Highly trained engineers and scientists at Chamberlain Laboratories work hand-in-hand with experienced, practical dairy farmers at the Transflow Test Farm to develop products and methods for improving sanitation and for saving time and work on America's dairy farms.

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What types of work do the labs and test farm carry out? Here's a typical example: Recently a series of tests was conducted in conjunction with leading manufacturers of cleaners and sanitizers to determine the most effective combination of time, temperature and solution concentration for use with TRANSFLOW. Tests were conducted under closely controlled conditions. For example, at the start of each period, new inflations, gaskets, air hose and TRANSFLOW milk tubing were put into service.

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Important data of many kinds are flowing from the mutual work of the test farm and the laboratory — data that will make an important contribution toward "better milk with less work."

Bulletins RM-60 gives complete information on TRANSFLOW Raw Milk Tubing. For your free copy, write Chamberlain Engineering Corp., Akron 9, Ohio.
Notice — December 24, 1963

To: Membership Only

The membership at the 50th Annual Meeting of the IAMFES voted to submit the proposed amendment to the Constitution to the membership for a mail vote. Following is the Constitutional Amendment on which members only of IAMFES are to vote.

All votes must be mailed within 60 days of the above date.

AMENDMENT

The proposed amendment to the Constitution, Article IV, Section 1 reads as follows:

Section 1. The officers of this Association shall be a President, a President-Elect, a First Vice President, a Second Vice President, and a Secretary-Treasurer who shall hold these offices for one year or until their successors are elected or appointed, as provided in the By-Laws. At the termination of each Annual Meeting the President-Elect, First Vice President, and Second Vice President shall automatically succeed into the offices of President, President-Elect, and First Vice President, respectively. A Second Vice President and Secretary-Treasurer shall be elected by majority ballot of votes cast.

The above change in the Constitution is necessary to make it consistent with the change in the By-laws. The amendment to the By-laws was adopted. Your vote is on the Constitution only.

The amendment to the By-Laws, Article IV, Section 2 reads as follows:

Section 2. Each year the President-Elect shall appoint a Nominating Committee at the Annual Meeting prior to next year's election, in ample time for them to meet at that meeting. This Committee shall consist of seven members other than officers of the Association. At least one member shall have been a member of the Nominating Committee of the previous year. The name of the Chairman of the Nominating Committee shall be published in the next issue of the Journal together with a date for submitting candidates for nominations for each office. The Nominating Committee shall submit the names of at least two nominees for the office of Second Vice President and at least one for Secretary-Treasurer to the Executive Secretary, as directed by the President-Elect. These names, with pictures and biographical sketches of the nominees, shall be published in the Journal not later than April 1 following the meeting. The next issue of the Journal shall contain a ballot listing the nominees. All ballots must be in the hands of the Executive Secretary by July 1 for checking against the eligible voter list and then forwarded to the Tellers Committee for counting. The person receiving the greatest number of votes for each office shall be certified to the President at least one month in advance of the Annual Meeting.

KARL K. JONES, Secretary-Treasurer
International Association of Milk, Food and Environmental Sanitarians, Inc.

YES □ NO □

Name ____________________________________________

Address ____________________________________________
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Report of the Coordinating Committee on Laboratory Methods

VOL. 53, NO. 8, A.J.P.H. 1305-1310, AUGUST, 1963

"It is recommended that media be used which has been tested by the APHA methods and bearing a label indicating that it has met the prescribed standards of the APHA."

This report of the Coordinating Committee on Laboratory Methods of the Committee on Evaluation and Standards (APHA) was approved by the Executive Board of the American Public Health Association on June 27, 1963.

[Image of a bottle of BACTO PLATE COUNT AGAR Dehydrated Standard Methods Agar]
BACTERIOLOGICAL SURVEY OF FILLETING PROCESSES IN THE PACIFIC NORTHWEST

II. SWAB TECHNIQUE FOR BACTERIOLOGICAL SAMPLING

WAYNE I. TRETISVEN

Bureau of Commercial Fisheries, Technological Laboratory
U. S. Department of The Interior, Seattle, Washington

Summary

A swab technique was developed for sampling fish and fish processing equipment. Selection of the swab used was based on the uniformity of weight, amounts of water absorbed, and water-holding capacity of the material of the swab. Size of sampling area was based on the limitations of the swabs used to pick up the material from the surface. The technique is simple, convenient, relatively accurate in use, and inexpensive for nondestructive sampling of wet surfaces in the field or in the laboratory. A nondestructive sampling technique was needed to provide comparable data on the bacterial loads of different products commonly encountered in the fishing industry. Various methods of obtaining bacteriological samples from fish were compared in this laboratory in a previous study (31) and the swab technique appeared to be the logical solution to the problem because it is relatively efficient in recovering a large and fairly uniform portion of the bacteria, is relatively simple, fast, and nondestructive. Before laboratory tests were started on the development of a swab technique, however, the literature from 1936 to 1963 was searched. Although no standard procedure was found, much helpful information was discovered to use as a guide for standardization. Based on this information, a swab technique was developed and then tested under a variety of conditions.

Literature Review

Until recently there was little information (1, 6, 18, 19, 33, 34) concerning the limitations of swabs, and practically all involved the sampling of relatively dry surfaces (with moistened swabs). Aside from American Public Health Association publications (4, 5) and the Milk Industry Foundation Manual (3) for sampling eating utensils and dairy equipment, there is little evidence of standardization of techniques or of equipment. Ayers, for example, used a variety of swab techniques in sampling meat (7, 8, 9, 10). Swabs were used to wipe surfaces to collect microorganisms for qualitative use, and later they were adapted for quantitative work (20).

Swabs

Swabs used consisted of such materials as pieces of cloth, gauze, paper, wool, glass-wool, absorbent (32) and non-absorbent cotton (11), and calcium alginate (2). They have varied considerably in size (32) and have been held by means of forceps, wires, and sticks (11).

Surfaces

The nature of the surface i.e., wet or dry, rough or smooth (1), load of organisms, and ease of their removal (11, 34) are important (31). The load of bacteria on the surface has been the primary factor considered in determining the size of the swab area (11, 15, 25, 32). Tiedeman (29) used a 4-sq inch area because a smaller area was difficult to swab accurately. Swab areas selected by other workers varied from 1 cm² to 1 ft² (26). Usually, unit areas were sampled, and the numbers of microorganisms were expressed as per cm² or per sq inch (18), or as per dish or glass.

Templates

Need for standardization leads to the use of templates for accurately delimiting the swab area, according to Vaughn and Stadtman (32). Use of a template for accurately limiting the swab area was considered to be more necessary when the bacterial load on the surface was high. Green and associates (20) developed a "swab-pression" technique for sampling surfaces of low bacteria loads without the need of a template.

Templates have been made of glass, cellophane, glassine, wrapping paper, filter paper, and various metals (8, 20, 22, 32). The swab area in a template is usually circular, presumably because it is easier to bore a round hole accurately than to make one of another shape, and it is easier to swab a circular area than to swab an area with corners. Rigid and thick templates were found to be impractical because they did not conform to irregular surfaces (22). Using templates that were individually wrapped and sterilized before use was less laborious than was flaming the template between use (11, 32).

Swabbing

Much work has been done on methods of obtaining samples (1, 10, 19, 29, 32, 33). Procedures varied from rolling a swab over the surface in four directions...
(7) to scrubbing the surface for 30 seconds (32). Buchbinder et al. (11) found that increasing the number of strokes of the swab increased the counts. In working with high-count surfaces, he found that reversing the direction of strokes decreased counts in all tests, whereas in working with low-count surfaces, he found that reversing the direction of the strokes between strokes increased the count from 5-15%. France et al. (17) compared the use of wet, moistened, and dry swabs in sampling glassware. Angle and pressure of the swab (1) variations in adhesiveness of organisms and mono-molecular grease films on the surface (34), and use of wetting agents (11) have been considered for their effect on the efficiency of swabbing. After a surface was swabbed, use of sterile forceps to remove the swab material from metal holders did not improve the accuracy of the counts and was an inconvenience. Use of wooden sticks, which are broken off after swabbing, was found to be adequate (11). Buck and Kaplan (12) developed a sterile cutting device to sever the stick. The efficiency of the pick-up of organisms by swabs and recovery of organisms from the swabs made of cotton were compared to those made of calcium alginate, which is soluble in a diluent, thereby releasing the entrapped organisms (2, 13, 22). The increased recoveries of the alginate swabs over that of the cotton swabs were reported as significant.

Dilution

The material picked up by the swab has been transferred directly to nutrient media or to solidified media by swabbing (14, 17, 23, 24) or indirectly to an intermediate diluent (19). Inconsistencies of dilutions have introduced variables; some workers reported using 99 ml solution (32), and others, 100 ml (17), yet each considered the dilution to be 10^{-2}. Although some of the factors mentioned are of little significance, the codes allow for great variations.

**Experimental Procedures and Results**

Based on the foregoing study of the literature, a tentative procedure was outlined and tested. The tests covered (a) water absorbency of various swabs, (b) water-holding capacity, (c) optimum sample area, (d) sampling impervious surfaces, (e) sampling absorbent surfaces, and (f) sampling surfaces of fish. In preliminary studies in which machine-made, cotton-tipped applicator sticks were used for swabbing 5-cm² areas, the swabs often reached the limit of their absorbency before the area had been adequately swabbed. Also, the bacteria counts obtained were inconsistent. Before an attempt was made to limit the swab area, the uniformity and the limitations of commercial cotton-tipped applicator sticks were studied. Different sources of applicator sticks were obtained. They appeared similar in shape but there was considerable variation in the amount of absorbent material comprising the various swabs (see Table 1).

Swabs from one source (B) were selected for the subsequent study primarily because of their uniformity. They consisted of a wooden stick approximately 6 inches long with an average of 0.035 ± 0.003 g of absorbent cotton wound tightly around one end, forming a cotton tip about 1/4-in. diameter and 3/4-in. long. The sticks were cut to approximately 4-in. in length and were inserted, cotton tip first, into glass test tubes fitted with screw caps. The swabs' water-absorbency and water-holding capacities were determined by weighing each swab before and after water was absorbed, and also after immersing in water. In order that conditions anticipated in the use of the swabs could be simulated, the tubes containing the swabs were autoclaved at 121° C. for 15 min the day before use and were cooled before the caps were tightened.

**Water-absorbency**

After each swab was weighed, the amount of water it would absorb was determined. The swab was held at an angle of approximately 45° for 30 sec with the cotton tip resting on a level piece of glass plate that was covered with a thin layer of water approximately 0.1 cm thick (5 ml of water was applied to an area of 50 cm²). The swab was placed immediately on a weighing support and weighed. The mean difference in weight (the amount of water absorbed) was 0.213 ± 0.053 g, (see Table 2).

**Water-holding capacity**

Immediately after the swab with absorbed water was weighed, its water-holding capacity was determined. Its cotton tip was immersed into a beaker of water for approximately 2 sec and then the swab was gently removed. The swabs were handled by

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<th>Source</th>
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<td>0.039</td>
<td>0.054</td>
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<td>0.034</td>
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<td>0.032</td>
<td>0.049</td>
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<td>0.029</td>
<td>0.040</td>
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<td>0.035</td>
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<tr>
<td>9</td>
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<td>10</td>
<td>0.035</td>
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Mean: 0.032 ± 0.004
S.D.: 0.008
foresceps only and were weighed rapidly on an automatic analytical balance. The difference between the weight of the dry swab and the weight of the saturated swab represented its water-holding capacity (see Table 2). The mean capacity of these swabs was 0.312 ± 0.018 g. The swabs absorbed water amounting to 68% [(0.213/0.312) x 100] of their water-holding capacity.

