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Editorials

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

THE ATLANTIC CITY MEETING

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

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

The members of the Committee are:

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

THE officers and members of the *International Association of Ice Cream Manufacturers* join President McKenzie in inviting the members of the INTERNATIONAL ASSOCIATION OF MILK AND FOOD SANITARIANS to attend the International Ice Cream Convention in Atlantic City on October 18, 19 and 20, 1950.

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

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

NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS

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

It was the outgrowth of a series of efforts, dating back to the 1946 Conference of State and Territorial Health Officers, to get the Public Health Service to develop a program for certification of interstate milk shippers, and this request was repeated in 1949. In December, 1949, representatives from several mid-western states met in Indianapolis to determine whether some program could be set up to meet this situation of inter-state milk shipments. As a result of the Indianapolis meeting, a conference of eleven mid-western states was held in Chicago in February 1950, where it was decided to appoint a committee to investigate the problems further and to arrange for a national conference on interstate milk shipments. This committe requested the Surgeon General of the U. S. Public Health Service to invite all the states to participate, including the milk industry.

At the St. Louis conference, Mr. J. L. Rowland set forth the objective, "The Best Possible Milk Supply for All the People". Visual aids were used to depict the machinery necessary to permit the free flow of milk from areas of production to those of consumption. Mr. A. W. Fuchs was called upon to present "the National problem and its background, review of action by the Public Health Service, and possible solutions." Members of official agencies and industry from both shipping and receiving states were called upon to present their specific problems. Messers. H Weavers and C. Luchterhand presented the Wisconsin plan for area supervision.

Delegates were assigned to several task forces whose reports follow. The Executive Committee was requested to develop plans for the institution of a permanent conference on interstate milk shipments, to be held again next year on June 4, 5, and 6. 1951, at the Hotel Statler, St. Louis, Mo.

Regulations. The 1939 edition of the U. S. Public Health Service *Milk Ordinance and Code* will be used as the basic regulation, and that compliance therewith will be measured by the U.S.P.H.S. milk sanitation rating method, as outlined in the U. S. Public Health Service Bulletin, No. 1970 "Milk Sanitation Rating."

Supervision. The receiving states were urged to recognize inspection and supervision by full-time local and state health department and state agricultural department personnel. Supervision shall be measured by the enforcement rating procedure outlined in *Public Health Service Reports,* Reprint No. 1970, "Methods of Making Sanitation Ratings of Milk Sheds."

Certification. Receiving states were urged to accept ratings made only by certified rating officials of either the U. S. Public Health Service or the State Health Department. Certification should include survey ratings on producing farms, receiving stations or plants, and the supervising agency. Area ratings shall be made not less than every two years. If an individual source is in a 90 percent rating area an individual rating is not necessary. Milk plants or individual sources not under an area survey and who are in areas less than 90 percent rating, shall be surveyed annually. If a request is received for a milk source not under recognized supervision, the survey will be denied.

The Public Health Service is to standardize rating procedures of its own personnel and of state rating officials, and to issue a certificate of competence to qualified state health department survey officers.

Laboratory. Strict adherence to procedures outlined in the latest edition of *Standard Methods for the Examination of Dairy Products,* published by the American Public Health Association. Where alternate methods are permitted by standard methods that milk intended for interstate shipment be examined by plate counts or direct microscopic counts. Samples from each dairy farm be examined not less than the frequency prescribed in the *1939 Milk Ordinance and Code.* The state may accept the results from official laboratories which they have approved as complying substantially with Standard Methods and checking closely with results obtained at least twice per year on split samples. The state may accept the results from officially designated laboratories that they have similarly officially checked periodically and found to be satisfactory.

The state approval of local laboratories should include an annual visit to the laboratory at which time evaluation of the quarters, equipment, procedures, results, and records will be made on appropriate survey forms of the U. S. Public Health Service or the equivalent. To insure uniformity, the U.S.P.H.S. is to spot check the laboratories of the state agencies participating in the certification of milk for interstate shipment and to certify their complaince with standard methods.

Channel for Requesting and Reporting Information. An individual in the receiving states desiring information on a milk supply should make the request to the state control official in his own state who will transmit the request to the Regional Office of the Public Health Service, to the state health department in the shipping state. The state health officer of the shipping state shall report the results of the survey to the regional officer of the Public Health Service, to the state official of the receiving state who will immediately notify the local health officer and/or the individual requesting the survey. Industry in a shipping state desiring a survey should likewise make the request to the regulatory official in his own state.

To expedite the requesting and reporting process, for the immediate future, requests and reports can be sent direct from one state agency to another state agency with carbon copies of all requests and reports being sent to the Regional Office of the Public Health Service. The latter shall consider the certified list of milk shippers and circularize all state agencies monthly who in turn are urged to advise their local health officers and/or industry.

Role of the Public Health Service. The state regulatory authorities should carry the work load involved in the interstate milk program, with the assistance of the U. S. Public Health Service. The latter shall be prepared to extend to state regulatory authorities and educational institutions such assistance in the training of field representatives of the state and local governmental units or of industry, field and plant personnel, and state survey officers as the respective state may require in operating the interstate milk shipment plan. The Public Health Service should also train or assist in training laboratory personnel of state, local, or industrial laboratories as requested by state authorities. The Public Health Service should act as a clearing house for the receipt and dissemination of information as indicated in the letter from the Surgeon General, dated December 31, 1946.

The P.H.S. should spot check the inspection and survey work of enforcement agencies to determine whether milk regulations are being correctly interpreted and enforced.

It should furnish state regulatory agencies periodically with interpretations and regulations based on questions submitted by such agencies and also that state authorities relay such interpretations to local enforcement agencies and/or industry.

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

Getting into Operation.

Receiving States:

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

Producing States:

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

Receiving and Producing States:

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

U. S. Public Health Service:

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

Thirty-seventh Annual Meeting
ATLANTIC CITY, N. J., OCT. 13–16, 1950
Hotel Dennis

BOTTLE-WASHING STUDIES UNDER PLANT CONDITIONS

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Little profits a milk dealer to enter an involved quality control program, pasteurize milk in the best of equipment, and handle it with extreme care if the milk is placed in containers which are not essentially sterile. On the other hand, any operator with an eye to economy will not want to use in his bottle washer caustic solutions which are stronger than necessary nor will he want to burden his washer with temperatures higher than are necessary to do an adequate job. For these reasons, it was believed that a thorough investigation should be made of the relationship between temperature, time of exposure, and caustic concentration in a soaker-type milk bottle washer.

The recommendations for caustic-temperature relationships which are in general use in the dairy industry throughout the United States are based on the work of Levine and co-workers [1, 2, 3, 4, 5] who investigated the washing of carbonated beverage bottles. Some laboratory tests were made on milk bottles and it is known that cleaning and "sterilizing" the milk bottle presents a radically different problem from the carbonated beverage bottle. The beverage bottle will contain residues of sugar, acids, and perhaps some extraneous matter; the milk bottle contains, in addition to these materials, a complex film of fat, protein, and minerals. This complex film provides a medium which is ideal for the growth of bacteria. The elimination of these bacteria from the bottle, together with the milk solids, presents the practical and important problem for the dairy plant. It is a problem comparable in importance with the pasteurization process itself. The plant operator must produce a bottle which is clean and spotless in order to hold the consumer's confidence, and which has a bacterial count low enough to be safe and to meet public health standards.

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

The Association of Bottlers of Carbonated Beverages has recommendations, based on the work of Levine and associates,[3] stipulating that "Unclean bottles shall be exposed to a 3 percent alkali of which not less than 60 percent is sodium hydroxide for a period of not less than 5 minutes at a temperature of not less than 130° F. or an equivalent cleansing and sterilizing process." It has not been proved that

the standards for cleaning and "sterilizing" beverage bottles are necessarily desirable or correct for milk bottles.

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

EXPERIMENTAL METHODS

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

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

TABLE 1

TEMPERATURE-CAUSTICITY RELATIONSHIPS STUDIED USING A 3-MINUTE SOAKING PERIOD

Temperature ° F.	*Causticity* %
120	4.2
130	2.9
140	1.9
150	1.3
160	0.9
170	0.9
180	0.9

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

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

For each given temperature, the tests usually were divided into three 5-day periods, in a few cases the periods were 10 days. During the first period the causticity of the solution was adjusted to the desired level by the addition of 76 per cent flake caustic (sodium hydroxide). During the second period an amount of trisodium phosphate equal to one-tenth of the required weight of the sodium hydroxide was added to the base solution. Finally during the third period tetrasodium pyrophosphate was added in the same proportion as the trisodium phosphate, while maintaining as a base the same solution that was used during the second period. A 32-pound sodium hydroxide charge was required to produce a solution having 1 percent caustic alkalinity in this machine. The indicated concentrations of these alkalis were maintained by several additions to the soak tank during the day's run. The required amount of sodium hydroxide was added and other materials, when in use, were added in correct proportion.

Causticity. When making causticity determinations, 5 ml. of the soak-tank solution were transferred with a pipette to a 100-ml. volumetric flask and made to volume; a 25-ml. portion of the diluted solution was transferred to a beaker and, if only sodium hydroxide was used in the soak solution, the aliquot was titrated directly with standard N/10 hydrochloric acid to the phenolphthalein and methyl orange end points to obtain causticity and carbonate alkalinity; if one or both of the phosphates were present in the basic sodium hydroxide solution, the carbonates and phosphates were precipitated by the addition of barium chloride and the solution was titrated to the phenolphthalein end point with N/10 hydrochloric acid.

Bacteria. To determine the bacterial count of the bottles, daily samples were selected. Three half-pint bottles were collected near the beginning, three near the middle, and three near the end of the run. As soon as the bottles were discharged by the washer, they were closed with sterile bottle caps which covered the pouring lip. These bottles were stored in a cooler at 40° F. until bacterial counts were made on them later in the day. The counts on these bottles were made according to standard procedures except that instead of using 100 ml. of sterile water, only 10 ml were used for the rinsing operation because of the small numbers of bacteria present. One ml. samples of this wash water were plated in duplicate on standard skim-milk agar and the plates were incubated at 35° C. On occasions, 5-ml. amounts of rinse water were plated as a further check on the low bacterial counts being obtained.

Visual cleanliness. Twelve quart bottles, collected at random daily, were used for making observations on visual cleanliness.

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

Surface tension. The surface tension of the solutions was determined with the same frequency as the specific gravities. Cenco-du Nouy precision tensiometer No. 10402, having a 4-cm. platinum ring, was used. The apparatus was properly calibrated and leveled; the platinum ring was washed with distilled water and dried with an alcohol flame before each determination. For surface tension measurements the temperature of the solution was adjusted to 20° C. in a water bath.

RESULTS

Sodium hydroxide soak solution. In table 2, recording the findings on tests made for 38 days using only sodium hydroxide in the soak tank, certain observations appear to be of interest. More than 55 percent of the bottles tested failed to show any colonies of bacteria. The average colony count per bottle never exceeded 15. The highest colony count of bacteria for any one bottle was 170; only once did the bacterial colony count exceed 100. Since the accepted standard requires less than 238 colonies per half-pint bottle,[7] all of the alkali concentrations and temperatures tested gave satisfactory bacterial counts.

