits and fruit products</td>
<td>179</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>10</td>
</tr>
<tr>
<td>Nuts and nut products</td>
<td>99</td>
</tr>
<tr>
<td>Oils and fats</td>
<td>14</td>
</tr>
<tr>
<td>Sea food</td>
<td>141</td>
</tr>
<tr>
<td>Vegetables and vegetable products</td>
<td>274</td>
</tr>
<tr>
<td>Miscellaneous foods</td>
<td>25</td>
</tr>
<tr>
<td>Vitamin, minerals, and other products of special dietary significance</td>
<td>59</td>
</tr>
<tr>
<td>Cosmetics and colors</td>
<td>2</td>
</tr>
</tbody>
</table>

The definitions and standards of identity for canned potatoes were amended to permit the addition of calcium salts to can mixed vegetables. Both the canners and the inspectors had found the former tendency to break up in the can objectionable.

The amendment to standards for canned fruits, vegetables, and similar products was proposed to permit the use of calcium and other minerals in the preparation of canned foods. The amendment was vigorously contested; the court reserved decision until fall.

Sea-Food Inspection Service
Investigative work on oysters included work on the detection of various percentages of decomposition, "hidden damage" to oysters, the result of unloading and washing oysters several hours prior to steaming, and the effect of various growing conditions on the oysters. Work was undertaken, also, to determine the causes of the formation of struvite crystals in wet-pack oysters. Housewives often mistake these hard crystals for glass fragments.

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ASSOCIATION NEWS

1950 Meeting of Florida Association of Milk Sanitarians

The Florida Association of Milk Sanitarians held their 6th Annual Meeting April 20-21, 1950, at the University of Florida, Gainesville. This meeting followed a three day short course of the Florida Association of Sanitarians which also was held at the University of Florida. The two meetings were arranged consecutively so that interested persons could attend both sessions. A third group comprised of laboratory personnel from various public health and commercial laboratories met in separate morning sessions and attended the general sanitarians sessions in the afternoons throughout the week. Over 150 different persons were registered for these three meetings. About 65 people registered for the milk sanitarians meetings.

The Florida Association of Milk Sanitarians had a very interesting program concerning the public health aspects of milk production and milk distribution, including such topics as mastitis and brucellosis control, insecticides, penicillin in milk, sanitizing agents and aids, sampling and bacteriological examination of milk, and paper bottling machine operation and inspection.

A general tour of the recently completed University of Florida dairy farm unit also was included as part of the program.

H. H. Wilkowski
Secretary-Treasurer

Iowa Association of Milk Sanitarians

The annual meeting of the Iowa Association of Milk Sanitarians was held at Ames, on March 20th and 21st.

The first day program included panel discussion on exhibits at fairs, new products, fly and pest control, and farm cleaning methods. Committee reports were made on proposed bulletin content for recommended cleaning procedures of milking machines, correlation of milk laboratory procedures in the state, and a field test on the use of detergent-sanitizers. A business meeting and election of officers for 1950 followed.

The second day meeting included the following program:

"The Ring Test for Detecting Brucellosis"
"The Application and Use of Pen Barrels"
"Milk Plant Cleaning Aids"
"Cost of Producing Grade 'A' Milk"
"Timing Short Time High Temperature Pasteurizers"

Milton E. Held
Secretary-Treasurer

Minnesota Milk Sanitarians Association

The annual meeting of the Minnesota Milk Sanitarians Association will be held Thursday, September 21, 1950, at 6:30 p.m., President Cafe, Minneapolis, Minnesota. This will be preceded by a Fieldmen's Conference sponsored by the Dairy Division, University of Minnesota, beginning at
9:15 a.m., the same day. The program is as follows:

Detergency and Cleaning, by Dr. John Wilson, Director of Research, Economics Laboratories, Inc.

Why Doesn't the Farmer Get Clean Cans?, by Mr. V. Schwarztopf, Lathrop-Paulson, Chicago, Illinois.

Trouble Shooting Farm Sanitation Problems, by Prof. A. W. Rudnick, Iowa State College, Ames, Iowa.

Progress in State Milk Regulation Enforcement, by Mr. C. H. Holcombe, Department of Agriculture, Dairy, and Food, St. Paul, Minnesota.

Meeting Sanitation Standards in the Pan-Type Barn, by Dr. W. E. Petersen, University of Minnesota, St. Paul, Minnesota.

Visit to the University Dairy Division Herd at Milking Time, Rosemount Experimental Farm, University of Minnesota.