Table 2. Weights of swabs before and after absorbing water from .1-cm layer on glass surface and after immersion in water

| Sample | Swab | Swab with absorbed water | Absorbed water (B-A) | Saturated swab | Water held (D-A) (Capacity) | Percent absorbency of Capacity | E
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<td>(g)</td>
<td>(g)</td>
<td>(g)</td>
<td>(%)</td>
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<td>.7170</td>
<td>.3008</td>
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<tr>
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<td>.3488</td>
<td>.5384</td>
<td>.1896</td>
<td>.6424</td>
<td>.2936</td>
<td>65</td>
<td>---</td>
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<td>.3866</td>
<td>.6207</td>
<td>.2341</td>
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<td>.3267</td>
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<td>.2315</td>
<td>.7246</td>
<td>.3228</td>
<td>72</td>
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Mean .213 .312 68
S. D. .053 .018

Sample areas

For sampling, the largest area that could be swabbed efficiently without the swab reaching the limit of its absorbency was desired. The amounts of material picked up by swabs in actually swabbing different size areas were determined. Templates made of aluminum foil 0.001 in. thick, with circular holes of various sizes, were used to outline the areas to be swabbed. At each sampling, a new template was used. The template was placed on the wet surface, and the swab, held at an angle of approximately 20° to the surface, was stroked once from each of four directions (at right angles to each other) across the swab area. Each time the swab was stroked, it was rotated 90° to expose as much swab material as possible. Care was taken to avoid stroking the swab against the opposing edge of the opening. The amounts of water absorbed, and the holding capacities of the swabs were determined as before.

Impervious surface

Water-covered glass, as previously described, was used to simulate the wet equipment used in processing fish. When an area of 3 cm² and larger was swabbed, it was found that the swabs had reached the limit of their absorbency before the entire area had been covered. Using templates of 2 cm² (1.6 cm diam circle) limited the amount of water absorbed by the swab to 0.187 ± 0.08 g (see Table 3).

These swabs had a water-holding capacity of 0.305 ± 0.036 g of water. The amount of water absorbed represented 62% [(0.187/0.305) x 100] of their water-holding capacity, or 87% [(0.187/0.213) x 100] of what they could absorb. It appeared that capillary action, or compression by the template, or both, were largely the cause of the variation in the amount of water absorbed from the water-covered glass.

Absorbent surface

A wet filleting board made of pine wood was swabbed using 2-cm² areas. The board was kept just wet enough so that it had a glossy appearance. After being swabbed, the swab area looked as though it had been blotted. The amounts of water picked up from adjacent areas were quite uniform, mean 0.098 ± 0.025 g. This was equivalent to approximately one-third [(0.98/0.302) x 100] of their water-holding capacity (see Table 4).

Fish surface

Whole fish were sampled by swabbing areas of 2 cm². Ten different areas from the head to the tail on each side of unwashed English sole (Parophyrys vetulus) of excellent commercial quality were sampled (see Table 5).

The mean weight of the material (slime) picked up by the swabs from the upper side of the fish was

Table 3. Weights of swabs before and after absorbing water from a 2-cm² area of wet glass, and after immersion in water

| Sample | Swab | Swab with absorbed water from 2 cm² | Absorbed water (B-A) | Saturated swab | Water held (D-A) (Capacity) | Percent absorbency of Capacity | E
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<td>.3992</td>
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<td>8</td>
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Mean .187 .305 62
S.D. .06 .036

Bacteriological Survey 385
TABLE 4. WEIGHTS OF SWABS BEFORE AND AFTER ABSORBING WATER FROM 2-CM² AREA OF WET FILLETING BOARD, AND AFTER IMMERSSION IN WATER

| Sample | Swab with absorbed water from 2 cm² | Swab with absorbed water from 2 cm² | Swab with absorbed water from 2 cm² | Swab with absorbed water from 2 cm² | Percent absorbency of material (Capa-
<table>
<thead>
<tr>
<th>No.</th>
<th>(g)</th>
<th>(g)</th>
<th>(g)</th>
<th>(g)</th>
<th>city) x 100</th>
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</thead>
<tbody>
<tr>
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<td>2</td>
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<td>.1024</td>
<td>.1349</td>
<td>.8434</td>
<td>.3701</td>
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<tr>
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<td>.3352</td>
<td>.0651</td>
<td>.6082</td>
<td>.2730</td>
<td>.3107</td>
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<td>4</td>
<td>.3419</td>
<td>.0697</td>
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<td>.3013</td>
<td>.2968</td>
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<tr>
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<td>.0770</td>
<td>.6403</td>
<td>.3013</td>
<td>.2968</td>
</tr>
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<td>.0720</td>
<td>.7393</td>
<td>.2433</td>
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<td>.3187</td>
<td>.0708</td>
<td>.6588</td>
<td>.3401</td>
<td>.4330</td>
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<tr>
<td>8</td>
<td>.4549</td>
<td>.0955</td>
<td>.7317</td>
<td>.2968</td>
<td>.4330</td>
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<td>.6344</td>
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<td>.3703</td>
<td>.1176</td>
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<td>.4330</td>
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<tr>
<td>Mean</td>
<td>.098</td>
<td>.302</td>
<td>.328</td>
<td>.302</td>
<td>.328</td>
</tr>
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<td>.025</td>
<td>.039</td>
<td>.039</td>
<td>.039</td>
<td>.039</td>
</tr>
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</table>

.0038 ± .0016 g and of the lower side, .0039 ± .0015 g.

Discussion

Although bacteriological sampling of surfaces of fish by the swab technique have given more uniform results than other methods of sampling, it is recognized that swabbing does not remove all of the material on a surface. Preliminary trials indicated that cotton swabs absorbed water efficiently from a wet, smooth, impervious surface until the swab itself became wet.

The maximum or potential water-absorption of similar swabs was determined, and this information was then used in determining the largest area of wet surface that could be sampled efficiently. Swabbing failed to pick up all the water from areas of wet glass as large as 3 cm². When 2 cm² of the wet glass were swabbed, the glass appeared to be wiped dry, and the swabs had additional absorbency. When similar 2-cm² areas of a wet filleting board were handled aseptically by means of sterilized forceps. Each swab was weighed, using a sterilized weighing support. The cotton tip was dipped into the fish slime and was pressed against the side of the tube to squeeze out some of the slime before it was reweighed. The swab was then placed into a bottle containing 100 ml of cold, sterile, phosphate diluent and shaken for 30 sec to dislodge and dilute the slime. Further dilutions were made, and the bacterial counts of the slime and from the swabs were determined by the procedure of Tretsven (31).

Of the bacteria in the slime, approximately 65% were recovered from the swabs.

Recovery from swabs

The recovery of bacteria from cotton swabs was determined by comparing the bacterial counts obtained from swabs having known amounts of a control slime. Approximately 4 g of slime was scraped from the surface of fresh English sole into a sterile tube. The slime was mixed by means of a sterile pipette that was then used as a weighing pipette. Slime (0.318 g) was added to 100 ml of cold, sterile phosphate buffer solution and shaken 30 sec. Serial dilutions were made before plating. Sterile swabs

were handled aseptically by means of sterilized forceps. Each swab was weighed, using a sterilized weighing support. The cotton tip was dipped into the fish slime and was pressed against the side of the tube to squeeze out some of the slime before it was reweighed. The swab was then placed into a bottle containing 100 ml of cold, sterile, phosphate diluent and shaken for 30 sec to dislodge and dilute the slime. Further dilutions were made, and the bacterial counts of the slime and from the swabs were determined by the procedure of Tretsven (31).

Of the bacteria in the slime, approximately 65% were recovered from the swabs.

TABLE 5. WEIGHTS OF MATERIAL PICKED UP BY SWABS FROM 2-CM² AREAS ON THE UPPER AND LOWER SURFACES OF ENGLISH SOLE.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Material swabbed from 2 cm² of upper surface</th>
<th>Material swabbed from 2 cm² of lower surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>(g)</td>
<td>(g)</td>
</tr>
<tr>
<td>1</td>
<td>.0030</td>
<td>.0049</td>
</tr>
<tr>
<td>2</td>
<td>.0057</td>
<td>.0030</td>
</tr>
<tr>
<td>3</td>
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<td>.0018</td>
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<td>.0058</td>
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<td>6</td>
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<td>.0056</td>
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<td>7</td>
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<td>8</td>
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<td>.0030</td>
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<tr>
<td>9</td>
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<td>.0049</td>
</tr>
<tr>
<td>10</td>
<td>.0029</td>
<td>.0050</td>
</tr>
</tbody>
</table>

Mean | .0038 | .0039 |
Mean | .0016 | .0015 |

.0038 ± .0016 g and of the lower side, .0039 ± .0015 g.

Recovery from swabs

The recovery of bacteria from cotton swabs was determined by comparing the bacterial counts obtained from swabs having known amounts of a control slime. Approximately 4 g of slime was scraped from the surface of fresh English sole into a sterile tube. The slime was mixed by means of a sterile pipette that was then used as a weighing pipette. Slime (0.318 g) was added to 100 ml of cold, sterile phosphate buffer solution and shaken 30 sec. Serial dilutions were made before plating. Sterile swabs

were handled aseptically by means of sterilized forceps. Each swab was weighed, using a sterilized weighing support. The cotton tip was dipped into the fish slime and was pressed against the side of the tube to squeeze out some of the slime before it was reweighed. The swab was then placed into a bottle containing 100 ml of cold, sterile, phosphate diluent and shaken for 30 sec to dislodge and dilute the slime. Further dilutions were made, and the bacterial counts of the slime and from the swabs were determined by the procedure of Tretsven (31).

Of the bacteria in the slime, approximately 65% were recovered from the swabs.

TABLE 6. RECOVERY OF BACTERIA FROM SWABS HOLDING FISH SLIME

<table>
<thead>
<tr>
<th>Sample</th>
<th>Swab with slime</th>
<th>Slime (B-A)</th>
<th>Standard plate count (per 100 x 100 ft²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>(g)</td>
<td>(g)</td>
<td>Average per swab</td>
</tr>
<tr>
<td>1</td>
<td>.3848</td>
<td>.4370</td>
<td>230</td>
</tr>
<tr>
<td>2</td>
<td>.3447</td>
<td>.3911</td>
<td>238</td>
</tr>
<tr>
<td>3</td>
<td>.4341</td>
<td>.4908</td>
<td>321</td>
</tr>
<tr>
<td>4</td>
<td>.3604</td>
<td>.4436</td>
<td>265</td>
</tr>
<tr>
<td>5</td>
<td>.3605</td>
<td>.4332</td>
<td>265</td>
</tr>
<tr>
<td>6</td>
<td>.3788</td>
<td>.4224</td>
<td>246</td>
</tr>
<tr>
<td>7</td>
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<td>326</td>
</tr>
<tr>
<td>8</td>
<td>.3783</td>
<td>.4541</td>
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<td>9</td>
<td>.3910</td>
<td>.4424</td>
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</tr>
<tr>
<td>10</td>
<td>.4023</td>
<td>.4645</td>
<td>276</td>
</tr>
</tbody>
</table>

Mean | .0596 | .451 |

*Standard Plate Count of fish slime, 6,900,000 per gram.
were swabbed, less water was picked up by the swabs, amounting to 46% \([0.098/0.213 \times 100]\) of their potential absorbency.