Due to the known resistance of bacterial endospores, many Grampositive cocci, and acid-fast organisms to strong

TABLE 2

THE BACTERIAL CONTENT OF HALF-PINT MILK BOTTLES SOAKED FOR 3.25 MINUTES IN SODIUM HYDROXIDE SOLUTION

Caustic soda %	*Soak temp.* ° F.	*No. of days run*	*No. bottles tested*	*Bottles showing no colonies* %	*Av. no. of colonies, any bottle*	*Highest no. of colonies, any bottle*
4.2	120	11	99	40	4	100
2.9	130	5	45	58	4	50
1.9	140	5	45	44	15	40
1.3	150	5	45	58	6	20
0.9	160	7	63	65	4	50
0.9	170	5	45	60	7	170

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

Sodium hydroxide and trisodium phosphate soak solution. If the addition of trisodium phosphate to the sodium hydroxide solution in the soaker tank of a milk bottle washing machine is an advantage, these tests should indicate it, since the trisodium phosphate was added to the same concentration of sodium hydroxide as used in the previously reported 42 tests. It is true that the amounts of trisodium phosphate added were small but they closely approximate the quantities commonly incorporated in bottle washing mixtures sold to the dairy trade.

In table 3, reporting the results of 29 days tests using sodium hydroxide and trisodium phosphate in the soaker tank, 32 percent of the bottles tested showed no colonies of bacteria by the

TABLE 3

THE BACTERIAL CONTENT OF HALF-PINT MILK BOTTLES SOAKED FOR 3.25 MINUTES IN A SOLUTION OF SODIUM HYDROXIDE AND TRISODIUM PHOSPHATE

Caustic soda + %	*Trisodium phosphate* %	*Soak temp.* ° F.	*No. of days run*	*Bottles tested* No.	*Bottles showing no colonies* %	*Average no. of colonies, any bottle*	*Highest no. of colonies, any bottle*
4.2	0.42	120	6	54	36	23	350
2.9	0.29	130	5	45	49	7	65
1.9	0.19	140	5	45	44	9	185
1.3	0.13	150	5	45	42	9	100
0.9	0.09	160	4	36	11	23	160
0.9	0.09	170	4	36	3	41	210

tests employed. Only one bottle exceeded the permissible standard limit, having a count of 350. The bacterial counts were more variable and some were higher. The results indicate that the addition of these amounts of trisodium phosphate adds nothing to the bottle-cleaning properties of the soak solution and may be detrimental.

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

TABLE 4

THE BACTERIAL CONTENT OF HALF-PINT MILK BOTTLES SOAKED FOR 3.25 MINUTES IN A SOLUTION OF SODIUM HYDROXIDE, TRISODIUM PHOSPHATE, AND TETRASODIUM PYROPHOSPHATE

Caustic soda + %	*Trisodium phosphate* %	+ *Tetrasodium pyrophosphate* %	*Soak temp.* ° F.	*No. of days run*	*Bottles tested*	*Bottles showing no colonies* %	*Average no. of colonies, any bottle*	*Highest no. of colonies, any bottle*
4.2	0.42	0.42	120	4	36	31	19	125
2.9	0.29	0.29	130	4	36	56	4	60
1.9	0.19	0.19	140	3	27	56	4	5
1.3	0.13	0.13	150	3	27	52	7	25
0.9	0.09	0.09	160	6	54	33	7	30
0.9	0.09	0.09	170	5	45	47	7	75

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

Several runs were made using only sodium hydroxide and tetrasodium pyrophosphate in the soak tank. The results obtained were as good or better than those in which all three alkalies were present, indicating that trisodium phosphate adds little or nothing to the cleaning qualities of the soak solution.

Physical cleanliness of bottles. In Table 5 it can be seen that tetrasodium pyrophosphate in the soaker tank was necessary to obtain properly rinsed bottles. This condition is the ideal desired, since the bacteriological findings show these glistening, clean bottles to contain only small numbers of bacteria. Rinsing was never satisfactory without tetrasodium pyrophosphate, although in other solutions the bottles did rinse somewhat better at the higher temperatures.

The surface tension readings were all low and varied only slightly due to different alkalinities, and the chemical composition of the soaker tank solutions. It was obvious that surface tension readings on the soaker tank solution do not measure, to even a slight degree of accuracy, the efficiency of the bottle washing process. A low

TABLE 5

OBSERVATIONS ON BOTTLE CLEANLINESS AS RELATED TO TEMPERATURE AND PERCENT OF CAUSTICITY

Added chemicals	*120° F.—4.2%*	*130° F.—2.9%*	*140° F.—1.9%*	*150° F.—1.3%*	*160° F. or higher—0.9%*
NaOH	Poor rinsing—water spots Blue-white film on bottom		Water spots	Slight cloudiness	
10 parts NaOH 1 part Na_3PO_4	Cloudy, white streaks Water spots, slippery when wet		Gradually become blue white		Few white spots Slight blue-white film
10 parts NaOH 1 part Na_3PO_4 1 part $Na_4P_2O_7$	Satisfactory Clean		Satisfactory Clean		Satisfactory Clean

surface tension is generally held to improve the penetration of the washing solution and to give better rinsing. These readings did not, in any degree, correlate with the rinsing results observed.

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

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

CONCLUSIONS

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

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

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

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

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

(Continued on page 205)

RELATIONSHIP OF COMPOSITION OF MILK TO METHODS USED FOR DETERMINING ADULTERATION

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Low solids milk has for years been a problem on many New England dairy farms. In addition to being required to accept Class II or surplus prices for all of their milk because of the low solids content, there have been instances where dairymen have been unjustly accused of adulterating their milk. Special emphasis on a study of the problem resulted from the senior author being consulted in connection with a court case involving accusation of milk adulteration by watering. An investigation of the involved herd showed that the cows were producing milk abnormally low in solids-not-fat for the fat content.

A review of the literature produced very little information on the tests commonly used for detecting added water in milk having an abnormal relationship of fats to solids-not-fat. A recent study made by the Dairy Department of the New Hampshire Agricultural Experiment Station has shown that many cows and herds do produce milk having an abnormal relationship of fat to solids-not-fat.

EXPERIMENTAL METHODS

The samples were collected over a period of nearly a year. For the most part samples used were from the cows in the University dairy herd on whose milk, data on fat and solids-not-fat had been accumulated over a period of several years. Herd samples were made by mixing from these individual cows. Any milk samples sent in for testing during the period of this study were included.

The samples were analyzed for percentage butterfat and total solids by the Mojonnier method. The percentage butterfat was determined also by the Babcock method. The refractive index of the acetic serum, sour serum, and copper serum, and the determination of the serum ash and the freezing point depression were determined by the methods outlined in *Standard Methods for the Examination of Dairy Products,* published by the American Public Health Association.

Table 1 summarizes in the form of group averages the results obtained on the 130 samples analyzed in this study.

TABLE 1

AVERAGE OF THE PERCENTAGE BUTTERFAT, SOLIDS-NOT-FAT, TOTAL SOLIDS; THE REFRACTOMETER READING OF ACETIC AND COPPER SERUM, AND THE PERCENT ASH SERUM

Number Samples	*Fat Babcock*	*Fat Mojonnier*	*S.N.F.*	*T.S.*	*Refractometer Reading: Acetic Serum*	*Refractometer Reading: Copper Serum*	*Refractometer Reading: Percent Ash Serum*
	Percent	Percent	Percent	Percent			
130.....	3.55	3.58	8.44	12.02	41.2	37.5	0.768
10[1].....	2.88	2.90	7.66	10.56	38.9	35.7	0.793
12[2].....	3.32	3.35	7.86	11.21	40.6	37.6	0.771
14[3].....	3.39	3.41	8.26	11.67	40.5	36.5	0.771
2[4].....	3.50	3.57	8.31	11.88	40.7	38.0	9.699
92[5].....	3.68	3.71	8.63	12.34	41.7	37.9	0.766

[1] low copper serum; [2] other samples having solids-not-fat of less than 8 percent not having low copper reading; [3] copper serum in 36's; [4] low ash serum; [5] all other samples.

Scientific Contribution #130 of the New Hampshire Agricultural Experiment Station.

TABLE 2

COMPOSITION OF THE TEN SAMPLES SHOWING COPPER SERUM READINGS BELOW 36

Sample Number	Fat Babcock	Fat Mojonnier	S.N.F.	T.S.	Refractometer Reading: Acetic Serum	Refractometer Reading: Copper Serum	Refractometer Reading: Percent Ash Serum
	Percent	Percent	Percent	Percent			
117.....	2.0	2.007	8.373	10.38	38.6	35.6	0.768
32.....	2.4	2.411	7.309	9.71	38.7	35.9	0.750
41.....	2.55	2.550	7.490	10.04	39.9	35.4	0.827
81.....	2.6	2.567	7.353	9.92	38.3	35.4	0.816
56.....	2.9	2.898	7.452	10.35	38.0	35.4	0.815
44.....	3.1	3.182	7.768	10.95	38.8	35.6	0.800
77.....	3.1	3.254	7.796	11.05	39.3	35.9	0.782
85.....	3.2	3.210	7.770	10.98	39.6	35.9	0.797
38.....	3.4	3.370	7.790	11.16	39.0	35.9	0.750
79.....	3.55	3.573	7.637	11.21	38.3	35.9	0.736

The freezing point is not included in this summary table as all samples showed a normal freezing point depression, indicating that none of the samples were adulterated with water. Ten of the samples had refractometer readings indicating added water. Acetic serum reading below 39 and copper serum reading below 36 indicated added water, according to Standard Methods. These 10 samples averaged 38.9 for the acetic serum reading and 35.7 for the copper serum reading. Acetic serum ash averaged 0.793 which did not indicate added water. The average solids-not-fat of these 10 samples was 7.66 percent, while another group of 12 samples with an average solids-not-fat of 7.86 percent did not indicate added water according to the refractometer readings.

Table 2 shows the detailed analyses of the 10 samples having copper serum readings below 36. The 3 samples having the lowest copper serum readings all showed an ash serum content of more than 0.8 percent which very likely was the reason why these samples did not give a freezing point depression indicating added water. Samples No. 117 and No. 79 were decidedly abnormal as to their relationship of fat to solids-not-fat. The experience of this Station is that individual cows and herds do produce milk that has an abnormal relationship of fat to solids-not-fat.

In Table 3 the samples were grouped according to per cent butterfat. The mean, range, and standard deviation are shown for the copper and acetic serum readings. A trend in increases of refractometer readings with increases in butterfat content of the milk will be noted.