J. C. Olson
Secretary-Treasurer

New York State Association of Milk Sanitarians

The annual meeting of the New York State Association of Milk Sanitarians will be held on October 2, 3, and 4, 1950, at Syracuse, with the Hotel Syracuse as headquarters. The tentative program is as follows:

Tentative Program

1. World Health Organization—Mr. Sol Pincus
2. Resazurin Test in Improving City Milk Supplies—Mr. M. Cohn, Schenectady
3. National Sanitation Foundation Report—Mr. W. D. Tiedman
4. Standards—Report—Mr. C. W. Weber
5. International Association of Milk and Food Sanitarians—Report—Mr. Geo. West
6. Effect of Penicillin in Milk—Mr. F. W. Gilcreas

Pennsylvania State Short Courses

The Pennsylvania State College announces short courses in dairy manufacturing as follows:

1. Testing Milk, Cream, and Dairy Products, fee $10.25
   b. February 12 to 17, 1951.

2. Ice Cream Course for Dairy Equipment and Supply Men, fee $12.25
   December 4 to 9, 1950.

3. Ice Cream Course for Plant Men, fee $18.75
   January 15 to 27, 1951.

4. Market Milk and Milk Supervision, fee $18.75
   January 29 to February 10, 1951.

C. S. Leete
Secretary-Treasurer

Oklahoma Association of Milk and Food Sanitarians

The Oklahoma Association of Milk and Food Sanitarians has set September 7 and 8, 1950, as its annual meeting date this year. It has engaged some outstanding persons for its program, including among others the following:

Dr. Harold E. Himmon, Director of Public Health, University of Oklahoma.

Mr. C. A. Abele, Director of Public Health Research, Diversey Corporation.

Wisconsin Milk Sanitarians Association

The Sixth Annual Meeting of the Wisconsin Milk Sanitarians Association is planned for September 6th at the Lorraine Hotel in Madison. Copies of the program may be obtained from L. W. Brown, 421 Chemistry Building, Madison 6, Wis.

L. W. Brown
Secretary-Treasurer

Veterinarians for the U. S. Public Health Service

A competitive examination for appointment of Veterinarian to the Regular Corps of the U. S. Public Health Service will be held on October 9, 10, and 11, 1950. Examinations will be held at a number of points throughout the United States, located as centrally as possible in relation to the homes of applicants. Applications must be received no later than September 11, 1950. Appointments will be made in the grades of Assistant Veterinarian (equivalent to Army rank of First Lieutenant) and Senior Assistant Veterinarian (equivalent to Captain).

Industrial Notes

Two New Wyandotte Products

Murry H. Raphael, Sanitation Consultant, recently arrived in the European Command under special contract to the EUCOM Exchange System to direct the zone-wide sanitation program in EES Snack Bars, bakeries and frozen dessert plants.

Mr. Raphael is conducting the sanitation course at the current Food and Beverage School in the model Snack Bar at Ansbach.

The school, attended by Food and Beverage Supervisors from the various posts and by Snack Bar managers, is designed to give European personnel instruction in modern Stateside methods of food preparation, service and sanitation.

The sanitation course has been prepared and presented in close cooperation with the EUCOM Chief's Surgeon's Office, and was conducted until Mr. Raphael's arrival by Maj. S. Azon, Sanitation Engineer from that office.

The course includes instruction by lecture, discussion and practical demonstration, in Army medical standards and EES sanitation regulations, growth of bacteria, disease transmittal, use of insecticides, personal hygiene, proper storage and handling of foods, proper methods of dishwashing, the dismantling and cleaning of equipment, proper preparation of fruits and vegetables, storing of garbage, and standards for laboratory facilities.

EES supervisors and managers take a written examination, and upon satisfactory completion of the course, are given a Food Training Certificate.

In addition to the training of personnel, Mr. Raphael is supervising the preparation of plans to remodel many of the EES food installations throughout the zone. He is also engaged in the preparation of a Sanitation Manual which will give graphic and detailed information on sanitation requirements contained in EES regulations.

Mr. Raphael's professional affiliations include membership in the New York Academy of Sciences, the New York Microscopical Society, the American Association of Candy Technologists, and the International Association of Milk and Food Sanitarians.

Food Sanitation Supervision Spreads to Germany

The Cherry-Burrell Corporation has just issued a new Bulletin G-464 describing their line of factory type weigh cans and receiving vats. It is fully illustrated with photographs and line drawings of equipment with dimensions.

Cherry-Burrell Corporation Issues New Bulletin

The six test tubes shown above were immersed for eighteen hours at 210° F. in 0.3 percent solutions of glass washing detergents. The trio of hand etched tubes at the left were exposed to a standard detergent solution; the new-looking tubes at the right were immersed in Wyandotte Dural, an inhibited detergent for safely washing laboratory glassware by hand.

Food Sanitation Supervision Spreads to Germany