Swabbing 2 cm² of the fresh fish surface resulted in picking up material (slime, scales, debris, and water) equivalent to 2% of the amount of water that similar swabs could absorb. These swabs were not immersed in water to determine their water-holding capacities, as the material picked up from the fish was both soluble and hydrophilic. Thus, additional water held could not be attributed entirely to absorption by the swab. There was considerable variation in the amounts of material picked up in sampling the fish. This variation was expected, as the amount of material on the fish was so irregular that the fish appeared to be mottled. In a subsequent bacteriological study, the numbers of bacteria from similar surfaces varied correspondingly. Old fish having much viscous slime were swabbed. The amount of material picked up by the swabs was greater than their water-holding capacity, indicating that there are factors involved in addition to those of absorption.

In a survey concerning the bacterial counts on the surfaces of fish and the processing equipment and their relation to one another, similar swabs, templates, and procedures were used so that the results would be directly comparable.

The following innovations were developed to facilitate the application of the procedure:

1. Swabs are selected on the basis of uniformity of weights of both the swab material and of the water absorbed. Ten or more swabs from a source are sampled.

2. Swab sticks are notched to facilitate breaking off the swab end when it is inserted into the neck of a container and the stick is bent. The stick with a notch cut around it approximately 1/64 in. deep above the swab tip is strong enough to swab the surface, yet break cleanly when and where desired.

3. Templates are made of 0.001-in. thick aluminum foil about 2 in. wide and 4 in. long with a circular hole 1.6 cm in diameter (2-cm² swab area) in the center. The length and width permits one to handle and hold the template in place without contaminating the outlining surface.

4. The template is wrapped around the swab stick (Figure 1).

5. The stick (swab end first) is inserted into a screw-capped tube, sealed, and sterilized.

6. To use, the cap is removed without contaminating the opening and the tube tilted so that the swab stick slides out.

7. The stick is grasped with the fingers.

8. The end of the template is held and unrolled from the swab stick.

9. The template is placed on the surface to be sampled and the ends of the template are pressed against the sample with two fingers.

10. The swab is held at an angle of approximately 20° to the surface, and stroked once from each of four directions (at right angles to each other) across the swab area. Each time the swab is stroked, it is rotated 90° to expose as much swab material as possible. Care is taken to avoid stroking the swab against the opposing edge of the opening (Figure 2).

11. The swab end is inserted into a container of sterile media, or diluent and the stick is bent to break off the tip.

As the amount of material on the swab is small, the dilution is considered to be equivalent to that of the liquid (i.e., if in 10 ml, the dilution is 10⁻¹). Less than 30 sec are required to obtain a sample from fish or equipment when this method is used.

12. The container is shaken rapidly for 30 sec to distribute the swabbed material throughout the liquid.
SUMMARY AND CONCLUSIONS

Because swab materials vary considerably in their weights and abilities to absorb and hold water, the swabs used in the present work were selected for their uniformity in these properties. Wet surfaces of various sizes were swabbed, and the largest area that could be swabbed efficiently was selected as the swab-sampling area. Dryness, as noted by the appearance of the surface after swabbing, and the amounts of water absorbed by the swabs were used in the evaluation of the efficiency. Using the cotton applicator sticks as swabs, a 2-cm² area was found to be the largest area that could be swabbed for obtaining samples from fish and fish processing equipment for a direct comparison of the bacteria counts. Approximately 65% of the bacteria of fish slime held by swabs was recovered. Innovations were developed that enhance the use of swabs for sampling.

REFERENCES

SUMMARY

Samples of retail pasteurized milk were allowed to stand at room temperature for 4 hr during which the temperature of the samples increased 33 F to an average maximum of 81 F. Coliform and standard plate counts were made during this period. Only 2 samples out of 16 showed extensive coliform growth. Increases in standard plate counts were surprisingly small.

In our area many in the dairy industry are convinced that a slight rise in temperature produces a marked increase in the coliform content of retail pasteurized milk. An increase of 1-2 to 4-5 F, in an indefinite but extremely short interval, is thought to induce such rapid growth of coliforms that the milk is by then beyond compliance with a coliform standard of not over 10 per ml. If this be the case, the sample collector or the laboratory may unintentionally allow samples to warm up enough to affect the counts. As a result, we decided to investigate bacterial growth in retail packaged milk in as simple and practical a manner as we could devise.

PROCEDURE

The samples consisted of Vitamin D homogenized, regular, and skim milk with the exception of one sample, No. 9, which was homogenized goat milk. They were processed by eight different plants, were pasteurized, and of one-quart size. Most were contained in paper cartons, but a few were in glass bottles. Samples were collected from trucks just prior to delivery and were kept well iced except during very cold weather. Upon reaching the laboratory, they were immediately placed in a refrigerator maintained at approximately 4 C until plated. Plating of the samples began at 12:00-12:30 p.m.

The coliform tests were made in duplicate as time permitted over a period of thirteen months. In addition, single standard plate counts were made on each sample. Violet red bile agar and plate count agar were used. Plating was carried out in close conformance to Standard Methods (1). Care was taken to see that clinging drops were not transferred with measured portions on the tips of pipettes. A mechanical convection incubator was used. Temperature readings averaged 35.0 C for the top shelf and 35.1 C for the bottom shelf.

Each sample was plated 10 times during a period of 4 hr. Platings were made initially and at the end of 5 min, 10 min, 15 min, 30 min, 45 min, 1 hr, 2 hr, 3 hr, and 4 hr. Samples were allowed to remain on the plating table at room temperature during the intervals between platings and care was taken to avoid undue warming from close proximity to gas burners. Only one sample was plated on a given day. The sample was shaken before each of the 10 dilutions was made, and 2 undiluted 1-ml portions were plated for coliforms and plates of 1:100 and 1:1,000 dilution were made for the standard plate count. The temperature of the sample was recorded as soon as possible after portions were removed for plating. The thermometer was allowed to stand in alcohol. Before use, the alcohol was removed by pouring two 99-ml quantities of sterile dilution water over the thermometer. After use, the milk was rinsed off the thermometer before it was placed in alcohol again. The room temperature was recorded at the beginning and the end of the 4-hr period.

The results are shown in Table 1 arranged according to plating intervals. In the interest of brevity, counts are reported for only the initial plating and as determined at the end of 15 min, 1 hr, and 4 hr. Sample and room temperatures and the month when each sample was examined are shown, also.

DISCUSSION

Only one sample showed a coliform count of over 10 on the initial plating, whereas four of the samples showed a standard plate count of over 30,000. On the final plating, after 4 hr of warming from an average temperature of 48 F to 81 F, four of the samples gave coliform counts of over 10 per ml and four gave standard plate counts of over 30,000 per ml. Sample No. 6 is assumed to be over 30,000 per ml on the final plating since a "spreader" resulted and the standard plate count at 3 hr was 44,000. Only one of the four samples having a high coliform count had a high standard plate count. One sample with an initial standard plate count of 38,000 gave a final count of 26,000. Thus, warming at room temperature for 4 hr

1 Formerly Chief, Public Health Engineering, Management Services, Inc., Oak Ridge, Tennessee.
Effect on Bacterial Counts

Table 1. The Effect of Gradual Warming of Milk Samples on Coliform and Standard Plate Counts

<table>
<thead>
<tr>
<th>Plating interval</th>
<th>Temperature</th>
<th>Room</th>
<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>80</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>3,400</td>
</tr>
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<td>15 minutes</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5,500</td>
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<td>65</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4,300</td>
</tr>
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<td>82</td>
<td>80</td>
<td>6</td>
<td>6</td>
<td>5,500</td>
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(Sample No. 1, Feb.)

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<th>Temperature</th>
<th>Room</th>
<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>80</td>
<td>45</td>
<td>1</td>
<td>1</td>
<td>1,400</td>
</tr>
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<td>15 minutes</td>
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<td>4</td>
<td>1</td>
<td>1</td>
<td>1,000</td>
</tr>
<tr>
<td>1 hour</td>
<td>64</td>
<td>28</td>
<td>21</td>
<td>21</td>
<td>500</td>
</tr>
<tr>
<td>4 hours</td>
<td>82</td>
<td>80</td>
<td>190</td>
<td>210</td>
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(Sample No. 2, Mar.)

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<th>Room</th>
<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>50</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td>15 minutes</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>400</td>
</tr>
<tr>
<td>1 hour</td>
<td>62</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td>4 hours</td>
<td>82</td>
<td>79</td>
<td>0</td>
<td>0</td>
<td>600</td>
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(Sample No. 3, Mar.)

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<th>Temperature</th>
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<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
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<tr>
<td>Initial</td>
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<td>3</td>
<td>3</td>
<td>33,000</td>
</tr>
<tr>
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<td>7</td>
<td>7</td>
<td>37,000</td>
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<td>13</td>
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(Sample No. 4, Apr.)

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<th>Room</th>
<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
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<td>51</td>
<td>2</td>
<td>4</td>
<td>16,000</td>
</tr>
<tr>
<td>15 minutes</td>
<td>62</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>14,000</td>
</tr>
<tr>
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<td>10</td>
<td>10</td>
<td>14,000</td>
</tr>
<tr>
<td>4 hours</td>
<td>87</td>
<td>86</td>
<td>180</td>
<td>190</td>
<td>20,000</td>
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(Sample No. 5, May)

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<th>Room</th>
<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>80</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>42,000</td>
</tr>
<tr>
<td>15 minutes</td>
<td>59</td>
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<td>0</td>
<td>0</td>
<td>44,000</td>
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<tr>
<td>1 hour</td>
<td>72</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50,000</td>
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<td>4 hours</td>
<td>82</td>
<td>81</td>
<td>0</td>
<td>0</td>
<td>Spreader</td>
</tr>
</tbody>
</table>

(Sample No. 6, May)

<table>
<thead>
<tr>
<th>Plating interval</th>
<th>Temperature</th>
<th>Room</th>
<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>78</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>29,000</td>
</tr>
<tr>
<td>15 minutes</td>
<td>59</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>24,000</td>
</tr>
<tr>
<td>1 hour</td>
<td>68</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>29,000</td>
</tr>
<tr>
<td>4 hours</td>
<td>84</td>
<td>83</td>
<td>2</td>
<td>2</td>
<td>28,000</td>
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</table>

(Sample No. 7, May)

<table>
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<th>Room</th>
<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>78</td>
<td>48</td>
<td>0</td>
<td>1</td>
<td>38,000</td>
</tr>
<tr>
<td>15 minutes</td>
<td>57</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>34,000</td>
</tr>
<tr>
<td>1 hour</td>
<td>64</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>32,000</td>
</tr>
<tr>
<td>4 hours</td>
<td>79</td>
<td>78</td>
<td>0</td>
<td>2</td>
<td>26,000</td>
</tr>
</tbody>
</table>

(Sample No. 8, May)

<table>
<thead>
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<th>Temperature</th>
<th>Room</th>
<th>Sample</th>
<th>Dup. coliform count</th>
<th>Single standard plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>78</td>
<td>47</td>
<td>3</td>
<td>0</td>
<td>3,600</td>
</tr>
<tr>
<td>15 minutes</td>
<td>56</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3,500</td>
</tr>
<tr>
<td>1 hour</td>
<td>66</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3,200</td>
</tr>
<tr>
<td>4 hours</td>
<td>81</td>
<td>81</td>
<td>2</td>
<td>4</td>
<td>4,200</td>
</tr>
</tbody>
</table>

(Sample No. 16, Feb.)

Caused three coliform counts to exceed 10 per ml but only one standard plate count to exceed 30,000 per ml.

During the first, or 5-min, interval none of the samples showed the alleged increase in coliforms; the temperature had increased an average of 2.6 F per sample. When warming was extended to 10 min, the temperature rose an average of 5.3 F and the samples failed to show marked increases in bacterial growth. The same was true at the end of 15 min when the temperature had increased an average of 8.4 F per sample. Even after 4 hours of warming during which the temperature rose an average of 33 F, from 48 F to 81 F, only six samples, Nos. 1, 2, 4, 5, 9 and 12, showed a distinct increase in coliform count. Only two of these samples, No. 2 and No. 5, both of which had a few coliforms initially, showed extensive coliform growth.