TABLE 3

COPPER AND ACETIC SERUM READINGS OF SAMPLES GROUPED ACCORDING TO PERCENTAGE BUTTERFAT

Butterfat Groups	Number of Samples	Copper Serum Readings: Mean	Copper Serum Readings: Range	Copper Serum Readings: Standard Deviation	Acetic Serum Readings: Mean	Acetic Serum Readings: Range	Acetic Serum Readings: Standard Deviation
–3.0	7	36.11	38.8–35.4	1.238	39.19	41.2–38.0	1.119
3.0–3.49	57	37.37	38.8–35.6	0.738	40.94	43.3–38.8	1.067
3.5–3.99	41	37.82	40.7–35.9	0.976	41.32	43.3–38.3	0.975
4.0+	25	37.76	38.8–36.7	0.592	42.18	43.3–40.7	0.871
All	130	37.52	40.7–35.4	0.910	41.20	43.3–38.0	1.191

TABLE 4

COPPER SERUM READINGS OF SAMPLES GROUPED ACCORDING TO PERCENTAGE SOLIDS-NOT-FAT

Solids-Not-Fat Groups	Number of Samples	Mean	Range	Standard Deviation
–8.00	25	36.72	38.8–35.4	0.960
8.00–8.49	42	37.57	38.8–35.6	0.737
8.50–8.99	46	37.75	40.7–36.4	0.897
9.00–	17	37.95	38.8–37.0	0.560
All	130	37.52	40.7–35.4	0.910

In Table 4 the samples were grouped according to percent solids-not-fat and the mean, range, and standard deviation are shown for the copper serum readings. While the copper serum readings increased slightly with the increases in solids-not-fat, this was not so noticeable as was the case of increases in the butterfat content, especially with samples containing more than 8 percent solids-not-fat. A statistical study comparing the probable error of the groups showed the results to be highly significant. This was especially true when comparing the low butterfat and solids-not-fat group with the next higher grouping, indicating that low copper and acetic serum results of these samples were normal for their composition.

SUMMARY

An investigation was made of the refractive indices of the acetic and copper sera and the ash of the acetic serum of 130 samples of milk. Some of the samples were chosen because of known low composition, especially abnormally low solids-not-fat content. All samples showed a normal freezing point depression, indicating that none of the samples were adulterated with water. Of all the samples analyzed, 38 had a low solids-not-fat content, and these either had refractometer readings that indicated added water or the readings were in the low range.

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

Bottle-Washing Studies

(Continued from page 201)

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

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

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IT IS unfortunate that milk, which contains so many of the essential constituents of the human diet, loses so much of its original reduced ascorbic acid during processing, distribution, and storage. The reduced ascorbic acid content of freshly drawn milk ranges from 20 to 25 mg. of ascorbic acid per liter, but a considerable portion of this is lost during the pasteurization process, during the vicissitudes of distribution, and during storage in the home. Holmes, Tripp, Woelffer, and Satterfield[7] and Holmes, Jones, Wertz, and Kuzmeski[8] reported a loss of over 18 percent of ascorbic acid during pasteurization of milk in the dark at 143° F for thirty minutes, and Woesner, Weckel, and Schuette,[16] Elvehjem,[3] and Mawson and Kon[14] observed a 20 percent loss of ascorbic acid.

A very considerable destruction of reduced ascorbic acid may occur during the commercial distribution of milk, particularly if the milk is allowed to stand for any length of time on the consumer's doorstep unprotected against sunshine or even bright light. Diemair and Fresenius,[2] Buruiana,[1] Hand, Guthrie, and Sharp[5] and others have discussed the effect of sunshine upon the stability of reduced ascorbic acid. Kraus[13] found that pasteurized milk exposed in a colorless bottle at room temperature lost its vitamin C in six hours, and Kon and Watson[12] reported that milk kept in June skyshine for an hour lost all of its reduced ascorbic acid.

Holmes and Jones[9] exposed milk in commercial flint milk bottles to the action of light of varying intensity and found that exposure for only thirty minutes on a rainy day destroyed all the reduced ascorbic acid in the milk.

Even if the pasteurizing and distribution conditions have been ideal and the milk contains a relatively large amount of reduced ascorbic acid when it reaches the consumer, there may be a serious loss of ascorbic acid while the milk is stored in the home refrigerator. Hand[6] studied the loss under such conditions and found that during six day storage at 1° C, the reduced ascorbic acid of commercial milk dropped from 19 mg. to 7 mg. per liter—a loss of over 60 percent. Gunsalus and Hand[4] observed a larger loss, namely, a drop of from 14.9 to 1.7 mg. of reduced ascorbic acid per liter, or an 80 percent loss during six day storage. Contrasted to these large losses, Holmes and Jones[10] found that mare milk stored in darkness at 10° C for six days lost only about 8 percent of its reduced ascorbic acid. In considering the large loss of reduced ascorbic acid from cow milk during processing, distributing, and storage, and the pronounced difference in the amount and rate of loss of reduced ascorbic acid from cow and mare milk, one notes that the ratio of riboflavin to ascorbic acid is radically different in the milk of the two species of animals. Accordingly, this study was undertaken to investigate whether added riboflavin would have any effect upon the permanency of ascorbic acid in raw cow milk.

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EXPERIMENTAL

The milk for this study was obtained weekly from the University dairy department. It was produced by the University herd of seventy cows of different ages and stages of pregnancy and lactation. The milk from the evening and morning milkings was thoroughly mixed in a large stainless steel vat preparatory to pasteurization. An aliquot of the raw milk was withdrawn and taken directly to the laboratory where it was divided into three identical portions. One portion served as a control. U.S.P. grade riboflavin was added to the other two portions at the rate of 4 mg. and 8 mg. per liter. Similar samples were prepared on seventeen Monday mornings between the middle of September and the first of March. They were assayed at once and at 24-hour intervals during the 5-day period from Monday to Friday inclusive. During the 96-hour experimental period, the samples were stored in a home-type electric refrigerator in darkness at 10° C. The length of the storage period doubtless exceeded the period that fluid milk ordinarily is stored in the home, but it was adopted to provide sufficient data for judging the rate of destruction of reduced ascorbic acid in riboflavin-fortified milk during early storage.

Reduced ascorbic acid was determined by the Sharp[15] method, which was modified by using 25 ml. of milk instead of 10 ml. and by using a mixture of 3 percent metaphosphoric acid and 8 percent acetic acid instead of sulfuric acid.

RESULTS AND DISCUSSION

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

TABLE 1

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

Sample	Ascorbic Acid mg./l				
	Monday	*Tuesday*	*Wednesday*	*Thursday*	*Friday*
Raw cow milk	17.9	14.3	11.9	8.7	6.3
Raw cow milk + 4 mg. riboflavin per liter	17.9	14.6	11.7	8.7	6.2
Raw cow milk + 8 mg. riboflavin per liter	17.9	14.6	11.9	8.6	6.0

8 mg. per liter, were essentially identical.

Holmes [11] obtained similar results in a study of the addition of riboflavin to pasteurized cow milk; namely, the addition of riboflavin to pasteurized milk did not increase the amount or the rate of destruction of reduced ascorbic acid. In fact, the total losses during storage of the pasteurized milk and the pasteurized milk fortified with 4 mg. and with 8 mg. of riboflavin per liter were 77 percent, 73 percent, and 69 percent respectively.

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

SUMMARY

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

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

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INTRODUCTION

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

Wildier after reading Pasteur's memoirs on alcoholic fermentation, began experiments of his own on the nutrition of yeasts. He concentrated a substance which he called "bios," necessary for the growth of yeast, and postulated that Pasteur's inoculum had been comparatively large and sufficient "bios" had been included in the inoculum to bring about growth and fermentation in an otherwise "bios"-deficient medium. Some of the active principles of Wildier's bios have been isolated and identified as biotin, 1-inositol and pantothenic acid.

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

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

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

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

Many vitamins have been shown to be essential portions of enzyme systems. Along with the recent discovery of the multitude of enzymatic processes, both degradative and synthetic, in the metabolism of all organisms has come a recognition of the important role which vitamins play in these enzyme systems.

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Certain amino acids are also essential to the nutrition of both microorganisms and animals. Just as in the case of the vitamins, the essential amino acids can be assayed, with great accuracy, by their effect on the growth of microorganisms in appropriately deficient media. The essential nature of certain amino acids in human and animal nutrition, as well as the desirability of supplying products rich in these essential amino acids, makes methods for their assay highly significant.

Microbiological Assays for Vitamins

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

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

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

Thiamine

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

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

Sarett and Cheldelin (1944) reported a method for B_1 determination based on the growth of *Lactobacillus fermentum*. The growth response is measured turbidimetrically. Good recovery has been reported by this assay method.

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

Riboflavin

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

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

Nicotinic Acid

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

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

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

Pyridoxine

Melnick, Hochberg, Himes and Oser (1945) reported that the microbiological assays of the B_6 content of materials with *Saccharomyces cerevisiae* underestimate the B_6 content because biologically active pyridoxine derivatives are less stimulatory for this organism. These compounds however show comparable activity for *Saccharomyces carlsbergensis* and for the rat (Hopkins and Pennington, 1947).

The method of hydrolysis in the estimation of the vitamin in natural materials is of importance as well as the choice of the organism. Oser (1949) reported that 0.055 N H_2SO_4 was more effective in extracting total B_6 than 2 N H_2SO_4. This has been confirmed by other workers, and autoclaving at 20 pound steam pressure for 5 hours in 0.55 N H_2SO_4 is generally recommended. Oser also reported that the most reliable method for determining the total B_6 in natural products is the microbiological method with *Saccharomyces carlsbergensis*. In pure pharmaceutical preparations in which pyridoxine is the only member of the B_6 complex present, *Saccharomyces cerevisiae* is satisfactory.

The animal assay for B_6 continues to play an important role due to this incompleteness of extraction. Pyridoxine, pyridoxal, and pyridoxamine when injected all have equal potency. When included in the diet, pyridoxine is more active (Sarma, Snell, and Elvehjem, 1946). Pyridoxine may be determined by the mold growth method. A pyridoxine-less mutant No. 299 of *Neurospora sitophilia* is employed, and the weight of mycelia, after drying, is used for calibration. Standard curves are produced by growing the mold on known levels of pyridoxine (Stokes, Gunness, Dwyer, and Coswell, 1943).

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

The determination of pantothenic acid by the method of Strong, Feeney, and Earle (1941) using *Lactobacillus casei* has been modified. Ives and Strong (1946) reported that the release of the vitamin by the use of an enzyme preparation Mylase P, and the use of *Lactobacillus arabinosus* 17–5 as

the test organism have advantages. Hoag, Sarett, and Cheldelin (1945) observed that *Lactobacillus arabinosus* was not as sensitive as *Lactobacillus casei* to the interfering effects of fats and starches. A greater response and more rapid growth of *Lactobacillus arabinosus* with the vitamin has been reported. Results may be read by turbidity in 14 hours or by acid production in 24 hours.

Biotin

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

Folic Acid

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

B_{12}

At the present time there is great interest in the development of a microbiological assay for the animal protein factor, now believed to be identical to B_{12}. Indications are that a strain of *Lactobacillus leichmanpii* will be a suitable test organism for this factor (Snell, Kitay, and McNutt, 1948).