Significant increases in coliform counts for intervals not reported occurred in only three samples.
Sample No. 1 showed significant growth at the end of 3 hr, Sample No. 2 at 30 min and 3 hr, and Sample No. 5 at 2 hr and 3 hr.

The increase in standard plate counts was less than was anticipated; this was especially true after warming for 3 and 4 hr.

When first plated, the samples were undoubtedly within the period after pasteurization during which counts decline. It has been shown (2, 3) that the decline continues for 24 hr in a large percentage of samples and for at least as long as 72 hr in a few samples. With the temperature gradually increasing, apparently varying lengths of time up to 4 hr or more were required to reverse the downward trend or resolve the static condition of the bacterial population by the production of viable cells with rapid growth characteristics.

Conclusions

The counts showed no immediate surge of growth resulting from the first few degrees of increase in sample temperatures.

Only two samples showed rapidly increasing coliform counts. The count of the sample having a high initial coliform content increased very slowly. Increases in the standard plate count were either surprisingly small or the samples showed small decreases in count. In view of these results, it is concluded that the after-effect of pasteurization (2) was responsible for holding the bacterial growth of the samples to the magnitudes encountered.

It appears that the warming of samples, under the conditions studied, has a somewhat greater effect upon the coliform count than upon the standard plate count. However, more data are needed to establish this relationship.

The results reported certainly are not license to be lax with samples. Rather, these findings are assurance that when samples are handled according to USPHS recommended ordinance regulations (4) the processor and/or distributor may justly be held accountable for the condition of the products as collected by the sanitarian and examined by the laboratory.

References

PHAGOCYTOSIS OF CERTAIN MASTITIS BACTERIA IN COLD MILK

GILBERT TAYLOR, R. T. MARSHALL, AND J. E. EDMONDSON

Dairy Department, University of Missouri, Columbia

SUMMARY

This experiment was designed to determine whether reductions in numbers of viable bacteria could be expected due to storage at 40 F in milk containing high numbers of leucocytes. There was a highly significant decrease in viable bacteria due to the storage for 2.4 hr at 40 F of milk containing from 6.5 to 16 million leucocytes per ml. There were no significant changes in viable count due to continued storage for up to 72 hr. The difference in susceptibility to phagocytosis was not significant for the organisms showing the greatest and the least decreases in count after 24-hr storage. Assays in raw milk using methods described herein appear to be superior to assays in sterile milk.

Mastitis researchers have often reported instances of apparently sterile milk samples obtained from inflamed quarters. Many explain that this is due to leucocytes and/or other conditions unfavorable for bacterial survival in the milk. That high leucocyte counts cause decreases in bacterial numbers within the udder has been shown by Hoffman (3) and by Cherrington, Hansen, and Halverson (2).

The emphasis upon decreasing the leucocyte content of market milk occasioned by "abnormal milk programs" and the paucity of information on the subject serves to emphasize the need for an assay of their role in controlling the flora of bulk milk.

There is limited agreement regarding the number of leucocytes which can be expected in the milk from a normal udder. However, there is little question but that a number of 500,000 per ml would be regarded as indicative of inflammatory response to either bacterial, chemical or physical stimuli (2, 4, 5).

To accentuate the possible effects of phagocytosis this experiment was designed to utilize comparatively high numbers of leucocytes and bacteria. Since certain types of bacteria might be more readily engulfed than others, both gram-negative and gram-positive bacteria were included as well as cocci and bacilli.

MATERIALS AND METHODS

Experimental conditions were chosen which would closely simulate the storage of bulk milk on the farm.

Milk was drawn aseptically from the cow and taken to the laboratory within 10 min. It was centrifuged at approximately 20,000 x g for 15 minutes to precipitate the leucocytes. Cream and skim milk were removed by decanting. The leucocytes were then placed in sterile skim milk.

Early in the study leucocytes were obtained from milk produced by an animal with a highly inflamed quarter. This proved a poor procedure since the leucocytes were often contaminated with bacteria. Therefore, a noninflamed quarter was chosen by means of California Mastitis Test records. Milk from this quarter contained less than 300 bacteria per ml. Leucocytosis was stimulated by injection through the teat canal of 3 ml of 0.9% sterile saline. This milk was collected, centrifuged and the leucocytes retained for the tests.

Phagocytosis was studied for six bacterial cultures which had been isolated from mastitic udders. These cultures were two species of micrococci, designated E1C and T6B, Staphylococcus aureus (WW1), Streptococcus agalactiae, Pseudomonas aeruginosa, and Escherichia coli.

Six 60-ml portions of sterile cold skim milk were inoculated with the cultures. After shaking, the milk was divided, and to six of the tubes leucocytes were added in high concentration. Each sample was plated immediately after mixing. Samples were then stored at 40 F.

Plate counts were made in accordance with Standard Methods for the Examination of Dairy Products (1).

Leucocyte numbers were determined by the direct microscopic method (1). Numbers varied from 6.5 - 16 million per ml.

Statistical comparisons were made using the paired value formula (6). Comparisons were made of the percentage change in bacterial count during each 24-hr period. For example, the mean percentage change in count caused by the presence of leucocytes at zero hr was compared to the mean percentage change caused by their presence over a 24-hr period.

RESULTS AND DISCUSSION

This experiment was designed to determine whether reductions in numbers of viable bacteria could be expected due to storage at 40 F in milk containing high numbers of leucocytes. The assumptions were made that effects of bacteria-leucocyte interactions would be accentuated by the addition of both in large

1 Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 2641.
2 Present address: District 4 Health Office, Popular Bluff, Missouri.
# Phagocytosis of Certain Mastitis Bacteria

## Table 1. Standard Plate Count, Percent Difference of Treated from Control, and Number of Leucocytes Added per Milliliter (Test Organism: *Streptococcus agalactiae*)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Hours storage</th>
<th>A SPC per ml control</th>
<th>B SPC per ml treated milk</th>
<th>Percent difference A-B</th>
<th>Leucocytes per ml (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1,500,000</td>
<td>1,500,000</td>
<td>0.00</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1,400,000</td>
<td>1,100,000</td>
<td>-21.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>830,000</td>
<td>740,000</td>
<td>-10.84</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>720,000</td>
<td>-55.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>400,000</td>
<td>340,000</td>
<td>-15.00</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>400,000</td>
<td>290,000</td>
<td>-27.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>290,000</td>
<td>310,000</td>
<td>+6.89</td>
<td></td>
</tr>
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<td>260,000</td>
<td>210,000</td>
<td>-32.25</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>280,000</td>
<td>300,000</td>
<td>+7.14</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>650,000</td>
<td>470,000</td>
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<tr>
<td></td>
<td>48</td>
<td>880,000</td>
<td>940,000</td>
<td>+6.81</td>
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<td>760,000</td>
<td>730,000</td>
<td>-3.94</td>
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</tr>
<tr>
<td>4</td>
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<td>-7.69</td>
<td>12.0</td>
</tr>
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<td>10,000</td>
<td>-16.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>8,700</td>
<td>7,200</td>
<td>-17.24</td>
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</tr>
<tr>
<td>5*</td>
<td>0</td>
<td>100,000</td>
<td>67,000</td>
<td>-33.00</td>
<td>8.0</td>
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<tr>
<td></td>
<td>24</td>
<td>74,000</td>
<td>56,000</td>
<td>-24.32</td>
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</tr>
<tr>
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<td>48</td>
<td>96,000</td>
<td>61,000</td>
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</tr>
<tr>
<td></td>
<td>72</td>
<td>62,000</td>
<td>37,000</td>
<td>-40.32</td>
<td></td>
</tr>
</tbody>
</table>

*Data represent observations made in the experiment in which raw milk was used instead of heat-sterilized milk. These data are not included in Figures 1 and 2.*

Numbers. Therefore, counts used are considerably higher than those commonly found in market milk. In addition, since the objective was to assay the effects of leucocytes, sterile skim milk was used as the basal medium to eliminate effects of other possible inhibitors or complementary factors such as opsonins.

The results should not be interpreted to reflect the exact effects of leucocytes on bacteria in raw milk due to the above cited conditions.

Both control and treated milk samples were plated at each storage interval to provide the greatest measure of control on the experiment. This procedure allows the direct comparison of treated and control samples. The response of the control to storage was not always the same. Generally, there was an increase in count. Often, however, the viable cell numbers would remain essentially static throughout the storage period, while in limited cases they decreased. This situation made it necessary to compare the percentage difference of the treated samples from the control samples. To do this the bacterial count of the treated sample was subtracted from that of the control. The product was divided by the count of the control and multiplied by 100. The result was a percentage, which, when negative, reflected a decrease in count or, when positive, reflected an increase in count compared to the control.

Data summarized in Figure 1 show percentage differences between control and treated sample counts for the four storage times and the six test organisms. Each line represents a series of four separate trials. Therefore, there were 24 trials with the test organisms. Of these 24 trials only two failed to show a comparative decrease in count during the first 24-hr of storage. In both tests the 0-hr treated sample count was abnormally low (more than 40% less than that of the control). Figure 1 shows that there was an average count decrease during the first 24-hr storage period of from 10.5% for *Pseudomonas aeruginosa* to 59.5% for the micrococcus designated E1C due to leucocyte additions.

Table 1 is an example of the data collected for each test organism and of the comparison method. This table includes data for *Streptococcus agalactiae* only.

Statistical analyses by paired comparisons revealed a highly significant change in bacterial count during the first storage period for each test organism due to the addition of from 6.5 to 16 million leucocytes per ml. There were no significant changes in count due to continued storage up to 72 hr. Figure 2, which shows the means of all percentage differences of treated from control counts illustrates this point well.
The fact that little change in percentage differences occurred after 24 hr storage suggests that phagocytosis occurs most rapidly during the first few hours after milking. The rate of phagocytosis during these early hours is now being investigated in this laboratory.

To determine whether differences in susceptibility to phagocytosis exist for the organisms tested, the mean percentage count differences were compared for the micrococcus, E1C and for Pseudomonas aeruginosa which showed the greatest and the least change in count, respectively. There was no statistically significant difference in susceptibility.

A preliminary experiment has been performed using raw instead of sterile skim milk as the medium for the phagocytosis test. Leucocytosis was initiated in a grossly normal quarter. Milk collections were made 15 hr after saline injections. Cream was removed by pipette and swab from the centrifuged sample. The upper 50% of the skim milk was removed by pipette to serve as the control. Precipitated leucocytes were remixed with the remainder of the sample to serve as the test solution. To the control and test milks were added 20 - 200 thousand bacteria per ml. All other methods were as given for the major experiment.

Responses appeared to be the same as those listed previously. A very definite decline in bacterial numbers resulted due to leucocyte addition and storage for 24 hr. The sample containing Streptococcus agalactiae showed a comparatively higher count after 24 hr storage (Figure 3). However, the initial treated sample count was about 32% below the control sample count.

Figure 1. Percentage Bacterial Count Differences of Treated from Control Samples due to Storage at 40°F. Raw Milk.

Figure 2. Mean Percentage Bacterial Count Differences of Treated from Control Samples due to Storage at 40°F. Sterile Milk.

Figure 3. Percentages Bacterial Count Differences of Treated from Control Samples due to Storage at 40°F. Sterile Milk.

A greater consistency of results, and the fact that other natural inhibitory or complementary substances in milk may render an effect suggests that the true measure of bacterial decreases can best be assayed by this latter method.

REFERENCES


MODIFIED SPECTROPHOTOMETRIC PHOSPHATASE METHOD

E. F. MCFARREN, L. A. BLACK, AND J. E. CAMPBELL

Milk and Food Research,
Robert A. Taft Sanitary Engineering Center,
Public Health Service, Cincinnati, Ohio

For study of the reactivation (1) of phosphatase in HSTS-and UHT-processed milk products, both a rapid test like the Scharer I (Rapid) Method (2) and a more accurate test like the Scharer II (Laboratory Method (2) was needed. To meet this need, a modified spectrophotometric procedure, combining portions of both tests, was developed and published (1). The present report demonstrates that results obtained by this method are reproducible in another laboratory and comparable with those obtained by other methods.

Most of the data presented here were collected during a collaborative study (3) of a test for differentiating reactivated from residual phosphatase. These data were not published at that time because most of the collaborators used visual methods and only reported whether the samples were positive (inadequately pasteurized) or negative (pasteurized) and whether they contained residual or reactivated phosphatase. A few collaborators used spectrophotometric methods for quantitatively measuring the amount of color developed and reported their results in micrograms of phenol. These data (Table 1 and 2) are useful in establishing the validity and reproducibility of the Modified Spectrophotometric Method. A description of all the samples tested can be found in a previous publication (3) and is not sufficiently important to warrant repetition here.

RESULTS

As shown in Table 1, Laboratory A and the Control Laboratory used the Modified Spectrophotometric Method (1). Laboratory A duplicated the results of the Control Laboratory to within 0.5 micrograms in 10 of the 12 tests. Tests on Sample 1 differed by 0.7 micrograms and Sample 9 differed by 1.2 micrograms. Laboratory B used the Official A.O.A.C. Method I (4) and although the results are not directly comparable, the same relative values were obtained.

In Table 2 are presented the results obtained after the differential test was conducted on these samples. Again, the results obtained in the Control Laboratory and in Laboratory A do not differ significantly from each other with the exception of the results on Sample 3 (probably experimental error) and the results on samples 9, 10, and 12. In the latter cases, the differences are somewhat greater because the results were calculated from solutions giving absorbancies greater than 0.90. For greater accuracy, the samples should have been rerun with a dilution being made with boiled milk or cream before the test (1) was conducted. As before, the results obtained by Lab-

### Table 1. Comparison of Phosphatase Test Results Obtained from Three Laboratories Using Two Different Spectrophotometric Methods

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Control laboratory*</th>
<th>Laboratory A*</th>
<th>Laboratory B*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.2</td>
<td>11.5</td>
<td>0.089</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.7</td>
<td>0.021</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>2.2</td>
<td>0.032</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>2.8</td>
<td>0.038</td>
</tr>
<tr>
<td>5</td>
<td>10.8</td>
<td>10.6</td>
<td>0.084</td>
</tr>
<tr>
<td>6</td>
<td>3.6</td>
<td>4.0</td>
<td>0.044</td>
</tr>
<tr>
<td>7</td>
<td>1.8</td>
<td>2.1</td>
<td>0.032</td>
</tr>
<tr>
<td>8</td>
<td>0.0</td>
<td>0.3</td>
<td>0.021</td>
</tr>
<tr>
<td>9</td>
<td>0.3</td>
<td>1.5</td>
<td>0.024</td>
</tr>
<tr>
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<td>7.2</td>
<td>7.3</td>
<td>0.054</td>
</tr>
<tr>
<td>11</td>
<td>4.5</td>
<td>5.0</td>
<td>0.051</td>
</tr>
<tr>
<td>12</td>
<td>4.8</td>
<td>5.1</td>
<td>0.045</td>
</tr>
</tbody>
</table>

* (Scharer) Modified Spectrophotometric Laboratory Method (1), micrograms phenol per ml.

### Table 2. Comparison of Differential Phosphatase Test Results Obtained from Three Different Laboratories Using Two Different Spectrophotometric Methods

<table>
<thead>
<tr>
<th>Control Incubated without Mg</th>
<th>1 to 6 Dilution with Mg</th>
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</thead>
<tbody>
<tr>
<td>No.</td>
<td>Control lab*</td>
</tr>
<tr>
<td>-----</td>
<td>--------------</td>
</tr>
<tr>
<td>1</td>
<td>12.4</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>3</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>2.6</td>
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<td>5</td>
<td>11.2</td>
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<td>3.6</td>
</tr>
<tr>
<td>7</td>
<td>1.8</td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td>9</td>
<td>24.5</td>
</tr>
<tr>
<td>10</td>
<td>27.2</td>
</tr>
<tr>
<td>11</td>
<td>9.0</td>
</tr>
<tr>
<td>12</td>
<td>21.7</td>
</tr>
</tbody>
</table>

*Micrograms phenol per ml; *Milligram phenol per 0.5 ml; *Micrograms phenol per ml of a 1 to 6 dilution; *Milligrams phenol per 0.5 ml of a 1 to 6 dilution.
oratory B, although not directly comparable, are of the same relative order.

In Table 3 are presented the results obtained on another series of samples tested by both the Modified Spectrophotometric Method (1) and by the Scharer II (Laboratory) Method (2) with a spectrophotometer. The differences here are somewhat larger than those previously discussed; however, it should be remembered that with the Scharer II (Laboratory) Method the samples are diluted in all cases by a factor of 2.3 as a result of precipitation of the proteins, filtering, and addition of color developing reagents. No dilution is involved with the Modified Spectrophotometric Phosphatase Method except when high absorbancies are encountered; it becomes necessary with either test to make dilutions so that the absorbancies of the solutions measured on the spectrophotometer fall below 0.90. If the results obtained (Table 3) are adjusted to a common base by division of each by the same dilution factor, the results do not differ significantly from each other. The error only appears large in some cases because of the large factor (dilution) by which it has been multiplied. In only three cases (samples D4 - 1/4, D4 - 2, and D4 - 4) are the differences greater than 0.5 micrograms.

### Discussion

In view of the similarity of results (Table 3) and of tests conducted on 0.1% raw milk (Table 1, Sample 4) it is evident that the criterion for adequacy of pasteurization with the Modified Spectrophotometric Phosphatase Method should be the same as with the Scharer II (Laboratory) Method; namely, 2.3 micrograms phenol or less per ml.

The proposed spectrophotometric test is simpler and less time consuming than either the Scharer II (Laboratory) Method or the Official A.O.A.C. Method I and does not involve the troublesome operation of precipitating the proteins to obtain a clear filtrate. Furthermore, since no dilution of the sample is involved and the absorbancy of a butanol extract is determined on the spectrophotometer rather than by visual comparison, the method is inherently more accurate.

### Acknowledgments

The assistance of Albert J. Wehby in examining nearly all of the Control Laboratory samples is acknowledged with appreciation. Our thanks also go to the following collaborators whose data are quoted in this paper: Alan Hotchkiss, Sanitation Service Section, Connecticut State Department of Health, 1179 Main Street, Hartford, Conn.; and James B. Hunter, Texas State Department of Health, 100 West 49th St., Austin, Texas.

### References

CLEANING INTERIOR SURFACES OF MILKING ROOMS

Martin E. Burkhardt, Donald C. Erbach, S. A. Witzel

Department of Agricultural Engineering

and

H. E. Calbert

Department of Dairy and Food Industries
University of Wisconsin, Madison

(Received for publication September 11, 1963)

Summary

The main objective of this work was to determine a management practice that would maintain a visibly clean wall surface in the milking room in the most practical manner. Walls could be kept clean by (a) wetting the surface at the beginning of the milking operation, (b) keeping the surface moist during the milking operations, and (c) immediate wash down of any manure or soil deposited on the surface. Satisfactory results can be attained at normal farm water system temperatures and pressures.

The milking room of the modern dairy farm should be considered a part of a food processing plant. Its interior surfaces must provide a satisfactory appearance as required by various sanitation standards (1). It is reasonable to expect that the discriminating consumer would have a direct interest in the cleanliness of the premises in which this important human food is produced. How to clean these surfaces so as to provide a satisfactory appearance is a problem of many milking room operators.

McQuitty et al. (2) have investigated the hydraulic removal of manure from concrete and concluded that an application of 6.3 gpm of water at 100 psi from a nozzle with a solid-spray pattern was the most effective on horizontal surfaces. Limited information on the removal of manure from vertical surfaces emphasized the need for study in this area. Observations under field conditions disclosed that a given surface on the walls of a milking room might be cleaned satisfactorily by one dairyman while another dairyman might do an unsatisfactory job. This indicated that the problem of having clean walls in milking rooms was primarily a management problem. Therefore, the purpose of this study was to determine a management practice that would maintain a visibly clean wall surface in the milking room in the most practical manner.

Procedure

Research by Harris (3) as well as that by Peabody et al. (4) has shown that it is possible to study surface cleanability under laboratory conditions. Therefore, this investigation was carried out in the laboratory. Initial exploratory work was carried out using small rectangular pieces of glass to represent wall surfaces as a means of determining management practices that would prevent manure stain. The 3% in. x 4 in. rectangles of glass (Kodak Lantern Slide Cover Glass) were dipped in different kinds of wall paint and allowed to dry two weeks before being subjected to fresh cow manure. A thin coating of manure was then applied to a series of the painted plates. Some of the plate surfaces were wet, others were dry when the manure coating was applied. The plates with the manure coatings were then allowed to dry 0, 2 or 24 hr before being subjected to a washing treatment. This exploratory work with the painted glass rectangles resulted in a procedure of wetting the surface before soiling with manure and washing before drying that was used in subsequent testing procedures.

In subsequent tests four foot square panels of ceramic tile, fiberglass and painted plywood were held in a vertical rack directly in front of and approximately six feet from a manually controlled nozzle (Figure 1). These panels were soiled with a material containing by volume 10% clay, 30% silt, and 60% sand. Washington of this material compared favorably with that of manure in both the wet and dried-on conditions. It did not, however, have the chemical or...
staining properties of fresh manure. Washing was done with an objective of getting the cleanest surface from the treatment used.

Combinations of water temperature, time, pressure, and application rate were used as treatments on the various panels. Temperatures were maintained with an electric water heater; time was controlled with an interval timer; pressure was varied by changing the speed of a gear pump; and, application rate was varied by changing calibrated solid-stream nozzles. This equipment is shown in Figure 2.

All washing was done with tap water without the addition of soaps, detergents or cleaning compounds. Temperatures of 60 F and 140 F were used to wash panels for intervals of 6 to 48 sec. Pressures were varied from 20 to 150 psi on five solid-stream nozzles.

Wet and dry panel surfaces were soiled with one-half pound of the soiling material moistened respectively with 60 ml and 70 ml of water. The soil was spread manually over the surface in its vertical position. Following washing, a grid was laid over the panel. Then a total of twenty 4½ in. x 4½ in. consecutive squares as shown in Figure 3 were visually examined and rated individually. Ratings of the cleanliness of each square examined in the panel were averaged to give the cleanliness rating of the panel. The following ratings were used:

1. Clean, no discoloration or foreign matter.
2. Some discoloration (due to fines) but no granular material.
3. Considerable discoloration and/or some granular material.
4. Much granular material.
5. Practically no soil removed.

**Results**

Studies on the 3½ in. x 4 in. painted glass rectangles indicated that a procedure of wetting the surface, soiling with manure and immediate washing produced the least adverse effects on the surfaces as shown in Table 2.