Amino Acid Assays

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

Substantial advances have been made during the past few years in revising and improving methods of microbiological assay for amino acids (Oser, 1949). Several systems of assay are based on the use of a single organism for a number of different amino acids. For example, a system of assay has been described by Gunness, Dwyer and Stokes (1946) for nine essential amino acids using *Streptococcus faecalis* as the test organism; the tenth, phenylalanine, is determined with *Leuconostoc delbrückii.* More recently a procedure for 13 amino acids has been reported by Boyd, Logan, and Tytell (1948) based on the use of *Clostridium perfringens* which is claimed to have the advantage of not requiring aseptic conditions. Oser (1949) reported that the present tendency is towards methods utilizing more than one organism for the series of amino acids, each designated to take advantage of the greater accuracy resulting from the specific requirements of the microorganism, special adaptations of media, conditions of incubation, etc. Aside from the conditions affecting bacterial growth the principal technique difficulties are concerned with the preliminary preparation of the protein hydrolysates without the destruction or racemerization to inactive forms. The *d* form of the acid has no biological activity, the *l* form being active. The *dl* forms have 50 percent activity.

Methionine, threonine, cystine, tryosine, and tryptophan must be enzymatically hydrolyzed as these amino acids are 20–100 percent destroyed by acid hydrolysis.

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

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

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

Conclusion

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

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CDC Laboratory Courses Revised

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

An additional 1-week course in laboratory diagnosis of enteric diseases, introductory enteric bacteriology, will be given March 20–24.

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

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

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

Information and application forms should be requested from the Chief, Laboratory Services, Communicable Disease Center, Public Health Service, Chamblee, Georgia.

CLEANING AND BACTERICIDAL VALUES OF DETERGENT SANITIZERS*

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The detergent sanitizers, also known as cleaner sanitizers, consist essentially of a quaternary ammonium compound and a nonionic wetting agent, with or without various combinations of polyphosphates and alkali cleaners. Their primary purpose has been to improve dairy farm sanitation by making it easier for the dairy farmer to clean and sanitize his equipment properly. Under certain conditions these detergent sanitizers have been found to be highly effective in eliminating or inhibiting the development of thermoduric bacteria on farm equipment. Studies carried out thus far to determine effectiveness of the compounds in maintaining low bacterial counts with a minimum of effort on the dairy farm also generally have been favorable toward detergent sanitizers.[1, 3, 4, 7] One study[4] indicated the detergent sanitizer to be comparable to conventional cleaning methods with standard cleaner and hypochlorite germicides.

Since the detergent sanitizers are relatively new to the dairy industry and some of their development has been based on uncontrolled studies, a number of details of procedure have been overlooked in published directions. Some examples of these are as follows: Certain directions have failed to specify a rinse with cool or lukewarm water to remove milk solids immediately after use of equipment. There has been great variation in composition of detergent sanitizers on the market and some obviously have been unable to carry out both an effective cleaning and germicidal treatment. Some reports have indicated a pH of 10.0 in use, dilution to be necessary to prevent excessive development of *Pseudomonas* species. Some directions have specified preparation of detergent sanitizer solution in cold water. Others specify storage of teat cups wet in solution racks rather than dry. Some directions also specify no rinse just before use of equipment, others, a rinse with water, others, with detergent sanitizer or quaternaries. Most of the methods specified have failed to make any mention of a periodic disassembling for a thorough removal of accumulated milk solids or other material.

Experimental

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

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and two with a mediocre or poor quality record on each procedure. Sanitation equipment was inspected at least twice during the two to three-week preliminary period before starting on the experimental procedures and every two weeks for the remainder of the experiment. Inspections were carried out by us in order to obtain first hand information on condition. Additional data covering biweekly raw and pasteurized plate counts on most of the producers for two years prior to the experiment were provided by the plant.

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

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

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

Standard Alternate Method. (1) Immediately after milking, draw one pailful of lukewarm water (100° F.) through the unit, raising and lowering the teat cup assembly so that some air is sucked in for scrubbing effect. Rinse milker pail thoroughly with this water. Rinse all other milking utensils with lukewarm water. (2) Follow this with a second flush of the milker unit, this time drawing up the standard alkaline washing solution at 125° F. (3) Brush the inflations, milk tube, milker pail, and all other milking utensils with the washing solution. Remove head gasket and wash it thoroughly. (4) After cleaning, draw through teat cups and milk tube one pailful of hot (170° F.) water. Keep teat cups submerged while drawing through water. Rinse milker pail and cover and all other milking utensils with hot water. Place pail and cover and utensils on rack to dry. (5) Place teat cup assembly on a solution rack with the organic acid detergent. (6) Just before milking drain solution out of teat cups. Assemble machine and draw about one pailful of 200 ppm hypochlorite solution through the unit. Rinse milker pail and all other milking utensils thoroughly with this solution.

Follow the above procedure for three days. Every fourth day the organic acid detergent at 125° F. should be used instead of the standard alkaline cleaner in the washing operation. Once a week completely dismantle machine, brush all parts and tubes with the proper brushes, using the organic acid detergent at 125° F. Reassemble machine and rinse and rack in the usual manner. Thoroughly rinse all milk cans before use with one pailful of 200 ppm strength hypochlorite solution.

Standard Detergent Sanitizer Method. Steps (1), (2), and (3) same as for the Standard Alternate Method except that alkaline detergent sanitizer is used instead of the conventional standard alkaline washing compound. (4) Hang or rack up to dry with no further treatment of any kind. (5) Just before milking rinse milker pail and other milking utensils with cold water to remove any dust particles. (6) Thoroughly rinse all milk cans before use with one pailful of 200 ppm strength hypochlorite solution.

Detergent Sanitizer Alternate Method. Same procedure as Standard Detergent Sanitizer Method except that every fourth day acid detergent sanitizer solution should be used instead of alkaline compound. Once a week completely dismantle machine, brush all parts and tubes with the proper brushes, using the acid detergent sanitizer at 125° F. Reassemble machine and rack to dry.

Detergent Sanitizer Short Method. (1) Draw one pailful of alkaline detergent sanitizer solution at 125° F. into unit, raising and lowering teat cup assembly for scrubbing action. (2) Rinse milker pail and all other milking utensils thoroughly with this solution. (3) Rack to dry with no further treatment. (4) Just before milking rinse milker pail and other milking utensils with cold water to remove dust particles. (5) Thoroughly rinse all milk cans before use with one pailful of 200 ppm strength hypochlorite solution.

Original Cleanup Procedure Used at Beginning of Experimental Period. In order to insure clean equipment as the farms went onto the experimental procedures, they were instructed either to equip machines with new rubber parts, or to subject rubber parts to the following acid detergent cleanup: (1) Place all rubber parts in a stone crock containing a solution prepared by adding two ounces of organic acid detergent per gallon of warm water. Soak all rubber parts in this solution from the morning milking until the night milking. (2) Before the night milking, take all parts thus soaked and brush them vigorously inside and out. After this has been done, rod out the milk tubes several times, or until the rod comes clean. (3) When all parts are completely clean and free of milkstone, wash them thoroughly in a balanced alkaline dairy cleaner solution. (4) Rinse all parts thoroughly with hot water. These parts are now ready to be reassembled.

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

Purpose of Various Procedures. The standard procedure was included in order to provide a standard control for the detergent sanitizer method. Previous studies had shown this method to yield satisfactory results when properly carried out. The second procedure, the standard detergent sanitizer method, represented a relatively simple standard detergent sanitizer procedure. The third, the detergent sanitizer alternate method, applied the alternate alkaline-acid principle to detergent sanitizers in order to prevent build-up of alkaline residues on equipment. The fourth method represented the simplest possible modification of the detergent sanitizer procedure. It was similar to some instructions for use of detergent sanitizers on the market and it was employed to determine effectiveness of the so-called flush-wash procedure under the most severe conditions of use. Field observations in the past have indicated the flush-wash procedure for milking machines to be far more common than generally realized. All producers were cautioned to follow the recommended procedure. They also were urged not to expend any additional effort above that needed for an average cleaning job. They were assured of immunity from Grade A requirements and inspections during the three to four months of the experiment (July 1 to November 1, 1948).

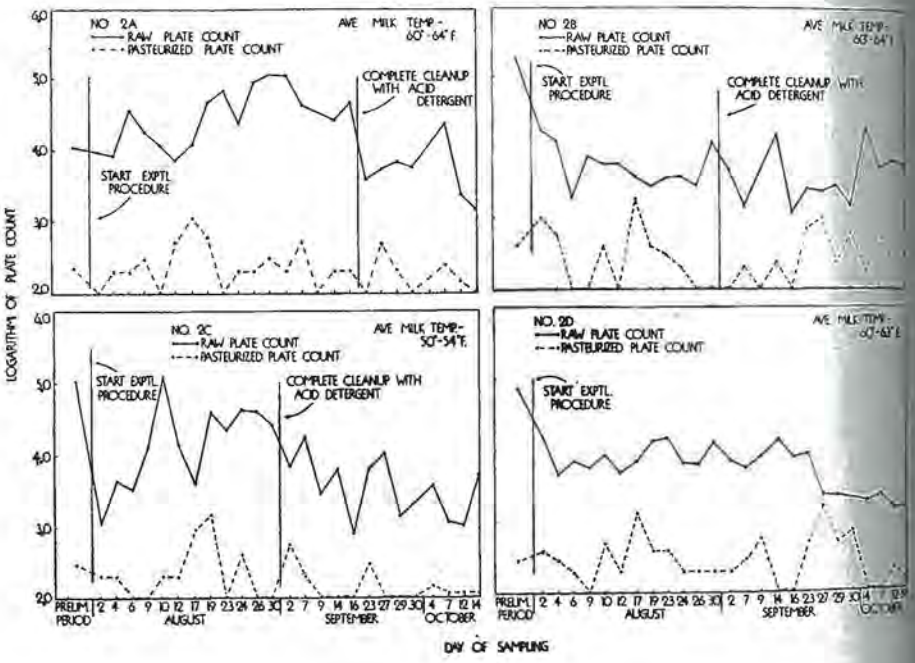
Laboratory Studies on Compounds Used in Farm Trials. Laboratory studies on the detergent sanitizers and hypochlorites used were carried out prior to and following the farm studies. These consisted essentially of trials to determine relative germicidal effectiveness of the different compounds. The methods applied included a modification of Johns slide technique[5] and more recently the method of Weber and Black.[8] Chemical determination of quaternary concentration in these studies was carried out by the method of Harper, Elliker and Moseley.[2]

Results with Standard Alternate Procedure. In general producers on the standard procedure were able to produce milk of satisfactory bacterial quality. One producer consistently averaged less than 5000 per milliliter raw count, another less than 10,000, and the other two less than 100,000 per

milliliter over the period of the experiment. Thermoduric counts were correspondingly low with the exception of one producer who gradually lowered his thermoduric count to a few hundred per milliliter of milk after several weeks on the alternate procedure. The one producer averaging less than 10,000 during the experiment was about to be degraded for high bacterial count when he went onto the experimental procedure. Obviously much of his previous trouble was due merely to a lack of knowledge of the steps required for proper cleaning and germicidal treatment. It became apparent after a few days on the experiment that every one of the producers on this procedure believed that twice-a-day disassembling and washing was unnecessary and in spite of our urging soon lapsed into a once-a-day washing with merely a rinsing after the evening milking. This was true even with those who showed the best records. Such a procedure proved sufficiently adequate to provide low bacterial counts in these trials.