Results of washing painted panels with 60 F water and various times and pressures are given in Table 3 for each of the solid-stream nozzles used. A washing quality of one is best, and is essential to obtaining a visibly clean surface. Table 4 summarizes the

---

**Table 1. Manufacturer's Calibration of the Nozzles Used.**

<table>
<thead>
<tr>
<th>Nozzle No.</th>
<th>15 (gpm)</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4 U0010a</td>
<td>.61</td>
<td>.71</td>
<td>.86</td>
<td>1.0</td>
<td>1.2</td>
<td>1.4</td>
<td>1.6</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4 U0020a</td>
<td>1.2</td>
<td>1.4</td>
<td>1.7</td>
<td>2.0</td>
<td>2.5</td>
<td>2.8</td>
<td>3.2</td>
<td>3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4 U0030a</td>
<td>1.8</td>
<td>2.1</td>
<td>2.6</td>
<td>3.0</td>
<td>3.7</td>
<td>4.2</td>
<td>4.7</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4 U0040a</td>
<td>2.5</td>
<td>2.8</td>
<td>3.5</td>
<td>4.0</td>
<td>4.9</td>
<td>5.7</td>
<td>6.3</td>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4 U0050a</td>
<td>3.1</td>
<td>3.5</td>
<td>4.3</td>
<td>5.0</td>
<td>6.1</td>
<td>7.1</td>
<td>7.9</td>
<td>9.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aManufacturer's nozzle number. Spraying Systems Company, Bellwood, Illinois.

*bSpraying Systems Company, Bellwood, Illinois.
Of the Cleaning and Washing Costs; and (c) as shown in Table 5, there is no significant difference in the cleanability of the surface materials studied.

An incidental observation made on a high pressure washer (2 gpm at 500 psi) indicated that this method was slow and potentially detrimental to painted surfaces covered with dried soil.

**CONCLUSIONS**

Milking room walls can be kept visibly clean through proper management. Proper management involves: (a) wetting the surface at the beginning of the milking operation, (b) keeping the surface moisture during the milking operation, and (c) immediate wash down of any manure or other soil deposited on the surface. Satisfactory results can be attained at normal farm water system temperatures and pressures but, the lowest water use is obtained with a solid-stream nozzle, a pressure of 50 psi and an application rate of 2.8 gpm.

Staining may be serious when manure comes in contact with a dry surface which will soak up stain laden moisture. Stain soakup can be prevented by keeping the surface saturated with clean water. Manure should not be allowed to dry on any surface as it is difficult to remove even with detergent and hand scrubbing.

**Table 5. Comparison of Surface Material Cleanability.**

<table>
<thead>
<tr>
<th>Material</th>
<th>Washing Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glazed ceramic tile</td>
<td>2.57</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.74</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.80</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.87</td>
</tr>
<tr>
<td>Fiberglass</td>
<td>3.12</td>
</tr>
<tr>
<td>Enamel A on plywood</td>
<td>2.82</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.90</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.95</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.85</td>
</tr>
<tr>
<td>&quot;</td>
<td>3.12</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.78</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.85</td>
</tr>
<tr>
<td>Epoxy on plywood</td>
<td>2.92</td>
</tr>
<tr>
<td>&quot;</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Suitable materials for milking room wall surfaces must be washable and prevent moisture from reaching the subsurface construction.

**References**


**Table 3. Results of Washing Painted Plywood Panels.**

<table>
<thead>
<tr>
<th>Nozzle No.</th>
<th>Pressure (psi)</th>
<th>Washing Time (sec)</th>
<th>Washing Quality (average values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>1/4 U0010</td>
<td>20</td>
<td>-</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>-</td>
<td>4.00</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>-</td>
<td>2.50</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-</td>
<td>2.10</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1.90</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.50</td>
<td>1.00</td>
</tr>
<tr>
<td>1/4 U0020</td>
<td>20</td>
<td>-</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>-</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.80</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.02</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.00</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1.05</td>
<td>1.02</td>
</tr>
<tr>
<td>1/4 U0030</td>
<td>20</td>
<td>-</td>
<td>3.30</td>
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<td></td>
<td>40</td>
<td>1.92</td>
<td>1.25</td>
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<td></td>
<td>100</td>
<td>1.05</td>
<td>1.02</td>
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<tr>
<td>1/4 U0040</td>
<td>20</td>
<td>2.75</td>
<td>2.80</td>
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<td></td>
<td>40</td>
<td>1.00</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.02</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.22</td>
<td>1.00</td>
</tr>
<tr>
<td>1/4 U0050</td>
<td>20</td>
<td>3.02</td>
<td>2.95</td>
</tr>
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<td></td>
<td>40</td>
<td>1.05</td>
<td>1.05</td>
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<tr>
<td></td>
<td>60</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.12</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Minimum conditions for each nozzle which yielded visibly clean surfaces.

Other results indicated (a) that elevated temperatures had no advantage in either washing speed or quality, and would (b) that when soil was permitted to dry on the surface no combination of pressure, time and volume of water cleaned satisfactorily without hand scrubbing; and (c) as shown in Table 5, there is no significant difference in the cleanability of the surface materials studied.

An incidental observation made on a high pressure washer (2 gpm at 500 psi) indicated that this method was slow and potentially detrimental to painted surfaces covered with dried soil.
Ivan E. Parkin, professor of dairy science extension, The Pennsylvania State University, will retire December 31 with 29 years of service to the Cooperative Extension Service and to the dairy industry of Pennsylvania.

Native of Connecticut, he left Meriden high school at the end of his freshman year, during World War I to help do farm work. In 1922 he took a two-year course in agriculture at the Connecticut Agricultural College, worked several years as a dairyman, and in 1928 entered the University of Connecticut where he earned a bachelor of science degree in dairy manufacturing. After graduation he returned to work in the dairy industry until he joined the dairy science extension staff at Pennsylvania as an assistant professor in 1934. He was promoted to associate professor in 1945, and to full professor in 1950.

Five major projects have been developed by Mr. Parkin since he came to Pennsylvania: managed milking routine and demonstrations; milking machine clinics; demonstrations on cleaning vacuum lines; bulk milk handling clinics, and establishment of the local Sanitarian groups and the Pennsylvania Dairy Sanitarians Association.

In his extension career in Pennsylvania, Mr. Parkin presented more than 1,200 demonstrations of managed milking to more than 36,000 dairy farmers; 400 demonstrations have been conducted on cleaning vacuum lines with 12,000 people in attendance; 66,000 persons have seen his demonstrations at milking machine clinics with manufacturers participating, and more than 200 meetings and 7 bulk milk handling clinics have been held for a total attendance of more than 10,000.

Mr. Parkin was instrumental in promoting the idea of herd management in mastitis prevention, and in making the Pennsylvania Extension Service the first institution to promote the idea.

In addition to 500 news stories in the dairy sanitation field, Mr. Parkin has also written many Extension circulars and leaflets for distribution throughout Pennsylvania. Titles include: “Quality Milk Production”; “Making Ice Cream at Home”; “Produce High Quality Milk”; “Managed Milking”; “A Pictorial Pattern for High Quality Milk Production”; “Milking Machine Care”; and “Farm Bulk Milk Cooling Tanks.”

Mr. Parkin has been president of the International Association of Milk and Food Sanitarians; chairman of the Extension section of the American Dairy Science Association; member of the 3A Sanitary Procedures Committee of the International Association of Milk and Food Sanitarians; member of the American Academy of Political and Social Science, and is listed in “Who's Who in the East.”

During a sabbatical leave from the Penn State staff in 1956, Mr. Parkin spent part of the year assisting the Dairy Society International under contract with the USDA's Foreign Agricultural Service in making a survey of the dairy situation in Columbia, South America. This mission was to determine if Columbia could feasibly support a growing dairy industry.

Mr. Parkin has participated in 21 State Dairy Sanitarians’ programs, as well as holding a directorship in the National Conference on Interstate Milk Shipments. In the last 10 years he has attended more than 150 meetings a year, presenting discussions in milk sanitation, bulk milk handling, dairy manufacturing, and milking programs on County Agricultural Extension programs.

In 1956 Mr. Parkin was given the title “Dairy Ambassador” by the Dairy Society International for his part in a three-man mission to Columbia. In 1959 the Pennsylvania Dairy Sanitarians’ Association awarded him the “Sanitarian of the Year” plaque, and in 1962 he was selected by the Pennsylvania Dairymen’s Association to receive the Pennsylvania Extension Dairy Award for outstanding contributions in the dairy field.

Mr. Parkin holds active membership in the Pennsylvania Dairy Sanitarians Association and its five local sanitarians' groups, the Pennsylvania Dairy-
men's Association, the International Association of Milk and Food Sanitarians, the Milk House Management Committee of the Pennsylvania Farm Show, American Dairy Science Association, the Extension Professors Association, Alpha Gamma Rho, and Epsilon Sigma Phi.

Mr. Parkin is married to the former Phyllis Birdsey, of Meriden, Connecticut, and has three children.

He was honored December 14 at "Ivan Parkin Day" by his associates and members of the dairy industry throughout the state. The banquet was held at the Hetzel Union Building at 6:30 p.m., on the Penn State Campus at University Park.

DR. C. K. JOHNS JOINS LAZARUS LABORATORIES AS CONSULTANT

One of the world's best known experts on milk sanitation, Dr. C. K. Johns, recently retired after 36 years of service with Canada's Department of Agriculture, has joined Lazarus Laboratories Inc., a leader in the field of dairy and farm sanitation, as a technical consultant. Lazarus Laboratories, originators of "Tamed Iodine" detergent sanitizers, is a division of West Chemical Products, Inc.

In accepting the invitation to associate himself with Lazarus Laboratories as technical consultant, Dr. Johns stated:

"My retirement from the government service does not mean the end of my interest in dairy sanitation. I want to maintain my contacts in this field and perhaps make some further small contribution to milk quality improvement. The invitation to become associated with Lazarus Laboratories as a consultant offers an excellent opportunity to accomplish both these aims. I have been greatly impressed with the performance of this Company's products, both on dairy farms and in plants. I have likewise been impressed with the character and competence of the Company's representatives, and with the very real interest they have shown in improving milk quality. It will be a privilege to work along with them toward the goal of top quality milk."

Dr. Johns, who has published more than 90 scientific papers and research bulletins, as well as numerous press articles, has earned an international reputation for his work in sanitation and quality control of milk and egg products.

VACCINATION AGAINST LEPTOSPIROSIS

A suspension of killed organisms that stimulates an active immunity in animals as a vaccination against leptospirosis is being produced by Globe Laboratories, a division of Chas. Pfizer & Co., Inc. The vaccine is being marketed under the label of Leptospira pomona bacterin, and is designed to provide immunity against the disease of leptospirosis in dairy and beef cattle, dogs, hogs, sheep, goats, and horses. Leptospirosis also occurs in deer and fox, and can be transmitted to humans.

Leptospirosis, according to Dr. Henry D. Carpenter, Director of Laboratories for Globe, affects the genito-urinary system, the blood system, and the cardio-vascular system. The leptospira is a long, closely-spiralled cylindrical organism with pointed ends. It is a flagellated spirochaete that can cause extensive damage to the kidney. Once an animal contracts the disease, the germs literally dig into the kidney tissue for a long, slow siege, producing tiny white scars on the kidney. The infected animals shed the germ in the urine, spreading it to non-infected animals.

Symptoms of the disease, according to Dr. Carpenter, are sudden abortion problems, depression, loss of appetite, fever from 104 to 107 degrees, anemia, weight loss, yellowish colored mucosa, drop in milk production, bloody milk, bloody urine, and thick milk of yellowish color.

Treatment with antibiotics will provide some relief to the symptoms, Dr. Carpenter said, adding that treatment with Terramycin will reduce the number of leptospira shed in the urine, and that treatment is more successful if begun early.

Dr. Carpenter said that it is advisable for animal owners to prevent the disease with vaccination rather than trying to treat it. The disease can occur anytime during the year, particularly if the animals are pastured and have access to streams flowing through other cattle or hog lots.
I AMFES Council of Affiliates Annual Meeting
October 22, 1963 — Toronto, Canada

Minutes of 1962 Meeting
Read and unanimously accepted.

Committee Membership Suggestions
It was the opinion of the affiliate representatives that names, with biographical material, continue to be submitted to the President of the International as possible candidates for IAMFES Committees. It was indicated also that the form, on which such names could be submitted, be provided and mailed to each affiliate association. (Dr. Olson suggested that names of possible members be submitted in plenty of time for appointment of committee members by the president who, in turn, submits all committees for publication in the January issue of the Journal.)

Affiliate Award
Mr. L. Wayne Brown and Mr. Orlow M. Osten reported that, in their opinion, the awarding of a plaque or certificate to an affiliate would not accomplish the main objective: stimulation to greater activity on the part of the affiliate organizations. It was their thinking that such an objective could possibly be better achieved by publishing any worthy activities, ideas or accomplishments in the Journal. They requested that they be dismissed from the committee, which request was honored by Chairman R. P. March.

After further discussion a motion was made to appoint a committee to formulate ground rules for an affiliate award, select a recipient, select the award and present it to the chosen affiliate at the next annual meeting. The motion was passed, and the following committee was appointed: Frank L. Kelly, Chairman, Robert C. Nelson and Shelby Johnson.

Badges
It was voted affirmatively that Mr. Thomasson be requested to continue to furnish the “Council of Affiliates” ribbons.

Speakers
The Council also went on record as favoring the procedure of contacting their association officers and members concerning program subjects and possible speakers and submit these to the council chairman.

Journal Articles
The need for “at least one grassroots article” in each copy of the Journal was discussed at length. (When this was presented to the Executive Board, Dr. Olson stated that any time an affiliate member heard a presentation which would make interesting reading and would get it organized and written out in an acceptable manner—he would be glad to consider it for possible publication.)

Time of Affiliate meeting
The affiliate council expressed a desire that the Executive Board consider the possibility of selecting a time for the Affiliate Council to meet during the regular Annual Program period. (The Executive Board felt that this would be extremely difficult to arrange in view of the numerous conflicts which would be encountered.)

Establishment of time and place of annual convention
Members of the Affiliate Council expressed a desire that the Executive Board consider the possibility of working with prospective host states or provinces in the firm establishment of the Time and Place for the Annual Meeting three years in advance.

Election of officers
The Council felt that, in order to maintain a democratic approach to election of second vice-president, nominations by individual members (other than those named by the Nominating Committee) be accepted and published in the Journal.

Item for 1964 Agenda
Members of the Council request that the Executive Board designate an individual of their choosing who will discuss with Affiliate Council at their 1964 meeting the present status, in respect to size, relationship and future of the three present sanitarians’ organizations. Especial concern was voiced as to what liaison might be instigated which could be beneficial to all concerned. The group would also like to have the subject of the contemplated National Sanitarians Registration Act discussed.

Election of Affiliate Council Officers for 1964
Prof. R. P. March, Dept. of Dairy and Food Science, Cornell University Ithaca, New York, and S. O. Noles, Florida State Board of Health, Jacksonville, Florida, were reelected Chairman and Secretary respectively.

Respectfully submitted
S. O. Noles, Secretary
Dr. George W. Shadwick receives award

Dr. George W. Shadwick (right), director of Technical Services, Beatrice Foods Co., Chicago, is presented with "Indy" award by Mitchell M. Badler, editor of "Industrial Photography" at annual honors banquet held here recently. George W. Colburn (center), president of George W. Colburn Laboratories, Inc., Chicago, holds certificate of merit received for technical production of film. Dr. Shadwick received a special citation on his film, "Milkman to Malaya" produced for Beatrice Foods' annual meeting. He also was honored for the "best one-man" film. The Selz Organization, Chicago, received a certificate of merit for script preparation. The "Indy" is the "Oscar" of the industrial film field.

FAT . . . CAUSE FOR PAUSE

Present concern over the fat content of the American diet has created considerable confusion in the minds of health-conscious Americans. Misunderstandings have led to changes in the daily dietary which are not justified by available facts.

Despite man's indispensable need for dietary fat as a carrier of fat-soluble vitamins and as a source of essential fatty acids, basic foods of unexcelled nutritional value are too often excluded because of a presumably high fat content.

Actually, how fat is milk? At the usually recommended level of two glasses per day for the adult, the fat in milk (3.5%) accounts for only 6% of the total calories in a well-balanced 2500* calorie diet providing the usual 40% of the calories from fat. Two glasses of milk contain between 44 and 58 mg. of cholesterol. Thus, the fat and cholesterol content of milk is lower than that in ordinary servings of most other high quality protein foods of animal origin.

The arbitrary exclusion of milk from the adult diet in the absence of specific medical indications is nutritionally unsound and forfeits an imposing source of nutrients essential for the health and welfare of man.

*Meets calorie and nutrient allowances recommended for sedentary adult male.

PUBLIC HEALTH SERVICE ANNOUNCES THREE TRAINING COURSES TO BE HELD IN FEBRUARY

The Public Health Service, through the Division of Environmental Engineering and Food Protection, announces three food-protection training courses scheduled for presentation February 3 through 28, 1964, to be conducted by the Training Program, Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio. Opening the series is the 1-week course, Food Microbiology, followed February 10 by the 1-week course, Pesticide Residue Analysis of Foods. The third course, Analysis of Radionuclides in Foods, begins on February 17 and is given jointly by the Division of Radiological Health.

Food Microbiology presents advanced technical information for laboratory and supervisory personnel concerned with the bacterial examination of foods to equip them to organize two types of programs — surveillance of the sanitary quality of foods and examination of foods implicated in foodborne disease outbreaks. The course, Pesticide Residue Analysis of Foods, is designed to enable professional personnel in control of regulatory laboratories to characterize the pesticides used in food production and processing.

More complete descriptions of the courses are given in the new Training Program Bulletin, which is available on request. Trainees may register for the courses singly or in series. Applications or requests for information should be addressed to the Director, Training Program, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, or to an appropriate PHS Regional Office. No tuition or registration fee is required.

FIRST NATIONAL CONFERENCE ON SOLID WASTE RESEARCH

With U. S. cities large and small reporting that the volume of refuse is steadily growing, that the expense of collecting and disposing of it is increasing, and that available space for disposal and storage is being exhausted, the First National Conference on Solid Waste Research, meeting December 2-4 at
the University of Chicago sought new ideas for research into ways of coping with the problem.

The size of America's solid waste disposal problem is an inevitable result of the trend to bigger cities and closer-together living of post-World War II United States. Since most refuse is collected and disposed in generally the same manner used in past centuries, municipal governments have had no choice but to accept the expense and trouble of disposal by these traditional methods — increased to meet the growing need.

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A WATER RESOURCES WALL MAP

An interesting and useful wall map of our nation's principal surface water resources is now available. Produced by its staff of professional hydrologists and cartographers, Water Information Center, Inc. offers a wall map of the major reservoirs, dams, lakes and rivers in the United States (not including Alaska and Hawaii).

The map, which measures 25 inches by 38 inches, is done in four pleasing colors. It also has an easy-to-read list of major dams and the capacities of major reservoirs. Printed on strong high-grade paper, all the important place names stand out clearly so that there is no need for squinting or searching to find what you are looking for.

This attractive and informative map will be delivered rolled so that it may be easily framed for wall hanging without creases. Framed, it will add an impressive note to the decor of any home, office, library or classroom.

The map is moderately priced at $2.95, postage paid. Orders should be sent to Water Information Center, Inc., Water Research Building, Port Washington, L. I., N. Y.

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BOVINE MASTITIS CONTROL EXHIBIT AVAILABLE

This Division of Environmental Engineering and Food Protection exhibit describes the Public Health Service interest in bovine mastitis control and shows cardinal points of a mastitis control program in a State or community. It explains that control of bovine mastitis requires a cooperative effort by State and local regulatory agencies, the dairy processor and producer and milk laboratories and shows by means of color transparencies four ways by which effective controls help eliminate the disease. Copies of a pamphlet on the subject are distributed with the display.

The exhibit is available on loan from the Special Projects Section, Milk and Food Branch, Division of Environmental Engineering and Food Protection, Public Health Service, U. S. Department of Health, Education, and Welfare, Washington, D. C. 20201. Requests should be made several months in advance of the date desired. The Branch will pay the costs of shipping and installing at large national and regional meetings. Instructions for assembling the exhibit are attached to the inside door of the single packing crate.

Specifications: (No. EEFP-12 Bovine Mastitis Control). Free-standing exhibit, 7 feet 6 inches high, 10 feet 2 inches wide, and 36 inches deep, total weight 210 pounds including the crate. Lighting fixtures require one outlet totaling 500 watts. Outside crate measurements are approximately 30 x 91 x 86 inches.

ANNOUNCEMENT ON STORAGE AND DISTRIBUTION OF SMOKED FISH

Smoked fish from the Great Lakes area will henceforth be stored and distributed as a frozen food, according to an announcement made by the National Fisheries Institute and the Food and Drug Administration.

The Institute, representing 90 to 95 per cent of the United States fish smoking and curing production and dollar value, advised FDA Commissioner George P. Larrick of this and other steps which the industry will undertake to insure against further instances of botulism poisoning by smoked fish. The botulism toxin does not develop below freezing temperature.

The Institute's action follows FDA's recommendation that all smoked fish products from the Great Lakes area should be destroyed. The FDA's warning applied only to smoked fish, NOT to fresh, frozen, pickled or canned fish. The Institute's action does not change the situation with respect
to smoked fish already distributed, FDA emphasized.

"The Institute is to be commended for its prompt action in dealing with an emergency situation," Commissioner Larrick said. "The measures they have agreed to adopt are consistent with recommendations of the FDA's advisory committee on botulism. They will be adequate to prevent botulism while technological studies are being made to develop practices for long-range application," the Commissioner said.

Following is the full text of the Institute's letter of commitment to the Food and Drug Administration spelling out changes which will be made in the industry's practices:

"The National Fisheries Institute and its members, representing 90 to 95 per cent of the United States fish smoking and curing production and dollar value, recognize the existence of an emergency in our industry. We agree with the intent of the Food and Drug Administration advisory of October 25, 1963.

"To enable the American consumer to continue to enjoy and we the producers to continue to market smoked fish, we agree, with respect to Great Lakes fish and fish processed in plants located in the Great Lakes area to:

"1. Review and improve all sanitation and handling practices throughout the entire chain of production from and including vessels, transportation, processing plants, distribution and retailing operations to the consumer.

"2. Smoke the fish in the best possible fashion, employing better technology and utilizing more scientific methods.

"3. Freeze the product upon completion of the smoking, whether film vacuum-packed or bulk-packed and to store and distribute the product in that condition to our customers.

"4. Plainly mark all packages containing fish 'Perishable — Keep Frozen.'

"In the case of the bulk-packed product, to supply the retailer with a placard which will read 'Perishable — Keep Frozen' to be displayed with the product."

The letter was signed by Lewis Goldstein, president of the National Fisheries Institute.

SHORT COURSE FOR LABORATORY PERSONNEL

Based upon the knowledge and appreciation that a well-equipped and well-staffed laboratory is an essential tool in modern dairy plant operations and in regulatory supervision, and that new developments in technology aid in the formation of new control programs to solve newly arising problems, a laboratory conference was held at Iowa State University on November 12 and 13, 1963 under the direction of the Department of Dairy and Food Industry. The program included demonstrations and discussions of various analyses and techniques of current importance and interest in the dairy industry, including dye-binding methods for determining protein in milk, the Gerber test for milk-fat determination, fat acidity and rancidity in milk, lactometric and cryoscopic detection of added water in milk, tests for mastitis, microbiological evaluation of dairy products using preliminary incubation, enumeration and significance of psychrophilic and thermoduric microorganisms in dairy products, and certification of laboratories for microbiological and chemical analyses.