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

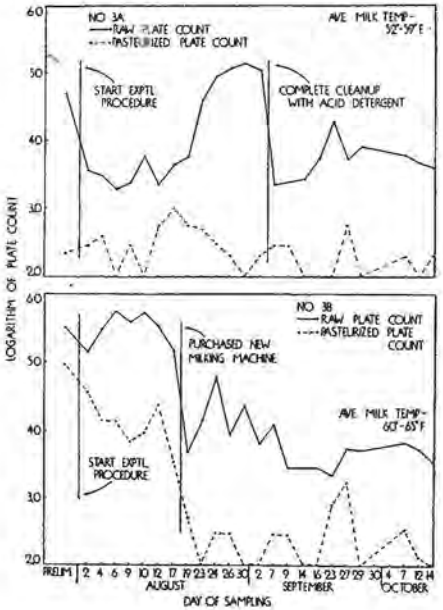
FIG. 1. Raw and pasteurized plate counts of producers on Standard Detergent Sanitizer Procedure. Note: Logarithm 2.0 represents plate count of 100 per ml., 3.0 represents 1,000 per ml., 4.0 represents 10,000 per ml., 5.0 represents 100,000 per ml. and 6.0 represents 1,000,000 per ml.



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

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

FIG. 2. Raw and pasteurized plate counts of producers on Detergent Sanitizer Alternate Procedure. (See Fig. 1 for values of logarithms.)

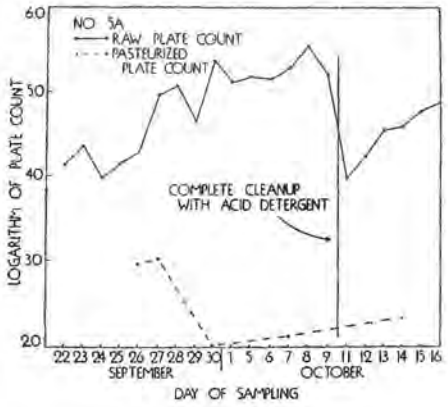


tionable. One producer experienced considerable difficulty with his air hose slipping off the metal nozzles when the detergent sanitizer was used. This was reported in some other instances but only with one make of milking machine.

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

The raw counts of producer 3A are interesting in that they indicate satisfactory results with this method for about the first three weeks and then a gradual increase in numbers due to accumulation of a soft deposit of milk

FIG. 3. Raw and pasteurized plate counts of one farm on Detergent Sanitizer Alternate Procedure. (See Fig. 1 for values of logarithms.)



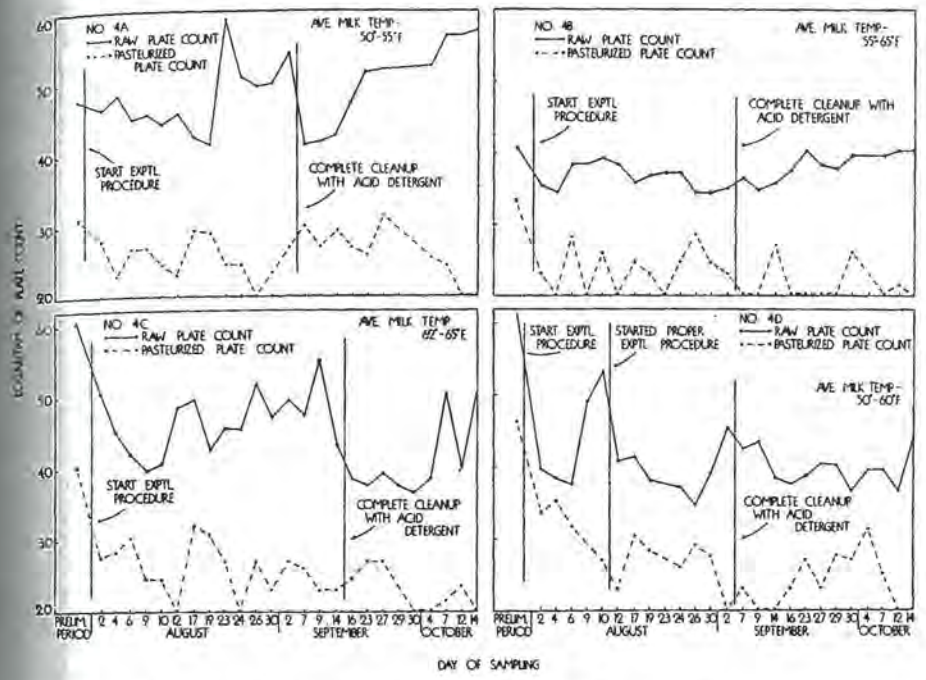
solids in the inflations. The thermoduric bacteria were definitely inhibited by the quaternary during this period. A complete disassembling and cleanup with organic acid detergent removed the accumulation and the count then remained low for the remainder of the experiment. Apparently under the conditions encountered in the experiment the alternate use of acid detergent sanitizer solution in the daily cleaning procedure was not entirely effective in removing accumulated deposit or in preventing such accumulation.

Counts of producer 3B are interesting for another reason and emphasize the necessity for frequent and careful inspection of each producer's equipment and operation in an experiment of this type. This producer started out with high counts and continued so on the experimental procedure. Inspection indicated that his milking machine, which was an old type, could not prevent escape of milk to the vacuum line. When this was pointed out to him, he immediately purchased a new machine with the results shown in Figure 2. His counts, both raw and pasteurized, remained low throughout the experiment. Both this producer and 3A reported that the acid compound appeared especially effective in removing milk solids from metal equipment.

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

Detergent Sanitizer Short Method. Results of this group, Figure 4, were the most interesting of the lot. It was expected that counts might run excessively high since the detergent sanitizer had to perform the operation of a water rinse, a cleaning, and a germicidal treatment in one single flushing

FIG. 4. Raw and pasteurized plate counts of producers on Detergent Sanitizer Short Method. (See Fig. 1 for values of logarithms.)



operation. Contrary to expectations most of the farms on this procedure produced a high quality milk and it was definitely established that they were following instructions relative to the details of the procedure.

Producer 4A did not replace his rubber inflations and milk tube at the beginning of the experiment, but instead carried out the original cleanup with acid detergent as outlined. Consequently the lack of a water rinse apparently placed too great a demand on the cleaner and there was a gradual accumulation of deposit in the slightly porous rubber. Examination at the end of the experiment indicated that the rubber was saturated with fat. The result was a gradual increase in bacterial count of the milk followed by a sharp drop after complete disassembling and acid detergent cleanup at the end of the first month. The accumulation again occurred with consequent increase in count of milk. The reason for the high count was quite obvious at the end of the experiment. In spite of the gradual increase in raw count, there was no marked increase in thermoduric bacteria at any time.

The next producer, 4B, maintained an average of well under 10,000 per milliliter raw count throughout the experiment. His thermoduric count dropped when new rubber equipment was installed at the beginning of the experiment and then remained low throughout. This producer had the benefit of a mechanical cooler which may have contributed in part to his low

raw count, but probably did not greatly influence his thermoduric count.

Producer 4C was slipshod in cleaning methods and was about to be degraded at the beginning of the experiment. His methods definitely continued slipshod through the entire trial. In spite of that fact, his raw counts averaged well under 100,000 the first month and, in spite of some increase in raw count, the thermoduric remained low. He finally disassembled his equipment after about five or six weeks and carried out the complete cleanup with acid detergent. His counts then remained quite low with only two exceptions for the remainder of the trial.

Some difficulty was experienced in getting producer 4D on the proper procedure, therefore the first two weeks on the experimental procedure are not representative. Following this period he carried out the procedure according to instructions and his counts on both raw and pasteurized milk were low for the remainder of the experiment.

All of the farms on the short procedure accumulated a brown precipitate in the wash vat. Three of the farms noted formation of such precipitate on metal and on the outside of rubber equipment. Two of these reported that it could be easily brushed off with warm water. The other producer, 4B, however, experienced rather heavy accumulation of the brown precipitate and found it quite objectionable. The brushes used for pails and similar equipment accumulated so much brown, gummy residue that they became unusable. In the case of this producer, it was found that rinsing all equipment and flushing teat cups with lukewarm water before washing with detergent sanitizer greatly aided in reducing the precipitate formation.

Results of Laboratory Studies. The laboratory trials in general indicated the germicidal properties of the detergent sanitizers to resemble those of the quaternaries employed in compounding them. The hypochlorite appeared to be a more active germicide in absence of organic matter, especially from the standpoint of destruction of coliform and *Pseudomonas* species. The alkaline detergent sanitizer powder preparation showed far superior germicidal activity against all types of organisms than the quaternary from which it was prepared when both were made up in distilled water. Part of the difference undoubtedly is due to the higher pH of the detergent sanitizer (about pH 9.4 with 200 ppm quaternary in solution). It is possible also that some other factor aids in potentiating germicidal activity of the quaternary.

DISCUSSION

This study has emphasized a number of factors not previously reported affecting application of detergent sanitizers. One of the most striking observations brought out was the cleaning ability of the detergent sanitizer powder preparation used. In spite of the fact that water hardness on these farms usually ranged only from 3 to 15 grains per gallon, almost all of the farms reported considerable milkstone accumulation on equipment with conventional cleaners previously used. Other studies carried out since this experiment was completed have emphasized the importance of the farm water supply in affecting success of detergent sanitizers. A detergent sanitizer preparation of high sequestering ability appears to be essential to cope with varying water conditions from standpoint of both prevention of milkstone deposits and bactericidal effectiveness of the preparation. It is believed on the basis of the studies reported here that the superior cleaning and sequestering ability of the alkaline detergent sanitizer employed in this experiment was in a large measure responsible for the low bacterial counts obtained with it. Examination of milking machine inflations on all procedures by cutting them open at the end of the experiment emphasized this fact. Those on the standard detergent sanitizer procedure were exceptionally clean and free of deposit. The results suggest that the detergent action may be as important as the quaternary action and that this factor of detergency and sequestration should receive chief emphasis in compounding a detergent sanitizer.

The effect of detergent sanitizers on thermoduric bacteria also was marked. The explanation must lie in the bactericidal or bacteriostatic activity of the quaternary against the gram-positive micrococci and thermoduric streptococci. In these studies as well as in other farm trials this action has been highly specific. In only one instance, and in this case only in one sampling, was there any evidence of accumulation of fluorescent *Pseudomonas* species of bacteria. This occurred on one of the farms on the detergent sanitizer alternate method. In most cases where high counts occurred with detergent sanitizer, the organisms resembled coliform types although others also were present.

The results rather definitely suggested that any type of detergent sanitizer procedure should include some form of periodic disassembling and soaking and cleaning preferably with a balanced organic acid detergent containing an effective wetting agent. In every case where raw counts increased due to gradual accumulation of milk solids, the soaking and brushing of rubber parts in the acid detergent removed the accumulation and immediately lowered the milk count. Results indicated that such a disassembling should be carried out at least every two weeks under the conditions employed in these studies. In many cases, weekly disassembly might be desirable. It may be possible to accomplish the same effect under proper conditions with an alternate alkaline-acid detergent sanitizer procedure, but this did not appear to be sufficient under conditions existing in these studies. The alternate procedure might be more effective in areas with extremely hard water.

Consideration of all results and observations points to the standard detergent sanitizer procedure as the most practical of the detergent sanitizer methods under conditions in the area studied. It was also observed that if the farmers were asked to expend only a minimum of effort daily as on the standard detergent sanitizer method, they usually were quite willing to disassemble the milking machine periodically for a more thorough cleaning with an acid detergent.

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

SUMMARY

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

2. Bacterial counts and observations on sanitary condition of farm utensils and milking machines indicated that the minimum steps necessary in a detergent sanitizer procedure included a

thorough rinse in cold or lukewarm water immediately after use, thorough brushing with a warm detergent sanitizer solution, and a periodic disassembling and thorough soaking and cleaning in a balanced organic acid detergent in order to remove accumulated solids.

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

4. The detergent sanitizer compounds exhibited a remarkable specificity against thermoduric bacteria. No accumulation of fluorescent *Pseudomonas* species occurred with procedures employed in these studies.

5. Formation of an undesirable brown precipitate occurred with some procedures but could be eliminated largely by thorough rinsing with cool or lukewarm water to remove milk solids immediately after use, and by thorough brushing subsequently in the warm washing solution.

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

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

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

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Wisconsin Winter Course in Dairy Manufacturing

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

Vermont Annual Conference

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

MILK and FOOD SANITATION

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LABORATORY DETECTION OF FOOD POISONING ATTRIBUTABLE TO DAIRY PRODUCTS *

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

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

CAUSES OF FOOD POISONING

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

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

Botulism. Symptoms of botulism usually occur in 12–36 hours although they may appear at any time within a period of 2–4 hours to 8 days. The toxin usually affects muscular coordination and the nervous system. The patient may or may not show symptoms of nausea, diarrhoea, vomiting. Poisoning usually is due to the eating of canned meats, fruits, vegetables, and fish which have been inadequately sterilized. It is rarely found in dairy products although a few, such as home-canned cheese and commercially-packed canned milk, have been implicated. Poisoning is caused by a toxin produced by the growth of the organisms in the absence of oxygen and within a relatively narrow temperature and pH range. Mortality: high.

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

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

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

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

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

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

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

In fluids, such as milk, *S. aureus* as a rule is killed by a 10-minute exposure to 58° C. (136° F.), and *S. albus* by a 10-minute exposure to 62° C. (143.6° F.). Pasteurization, therefore, is a bit more than a reasonable safeguard aginst *S. aureus* as an organism. However, if *S. aureus* has succeeded in producing enterotoxin before pasteurization, this toxin will survive pasteurization temperatures and, if sufficient toxin has been formed, will cause symptoms of food poisoning even though no living staphylococci can be recovered. Toxin may be boiled for 30 minutes or more with little if any loss of potency. In fact, so heat stable is this enterotoxin that it is common practice actually to boil it prior to cat inoculations in order to eliminate the possible presence of other less stable toxins which also may be present in the preparation.

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

* Presented October 25, 1949, at the Annual Meeting of the CALIFORNIA ASSOCIATION OF DAIRY AND MILK SANITARIANS, San Francisco.

* *Micrococcus pyogenes* var. *aureus* (6).

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

STAPHYLOCOCCI IN COWS

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

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

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

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

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

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

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

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

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

STAPHYLOCOCCI IN FOOD HANDLERS

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

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

home late Sunday afternoon and the elder daughter served all of them the remainder of the Boston—all save the one slice that was brought in to the laboratory. All of them ate of it but, strangely enough, no one suffered any symptoms whatever.

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

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

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

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

LABORATORY PROCEDURE IN FOOD POISONING

Solving a food poisoning case is not always a simple routine affair. There may be an absence of definite information on symptoms and the time of onset of these symptoms following ingestion of the implicated food. Or, even if this information is supplied, the local physician may call for a *Salmonella* determination when all the facts point toward a *Staphylococcus* case. Or, the sample itself may be so old and badly contaminated with other organisms that any attempt at isolating the real culprit is like looking for a needle in a haystack. Occasionally, too, one will find a mixed contamination in which both a *Staphylococcus* and a *Salmonella,* or possibly a *Shigella,* may be involved. For this reason, and unless one is fairly certain what the organism really is, it is best to begin with a rough screening of the sample which will segregate all likely genera. This involves: *

* It is understood, of course, that these basic procedures should be modified or supplemented where the need is indicated.

1. Inoculation of tetrathionate broth with 1 gm. (or 1 ml.) of sample per 15 ml. of broth; and also a 5 gm. (or 5 ml.) portion of sample in 50 ml. of the broth. These are placed in the 37° C. incubator overnight.

2. At the same time, streak 1 gm. (or 1 ml.) of the sample over the surface of freshly poured and hardened plates of bismuth sulfite agar and of SS agar. These plates are placed in the incubator along with the tetrathionate cultures. Their purpose is to serve as a short-cut in the isolation of any *Salmonella-Shigella-Proteus*-paracolon organisms which might be present in sufficient numbers to grow without preliminary enrichment.

3. The following morning, if no colonies are found on these differential plates, 1 ml. or more of the tetrathionate broth culture is spread over several bismuth sulfite agar and SS agar plates and these plates incubated for 12–18 hours.

4. If colonies typical of *Salmonella-Shigella*-paracolon are present, transfer several of them to tubes of Kligler's medium which should indicate whether they belong to any of the above genera and if so, roughly to which one. If our test indicates that we have a *Salmonella,* and after taking great pains to purify our culture including inoculation into Rustigian and Stuart's medium (2), we again test it on Kliger, on bismuth sulfite and SS agar; we stain it by Gram's method; determine the presence or absence of motility; its effect on gelatine; whether it does or does not form indole; whether it is able to produce visible growth in Koser's citrate medium, and whether it is able or unable to ferment certain carbohydrates. By this means, if indeed we do have a *Salmonella* contaminant, we are able to arrive at a fairly close approximation of its identity. Final confirmation, if required, is had by agglutination with appropriate anti-sera.

If we are dealing with a *Shigella* or with a paracolon, much the same procedure is followed, identity being determined by the nature of the response to the test substances.

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

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

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

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

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

In the meantime, we have not lost sight of the possible presence of staphylococci in our sample.

1. Parallel with the above work we also have plated out a 1 gm. (or 1 ml.) portion of the sample using lactose

agar or tryptone-glucose-extract-milk-agar. The purpose of this is two-fold: (1) to determine the presence or absence of *S. aureus* or *albus* and (2) to gain an idea of their relative concentration.

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

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

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

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

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

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

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

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

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

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

* An exception is noted for *M. ureae* which not only grows extremely luxuriantly on this medium but which, contrary to Bergey (6), also develops a buff to yellow pigment when freshly isolated.

PREVENTION

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

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

There must be:

1. Proper cleaning and sterilization of milk cans, milking machine parts and other equipment.
2. Careful watch over the physical condition of cows in the herd, supplemented by microscopic examination of incubated milk samples where indicated.
3. Careful observation of colony types on agar plates of all routine samples of milk and cream. When staphylococci increase to the point where they predominate on the plate, an individual check on all cows of the implicated herd is indicated.
4. When physical examination of the cows yields negative results despite the continued presence of these cocci in large numbers in the milk, suspicious colonies should be examined to learn if they are potentially dangerous.
5. *There must be proper cooling* and protection of milk until it is consumed or pasteurized.
6. A check on food handlers and dispensers definitely is indicated.
7. In order that a regulatory body or health agency may operate efficiently, it must be backed by an effective laboratory.
8. As these organisms in milk are dangerous only when they have been allowed to grow and to form enterotoxin, it is believed that the above suggestions should prove practical and effective in preventing food poisoning attributable to market milk and cream. That such poisonings have not been more numerous is evidence of the effectiveness of cooling and sterilization. Prompt elimination of infected cows should reduce all danger to a minimum.

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REPORT OF COMMITTEE ON DAIRY FARM METHODS

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

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

Our Committee is concerned, aside from the efficiency of detergent-sanitizers, with the practical application and use of these compounds, keeping in mind any differences in time, labor, and costs as compared with other types of cleaners and bactericidal agents. One member of our Committee writes: "In normal procedure the utensils are rinsed with cold water immediately after use and then washed with hot water and washing powder, following which they are rinsed with clean hot water or water in which a suitable germicidal agent has been placed. The water in which the utensils are washed usually becomes dirty before completion of washing of all the utensils, and rinsing with clean water is necessary for the utensil to be clean after it dries. If a cleaner-sanitizer is used in the wash water, will rinsing counteract the sterilizing properties of the cleaner sanitizer agent?"

In answer to this question, it has been suggested that the cleaner-sanitizer should be used again in the rinse following washing, or another rinse used just prior to use.

Satisfactory answers to the above, and a determination of proper procedure for most effective use, will be helpful in establishing comparisons of cost and labor involved. It is hoped that the Committee will continue study of this subject next year and that sufficient factual information will be available to warrant some definite conclusions.

Some members of the Committee thought that more information should be had on the part, if any, that unclean vacuum hoses and dirty pipe lines play in the contamination of milk. This is something that has concerned many milk control officials and other individuals interested in quality milk programs throughout the country for quite some time. Dr. Bober reports that a field study of more than fifty farms in the Detroit, Flint and Saginaw area has been made in cooperation with the Health Departments of these three cities. Laboratory work was done under the supervision of Dr. Mallman of Michigan State College. It is believed that Professor Churchill will make a report of the work at this year's meeting.*

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

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

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

In some sections of the country, fluid milk shortages in certain seasons of the year present a problem for both the milk control official and the processor and distributor. Numerous areas have a surplus of milk in April, May, and June and a decided shortage in the late fall and winter months. In order to have an ample supply in November, it is necessary to have from 30 percent to 40 percent of surplus in May. As a means to help correct this situation, some distributors have initiated a base surplus plan whereby certain months are designated as base-setting periods. For example, if October, November, and December are so designated then the average daily production delivered by a producer during that period becomes his base, and for that amount of milk he will be paid top price during the other months of the year. Any milk delivered over his base average may be

* This paper was published in the May-June issue of this Journal, page 137—Ed.

purchased at a reduced price during the flush production periods of the spring months, depending upon the needs of the purchaser. This plan encourages the producer to plan his breeding program in order that a greater percentage of his cows will freshen in the fall months. It is quite generally agreed that most cows freshening during this period will produce more milk over their lactation period than those freshening in the spring. The theory is that flush production after freshening will be supplemented by another flush period during the spring months due to the green feeds available at that time, this being true where scientific feeding programs are not followed.

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

Ventilation of dairy barns has been given much thought in the past and different solutions have been recommended and put into effect. Some think that the trend is toward lounging sheds and that the old type barn will gradually be used to a lesser degree. Exhaust fans have been suggested as an answer to the ventilation problem. In certain sections one observes the installation of fans in increasing numbers for use in the summer months to add to the comfort of the milkers during milking operations. Perhaps more thought should be given to milk house ventilation. Some observers believe that there is a relationship between humidities in milk houses, where equipment is stored, to the development and control of thermoduric bacteria.

The practice of using paper towels in washing teats and udders is on the increase. Reports indicate that this type of towel has proven quite satisfactory, provided the towel will not tear apart easily when wet nor peel while being used in the washing operation. Where used, the producers indicate that the cost is compensated for in the elimination of the chore of washing cloth towels.