Iowa State University staff members participating in the program were: Emerson W. Bird, Warren S. Clark, Jr., William S. La Grange, Verner H. Nielsen and George W. Reinbold of the Department of Dairy and Food Industry and John B. Herrick and R. Allen Packer of the College of Veterinary Medicine. Also participating in the program were Mrs. H. Fellund and Mr. J. Schoop of the State Chemical Laboratory, Iowa Department of Agriculture.

The conference was attended by 50 persons representing management and laboratory factions in Iowa and surrounding states.

INSTITUTE OF SANITATION MANAGEMENT ANNUAL CONFERENCES

The Institute of Sanitation Management completed its most successful annual Conference and Show in Cleveland, Ohio, October 13-16. The reported registration of some 740 was a high mark for the Institute's Sanitation Maintenance Conference and Show, of which the Cleveland meeting was the 8th.

The Conference and Show had an international flavor this year, as well as attracting registrants from some 38 states: One of the featured speakers was Mr. James Bailey, Hygiene Officer for British Overseas Airways Corp., London, and one of the biggest single groups to go through the Conference and Show was the group of 30 men and women from the German Association of Building Cleaners, a group of businessmen and women from various cities in West Germany.

Underscoring the growing international movement in sanitation management, the Institute entered into a cooperative agreement with the British Institute of Cleaning Science during its annual meeting. The two groups will exchange all technical information, publications, and official proceedings of their respective associations. The British Institute is comparable to ISM in Great Britain, and is now engaged in establishing chapters throughout the United Kingdom.
The Institute presented a broad program ranging from the basic theories and principles of sanitation management to day-to-day techniques and scheduling of labor and use of materials. As in the past three years, one of the outstanding features was the "Cracker Barrel" Sessions — informal meetings held in the evenings where every registrant has the opportunity to ask his questions or have his say. These were probably the most crowded of the ISM Conference sessions, the Institute estimated.

Another innovation was the Workshop Sessions held on the final morning of the meeting. Here again, the emphasis was on the informal, shirt-sleeve atmosphere, with everyone given an opportunity to talk in across-the-table discussions.

The Institute also announced that for the first time, it will publish a "Highlights" of the 1963 Conference, which will include the major papers delivered there, covering all the fields represented by the Institute. The price for the Proceedings will be $3.00 for ISM members, $5.00 for non-members. The Highlights will be published the first week of January, 1964.

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**PORTLAND, OREGON TO HOST 1964 FOOD SERVICE EXECUTIVES CONVENTION**

International President Lawrence B. Wong of the Food Service Executives Association has announced that the FSEA's 1964 convention will be held in Portland, Oregon, August 9-12. Portland's Memorial Coliseum will be the headquarters for the exposition and scene of all major meetings and special educational events which will continue the FSEA's theme of "Accent on Education".

Official hotel headquarters for the FSEA's big annual event will be the beautiful new Portland Hilton. Following the new policy begun in 1963, the Portland convention will be open to all management level personnel in the food service industry. Other industry associations are also being invited to participate in the professional program. Educational and commercial exhibits are expected to emphasize "Youth and Progress in the Food Service Industry". The culinary arts show is expected to draw many outstanding entrants from the U. S. and Canada and will feature, for the first time in a National exposition, entries from the "Junior Stewards" division of the FSEA.

Special activities will include special programs for the ladies and children and exciting "Northwest" events during this convention which is expected to draw the largest membership attendance in the FSEA's 63-year history.

General Chairman of the 1964 convention will be FSEA 1st Vice President Pete Jones of Portland's Rose Manor Inn and The Hilltop House restaurants. He will be assisted by Portland FSEA Branch President Eddie Mays and other prominent area food service executives, and by FSEA Executive Vice President Ray S. Ewing of the organization's International Headquarters in Washington, D. C. Mr. Frank Masters of Trade Associates, Incorporated, will again coordinate exposition space sales and show management.

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**A HEADQUARTERS FOR BOOKS ABOUT WATER**

To provide a means of procuring books and reports relating to water not readily available in regular book outlets, Water Information Center, Inc. has established a book division, WATER INFORMATION LIBRARY.

Water Information Center, Inc., publishes WATER NEWSLETTER and the new and unusual WATER ATLAS OF THE UNITED STATES, a geography of water resources.

The library keeps on hand in its own warehouse, important publications in the water field selected by the technical staff of Water Information Center, Inc. from the viewpoint of maximum usefulness and interest to everyone concerned with water.

Brochures listing the selected books are prepared periodically for distribution to individuals, public and private institutions, government agencies, libraries, colleges and industrial corporations.

Free copies of the latest list are available from Water Information Center, Inc., Water Research Building, Port Washington, L. I., N. Y.

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**MAJOR MOVE IS PLANNED BY BABSON BROS. CO.**

Thomas W. Merritt, Sr., President of Babson Bros. Co., has announced that the company, builder of Surge dairy farm equipment, is erecting a large general office building in Oakbrook, Illinois, a suburb west of Chicago. The manufacturing and shipping facilities will remain at the present location in Chicago.

A national leader in the sale of milking machines, Babson Bros. Co. products have international distribution. In addition, the firm is the only one in Illinois which builds milking machines and is the only major milking machine company in the United States that devotes all of its facilities to the business of milking cows.

The new building will be curtain-wall construction with gray glass and gray spandrel panels. Vivid contrast to the subdued basic color is provided by white trim and a colonnade of white columns around three sides of the building.
Completely air-conditioned, the two-story edifice will contain not only offices but also experimental facilities, a kitchen and a cafeteria for employees. Design is for overall comfort as well as efficiency. The architect is Shaw Metz & Associates. Completion of the building is scheduled for fall, 1964.

The new building will play a vital role in Babson Bros. Co.’s long range planning. The firm was founded in 1906 and started in the dairy farm equipment field in 1913.

Decision to build in suburban Oakwood was made partly because the location provides plenty of room for expansion. Also, it is an ideal location from a transportation standpoint and affords sufficient parking space.

Oakbrook is a 20-minute drive west of Chicago’s business district on the East-West Tollway. It is a carefully planned complex of choice industrial-commercial sites, a large shopping center, a major motel, an airstrip and an exclusive residential section. The entire development is situated in wooded, rolling hillside.

DIVISION OF INDIAN SANITARIANS HOLD FOOD SERVICE TRAINING COURSE

Three environmental health officers of the Division of Indian Health, U. S. Public Health Service, conducted a two-day food service training course December 3 and 4 at the Gallup, New Mexico, Public Health Service Indian Hospital.

Some 40 people, employees from Bureau of Indian Affairs’ schools and dormitories, and from PHS Indian hospitals attended the course.

Instructors were Vernon Bergman, Chief Sanitarian, Albuquerque Indian Health Field Office; J. W. Stacy, Chief Sanitarian, Window Rock Indian Health Field Office, and John G. Tood, Field Sanitarian, Winslow, Arizona.

Arrangements for the training were made by the sanitarians in cooperation with the Gallup hospital staff, Rebecca Roseberry, Dietary Consultant, Division of Indian Health Albuquerque Area Office, and Wilbur Sperry, Food Service Officer, Bureau of Indian Affairs.

LIQUID CARBON DIOXIDE REFRIGERATION TESTED BY USDA

A test to evaluate the use of liquid carbon dioxide (CO₂) for refrigeration and to determine its suitability for use in the transportation of frozen foods has been made by the U. S. Department of Agriculture.

The test by researchers of USDA’s Agricultural Marketing Service was conducted with two trailers—one with CO₂ refrigeration, the other with mechanical refrigeration.

Each trailer was loaded with 26,000 pounds of frozen meat and tested on a “piggyback” (trailer-on-flatcar) run between Colorado and Pennsylvania.

Both trailers maintained about the same average commodity temperature, with the CO₂ refrigerated trailer generally at 3°F and the mechanically refrigerated trailer at 1°F.

However, the test indicated that the CO₂ refrigeration system presently has disadvantages related to weight, cost, necessity to recharge, availability of carbon dioxide, and the inability of loading crews to enter the trailer until CO₂ gas has been vented from the trailer.


HOW TO IDENTIFY EDIBLE MUSHROOM

The safest way to identify a mushroom for eating is to use a microscope.

The next best thing is to buy “The Mushroom Hunter’s Field Guide” by Alexander H. Smith, published by The University of Michigan Press.

First published in 1958, “The Mushroom Hunter’s Field Guide” established itself as the foremost handbook on the hunting and collecting of wild mushrooms. It earned a reputation for being both authoritative and practical.

A revised and enlarged edition (324 pp., $6.95), enriched by 89 new color plates plus 243 black and white photographs, has just been published.

The handsome, pocket-shaped book has been enlarged to include descriptions of 188 species. It tells when, where, and how to find edible mushrooms. It tells how to avoid poisonous ones.

People who appreciate the mushroom as a choice food, a natural delicacy of the field, will find the book indispensable.

The author is a professor of botany at Michigan and director of the University Herbarium. He writes: “For years I refused to write a field guide to the common edible and poisonous mushrooms, because field characteristics alone are not sufficient for accurate recognition of our native species. This is still true, but another factor has caused me to change my attitude.

“During the course of more than 20 years as a student of mushrooms, I have realized that many people persist in collecting fleshy fungi for food who
do not or will not follow the procedures leading to a scientifically accurate identification of the plants. In conversation such people almost invariably ask me if there is any book that will serve as a field guide to mushrooms."

While no field guide will enable a layman to make scientifically accurate identifications of all species, a well-devised work can protect against some serious mistakes, Smith explains. His book has this purpose.

"The usual scientific procedures, those involving laboratory work or serious home study are not required," he points out. "We are concerned here with the mushrooms most easily identified by their pictures. It follows, therefore, that the illustrations are the backbone of this book. Formal descriptions of the species are omitted, and in place of them the important identification marks are given."

The book illustrates most of the truly fine edible mushrooms, whether common or not. The really dangerous species are included in order that the mushroom hunter may learn about them for his own protection, and so are some marginal species which may agree with some people but not with others.

The author warns that there are no practical tests which will enable the layman to distinguish "good" mushrooms from "bad" toadstools:

"The idea that there are such tests is the worst booby trap into which a collector can fall . . . The only safe approach is to know the different kinds just as you learn to distinguish between the different kinds of fruits and vegetables."

The book also includes hints on how to cook and eat the mushrooms one can safely identify.

**DAIRY REMEMBRANCE FUND ANNOUNCES STUDENT LOAN**

The Dairy Remembrance Fund Executive Committee, which met in Dallas during the autumn dairy conventions November 3-8, has announced its latest student loan, to an undergraduate dairy science student at the University of Nebraska.

From March 1 to October 31 of this year, contributions to the Dairy Remembrance Fund showed a substantial increase over the same period a year ago, according to the financial report presented by P. R. Ellsworth, Assistant Treasurer.

Two student loans were fully repaid this year. One had assisted a student working toward his Ph.D., and the other had assisted a student working toward his Master of Science degree.

The Dairy Remembrance Fund, a national organization to which donations may be made to memorialize persons or mark events, is sponsored by nearly every national, state and local dairy industry association or group in the United States. Funds are used for projects beneficial to the entire dairy industry. Its offices are at 1145 - 19th Street, N.W., Washington 36, D.C.

Those knowing of a student who might benefit from a loan should write to the Fund offices for application information. Contributions may also be sent to the office address.
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