Mechanical barn cleaners do a very satisfactory job when ample bedding is used, and operators report that they are real labor savers. They do not, however, clean gutters satisfactorily without bedding or other similar material in the gutter. Some operators, on this account, discontinue their use temporarily during the summer months. This is done by taking out that part of the apparatus operating in the gutter proper. No doubt the manufacturers of these mechanical cleaners will be able to overcome the defects now existing and thus add to the usefulness of this type of labor saving device.

R. C. Ross, *Chairman*
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Abstract of Paper Entitled

SIXTY YEARS OF PROGRESS IN SANITATION, 1890–1950

Presented by Dr. Murray P. Horwood

at the Meetings of the
Massachusetts Public Health Association
Boston, April 25, 1950

ALTHOUGH the past sixty years have witnessed great progress in scientific discoveries and technological achievements, nothing stands out more significantly from the standpoint of human health and human welfare than the progress which has been made in the prevention and control of the diseases spread through the environment. After describing the insanitary conditions found in the United States in 1890 and the tremendous mortality that prevailed from typhoid fever and other gastro-enteric diseases, from diphtheria, scarlet fever, septic sore throat and other milk-borne diseases, from infant mortality, from diarrhea and enteritis under 2 and from tuberculosis, Dr. Horwood indicated that great changes began to occur in the control of the environmental diseases at about the time that the M.P.H.A. was organized. The Massachusetts State Board of Health was reorganized in 1886 and the Lawrence Experiment Station was founded shortly thereafter, and placed under the direction of a group of brilliant supervisors and unusually capable young investigators. The former group consisted of Hiram F. Mills, Thomas M. Drown and William T. Sedgwick; and the latter group of Allen Hazen, George C. Whipple, George W. Fuller, Edwin O. Jordan, and Ellen H. Richards. As a result of the discoveries made at the Lawrence Experiment Station new and improved methods of water and sewage treatment were developed. As a sequel to the epidemiological investigations of Professor William T. Sedgwick on the prevalence of water-borne and milk-borne typhoid fever, a slow sand filter plant was established at Lawrence for treating the sewage polluted Merrimac River water which was being used as a source of drinking water; and subsequently, public interest was stimulated in pasteurization as a means of preventing milk-borne disease.

The period from 1890–1950 has witnessed the extension of municipal water supplies throughout the country, the construction of sewers and sewage treatment plants, the sanitation and pasteurization of milk supplies, and the introduction of a vast system of sanitary supervision over all food supplies. Partly in consequence of these and other improvements in environmental sanitation, the general death rate in the U. S. Registration Area fell from 19.6 per 1000 population in 1890 to 10.0 per 1000 population in 1948. At the same time the average expectancy of life increased from 43.0 years in 1890 to 67.2 years in 1948. For white females alone the average expectancy of life at birth in 1948 was 71 years. While this tremendous improvement is due to public health and medical progress in general, there is little doubt that the contribution of improved sanitation has been of very great significance. The typhoid fever death rate in the U. S. Registration Area in 1900 was 35.9 per 100,000 population. In 1947 it was reduced to 0.2 per 100,000 population. Similarly, the infant mortality rate in Massachusetts in 1890 was 166.6 while in 1948, it was 26.4. The death rate from diarrhea and

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

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

While tuberculosis is a respiratory disease and its incidence does not have any direct relationship to infected water or improper methods of sewage disposal, it is reasonable to conclude that the prevention of serious enteric diseases help to maintain the vital resistance high enough to prevent the development of tuberculosis in many instances. The death rate from tuberculosis in the U. S. Registration Area declined from 201.9 per 100,000 population in 1900 to 30.0 in 1948.

The period from 1890–1950 also witnessed the establishment of the fact that insects are involved in the dissemination of certain diseases. Thus, ticks, mosquitoes, flies, fleas, lice, and mites have been incriminated in this way. The diseases spread by these insects include the gastro-enteritic diseases in the case of certain flies; malaria, yellow fever, dengue fever and filariasis in the case of mosquitoes; epidemic typhus fever in the case of lice; endemic or murine typhus fever and bubonic plague in the case of rat fleas; African sleeping sickness in the case of the tsetse fly; Rocky Mountain Spotted Fever in the case of the tick, tsutsugamushi fever and rickettsialpox in the case of mites; and other diseases in the case of the deer fly, the sand fly and other insects. The development of successful methods of combating these insects by eliminating their breeding places or by the use of larvicides and insecticides like DDT represents one of the greatest achievements of sanitary science. This success has improved the public health throughout the world tremendously and has opened up vast areas for human habitation.

Unquestionably, the contributions of sanitary science during the past 60 years represent some of the greatest achievements of the human race. Much remains to be done however. Greater emphasis is being placed today on adequate protection of food supplies, the proper cleansing and sterilizing of eating and drinking utensils, improved housing conditions, the control of smoke, smog, toxic gases and toxic fumes, and many other aspects of the environment. Much remains to be done also in maintaining the gains in sanitation that have already been accomplished and in extending them to areas that have not yet felt their benign influence. The protection that sanitation provides must be made permanent and must be maintained uninterrupted if the gains of the past 60 years are to be retained. With the increasing proportion of the population in the age group beyond 45, greater emphasis in the public health of the future will unquestionably be placed on human comfort and convenience. In consequence, the attention which sanitation will receive is destined to be greater rather than less.

Abstract of Report of Bureau of Dairy Industry 1949

Accomplishments of Bureau in Twenty-five Years

On July 1, 1949, the Bureau of Dairy Industry was 25 years old, having begun to function July 1, 1924. . . .

Throughout the intervening years, the major efforts of the Bureau have been devoted to the development of scientific and practical information relating to dairy problems. . . .

One of the Bureau's contributions to better dairying in the early part of this 25-year period was the introduction of the proved-sire system of breeding. The proved sire is now recognized as the most important factor in any breeding program. . . .

The use of more roughage on the dairy farm, with less dependence on grain crops, is a contributing factor in the gradual shift to grassland farming and the accompanying soil-conservation practices.

Evidence of the improvement in the dairy-farming industry as a result of better breeding, feeding, and management methods, is the increase in average milk production per cow from 4,167 pounds in 1924 to 5,036 in 1948. Only about 7 percent more cows were milked in 1948, but total milk production for the year was 29 percent more than in 1924.

Early work in the Bureau established the time and temperatures required to kill disease-producing organisms in milk, and this laid the foundation for the successful development of the pasteurized-milk industry. More recent research has resulted in the use of pasteurized milk in the manufacture of many dairy products, notably Cheddar cheese. Bureau workers also showed that feed flavors and odors are transmitted to milk largely through the body of the cow, and this information had the practical effect of reversing the usual time of feeding and milking so as to avoid off-flavored milk. . . .

Bureau workers also showed the importance of feeding adequate quantities of carotene-rich forages to the milking herd, not only to maintain the health and normal reproductive functions of the cows but also to increase the vitamin A content of their milk and butterfat. The need for better winter feeding practices was emphasized by the results of the Nation-wide survey conducted in 1943–45, in cooperation with 21 states, which showed that winter milk and butter had a lower average vitamin A content than milk and butter produced on pasture and other green feeds in spring and summer. More recently, the Bureau's forage-harvesting experiments have shown the possibilities of conserving significantly larger amounts of carotene (pro-vitamin A) for winter feeding by making silage, barn-dried hays, or artificially dried hays than by curing the crop in the field.

Also, it is interesting to note that, on its twenty-fifth anniversary, the Bureau found milk and nonfat milk products to be rich sources of the newly discovered vitamin B_{12}. . . .

The increasing use of nonfat milk solids in the human diet is indicated by the relative amounts of cream and whole milk now delivered by farmers to the processing plants, as compared with 25 years ago. In 1924, cream and whole milk deliveries were 29 and 26 billion pounds, respectively; in 1948, the relative amounts were 19 and 69 billion pounds. . . .

DHIA Herds

Members of dairy-herd-improvement associations have been making rather steady improvement in the production level of their herds for many years. In 1948, the average for all cows in the association herds was 8,675 pounds of milk and 350 pounds of butterfat per cow. These averages are an all-time high.

In 1926, about 45 per cent of the association cows produced less than 275 pounds of butterfat per cow and only 15 percent produced more than 375 pounds. Now only 23 percent produce less than 275 pounds and 35 percent produce more than 375 pounds. Approximately 160,000 cows in these herds, or about 18 percent, produce more than 425 pounds of butterfat a year.

In contrast to the high production level in the association herds, the average for all cows milked in the United States in 1948 was 5,036 pounds of milk and 201 pounds of butterfat per cow. . . .

The association cows that averaged 8,675 pounds of milk per cow per year consumed $1.90 worth of feed for each 100 pounds of milk they produced, whereas the cows that averaged 5,000 pounds of milk (or about the same as all cows milked in the United States) consumed $2.65 worth of feed for each 100 pounds of milk they produced.

Streptomycin Less Effective Than Penicillin

Preliminary tests with streptomycin in treating mastitis infections in the Bureau of Dairy Industry's herds at Beltsville, Md., indicate that this drug is not as effective as penicillin in eliminating the disease but that, like penicillin, it is more effective against certain organisms than others. . . .

The relative effectiveness of penicillin against different mastitis organisms was

90.8 percent for all streptococci, 85.4 for staphylococci, 93.3 for coliform bacteria, and 76.0 percent for pseudomonades. The relative effectiveness of streptomycin was 78.3 percent for all streptococci, 60.0 for staphylococci, 87.5 for coliform bacteria, and 72.7 percent for pseudomonades. . . .

DDT Residues on Forage

In a study to determine the effect of feeding milking cows on forages that had been sprayed 2 to 3 weeks earlier with DDT (0.6 pound to 2.4 pounds per acre), the Bureau of Dairy Industry found that 5 to 30 percent of the DDT intake appeared in the milk and that some appeared 30 to 170 days after the feeding of the treated forage was discontinued, the variations being in direct proportion to the amount of DDT intake. When hay containing 34, 12, and 9 parts per million of DDT residue was fed to calves for 6 to 8 months, the kidney fat was found to have 15, 4, and 4 parts per million of DDT, respectively.

In other tests, a field of alfalfa was sprayed with 1 pound of DDT per acre, or 1 pound of chlordane, or 1.5 pounds of toxaphene. When the hay was cut about a week later, the concentration of DDT, chlordane, and toxaphene (based on organic chlorine content) was 27, 10, and 36 parts per million, respectively. Ordinarily, the insecticides would be applied considerably farther in advance of harvest than this. Cows receiving the treated hays showed significant amounts (up to 9 parts per million) of DDT in the milk but very small amounts (less than 1 part per million) of the other two insecticides.

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

Milk and Nonfat Milk Products Contain a Material Having Same Growth-Promoting Properties as Vitamin B_{12}

Research in the Bureau of Dairy Industry this year showed that pure crystalline vitamin B_{12}, which is the name commercial chemists gave to the antipernicious anemia factor they isolated from liver in 1948, has the same physiological effects in normal mammals as the unidentified growth factor (nutrient X) which Bureau workers had previously found in milk, skim milk, nonfat milk products, liver, and certain other foods.

Unlike pure vitamin B_{12} or liver extracts, however, milk and other food sources of nutrient X are not effective in treating pernicious anemia, probably because of the form in which the factor may exist in these foods.

When fed in maximally effective doses to rats, both the pure vitamin B_{12} and various foods known to be sources of nutrient X were equally effective (1) in promoting growth; (2) in overcoming the deleterious effects of high levels of protein in the diet; (3) in correcting the harmful effects of lactose; and (4) in bringing about early sexual development. It also appears from the preliminary results of experiments now in progress that vitamin B_{12}, like nutrient X, plays an important role in reproduction.

The comparison to date indicates that milk, nonfat milk solids, and cheeses of various kinds, although not directly effective in treating pernicious anemia, supply either vitamin B_{12} or a substance which functions like this vitamin in the normal mammal. The research has also demonstrated that the oil meals, wheat flours, cereal grains, and cereal grain products do not have vitamin B_{12} activity, but may be effectively supplemented in this respect by milk and other dairy products that contain nonfat milk solids. . . .

New Type of Milk Heater

A new-type milk heater designed and built by the Bureau of Dairy Industry several years ago, for use in studying the effect of high-temperature, short-time heating procedures, was further improved this year and demonstrated to the dairy manufacturing industry.

In addition, a new-type flow-diversion valve was also developed and demonstrated for use with various kinds of milk-heating equipment or pasteurizers. The valve prevents the discharge of any inadequately heated milk by automatically diverting it back to the heating unit. (See this Journal, Mar.–Apr., 1949, p. 127.)

Early investigations in the laboratory indicated that the rapid heating of milk to temperatures above boiling would produce changes that might be advantageous in certain manufacturing processes. The new heater is capable of heating fluid milk, in a continuous flow operation, to temperatures as high as 300° F. in about 1 second. . . .

When the milk is forewarmed at a higher temperature for a shorter time than is usually done, the canned product is better able to withstand the heat of sterilization and, therefore, stabilizing salts can be omitted or reduced in amount. A similar forewarming treatment for sweetened condensed milk retards thickening of the product during storage.

Additional studies have indicated that the high-temperature, short-time procedure, when used to sterilize concentrated milks before they are placed in the can, will improve the color and flavor of the final product. It is now possible to sterilize and then aseptically package concentrated milks, but changes occur during storage which affect the keeping quality. . . .

At least two commercial companies are now making plans to manufacture the heater, and others have adopted certain features of both the heater and the flow-diversion valve to modify present equipment. The design of the heater is such that it could easily be adapted for use as a pasteurizer in small market milk plants or cheese factories, where less expensive equipment is needed.

Milk Pasteurization Makes Better Cheese

Some cheesemakers who use raw milk in making Cheddar cheese believe that good quality cheese cannot be obtained by following the time-schedule method advocated by the Bureau of Dairy Industry. . . .

To test the validity of this belief, the Bureau this year conducted some experiments with both methods of manufacture. . . .

Practically, pasteurization produced consistent uniformity in most of the properties of the milk regardless of its original quality. The pasteurized-milk cheeses, like the milk, were much more uniform in quality and other properties than the raw-milk cheeses. The greatest improvement in the quality of the cheese, as a result of pasteurization, occurred when the original milk was of poor quality.

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

Phosphatase Test

The Bureau of Dairy Industry's improved phosphatase test for determining the adequacy of pasteurization in cow's milk, and in dairy products made from cow's milk, has now been modified for use with goat's milk.

Studies during the year showed that the enzyme in goat's milk is the same as the enzyme in cow's milk, on which the test depends. Since goat's milk was found to contain only about 10 percent as much phosphatase activity as cow's milk, however, it was necessary to modify the procedure in order to increase the magnitude of the results.

When the regular test, as used with cow's milk, is applied to goat's milk it will detect the addition of 1.5 percent of raw milk to pasteurized milk. The modified goat's milk test will detect the addition of as little as 0.3 percent of raw milk.

Although still not as sensitive as the cow's milk test, the goat's milk test is now considered sufficiently sensitive for all practical purposes. A reliable test for goat's milk is especially important because of the frequency of brucellosis organisms in the raw milk.

How Light Causes Oxidative Deterioration in Milk

In the processing of milk and its products, oxidative deterioration occurs. One of the substances oxidized is ascorbic acid, and light is known to be one factor involved in its oxidation. Riboflavin absorbs light of the visible portion of the spectrum, and this light promotes the oxidative breakdown of riboflavin and of other reducing substances, such as ascorbic acid. . . .

Ascorbic acid in pure solution oxidizes slowly in the dark. Light of 436 millimicrons wavelength accelerates this oxidation in presence of riboflavin. The most rapid oxidation takes place at pH 6.5, which is approximately the pH of fresh milk. At more alkaline reactions, riboflavin decomposes and hence less oxidation of ascorbic acid occurs. At pH 3 the rate of oxidation of ascorbic acid is very slow. High concentrations of ascorbic acid and low concentrations of riboflavin are conducive to high retention of ascorbic acid. 10° rise in temperature increases the rate of oxidation by 35 percent.

These results indicate the importance of excluding light from milk, especially from milk undergoing heat treatment.

Two New Tests Devised for Determining Lipolytic Activity in Milk

. . . The most promising method consists of adding naphthyl fatty acid esters to the milk and incubating it for 2 hours at 37° C. Precipitation of the proteins gives a clear filtrate and the addition of 2–6 dibromoquinone produces a purplish blue color, the intensity of which is determined with a colorimeter. The intensity of the color is proportional to the amount of lipase.

The other method is similar except that resorcinol esters are used, the resorcinol yellow color being developed by the addition of sodium nitrate. . . .

Carbon Dioxide Under Pressure Improves Keeping Quality

. . . The present commercial procedure is to evacuate the air for about an hour and then let nitrogen in to restore atmospheric pressure before sealing the cans. The substitution of carbon dioxide for nitrogen in this procedure gave improved keeping quality, but even better results were obtained by forcing the carbon dioxide in until it built up a pressure of 50 pounds, holding at that pressure for 3 hours, then releasing it and sealing the cans. Nitrogen was also tested under 50 pounds pressure, but it did

not have the same effect as carbon dioxide. . . .

Particle Size of Milk Proteins

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

They found that all the particles are extremely large in comparison with the molecules of other common proteins and that at least most of them are composed of a single unit having an apparent molecular weight of about 33 million. This work also showed that the protein is chemically a combination of calcium caseinate and calcium phosphate, apparently in a very definite proportion. There appears to be no free calcium caseinate, free casein, or colloidal calcium phosphate normally in milk. . . .

Flavor and Body Stability of Frozen Milk

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

In related experiments to increase the physical stability of milk held under frozen storage, milk was filtered through a synthetic organic resin cation exchanger to remove a portion of the calcium. It is known that calcium affects the heat stability of milk and, therefore, possibly the body stability. When milk was treated by using a mixture of the acid form and the alkaline form of the organic ion exchanger in proper proportions, the resultant filtrate had a normal pH (6.5), or acid intensity. It is not necessary to acidulate the milk before treatment with organic exchangers as is the case with the inorganic exchangers.

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

Preservation of Goat's Milk by Freezing

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

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

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

Sherbets Made with Cheese Whey

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

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

Abstract of Report of the Food and Drug Administration 1949

Actions on Food

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

The 1948 report recorded the passage late in June of the Miller amendment to the Food, Drug, and Cosmetic Act. This measure, which defined Federal jurisdiction over articles after interstate shipment, was of particular significance in the food field because it restored control over food stored under insanitary conditions following shipment in interstate commerce. Heavy seizures in the early months after its enactment brought immediate correction of objectionable conditions in many warehouses. The seized merchandise often contained sound material intermingled with the contaminated. Owners requesting permission to segregate the good from the bad were, as a rule, required by the courts to show to the satisfaction of the Administration that their warehouses were being so operated that further contamination would be avoided. Those who lost foods through seizure because of public warehouse contamination brought pressure to bear on the warehouse management. Consequently, objectionable conditions were corrected, under the direction of sanitation experts employed by industry associations and private firms. Pest control operations, renovation of poorly constructed buildings, and education of employers in the essentials of sanitary operations all played an important part. . . .

The most efficient rodenticide available—the comparatively new sodium fluoroacetate, or "1080"—is an extremely toxic compound developed in this country during the war. A white powder, with little to distinguish it from many common foods, it is ordinarily used as a clear water solution dispensed in small paper cups. Although this poison is sold under restrictions limiting its use to trained pest control operators and public health personnel, inspectors have encountered many instances of alarmingly careless handling in food plants. The paper-dispensing cups were frequently found within a few inches of unprotected foods, and the employees had not been informed that they contained a deadly poison. Several deaths occurred accidentally from bringing this poison into the private homes—among the victims were children who drank the liquid from the paper cups left for rats or who chewed empty paper cups containing a residue from this poison. There is no satisfactory antidote for it and its effects are rapid. So far, no injury is known to have resulted from the consumption of a food, drug, or cosmetic contaminated with 1080. The Food and Drug Administration has made available to interested parties rules drawn up by the National Research Council for the use of this rodenticide without jeopardy to the safety of the articles it is intended to protect from rodents and has maintained surveillance throughout the year to detect and warn food plants against careless practices. . . .

Bakery Products

Militant consumer interest in bakery sanitation developed in a large Middle West city when four criminal informations filed against bakeries operating under insanitary conditions were reported in front-page, editorial, and illustrated feature articles in the local papers. Organized housewives, guided by a state inspector, were soon making their own sanitary inspections of bakeries throughout the city. Health groups became interested. The Federal grand jury subpoenaed the district chief of the Food and Drug Administration, obtained a first-hand report on rodent and insect contamination in the plants, and recommended that the program of criminal actions against the owners of insanitary factories be continued. . . .

Two large food poisoning outbreaks were traced to cream-filled pastries. In one case 100 persons became ill and one died. In the second, the outbreak occurred after a criminal action had been filed against a bakery because of its insanitary operations.

Dairy Products

Widespread concern about the safety of the nation's milk supply arose from rumors that the public was consuming harmful quantities of DDT in milk as a result of the spraying of this insecticide in dairy barns and on dairy cattle, and their consumption of fodder treated with it. The Food and Drug Administration's spot check of market milk throughout the United States in the spring of 1949 showed that this rumor was unfounded. It had started after the Bureau of Entomology and Plant Quarantine recommended that the dairy industry change from DDT to a safer insecticide to control flies in dairy barns. This recommendation was very properly made when investigations showed that experimental animals gradually

accumulate this compound in fatty tissues and secrete it in their milk. . . . No tolerance for DDT in milk will be set up because it is a poison that is not required in good dairy farm practice. . . .

OILS AND FATS

. . . When a Government chemist discovered that squalene content was a distinguishing feature of olive oil, and cheaper oils masquerading as olive oil were thus detected and exposed in court, a large-scale adulteration scheme began. Edible oil mixtures, labeled as containing 10 to 20 percent of olive oil, were fortified by small amounts of purified squalene to mask the omission of olive oil and preclude chemical detection. Food and Drug chemists countered by putting secret markers in squalene going through intermediaries to outlets in the oil industry in the summer of 1947. A long, patient vigil followed. Finally the hidden marker was found in so-called olive oil blends shipped in interstate commerce and 14 seizures were made. Certain that the Government had no way to identify the squalene, the shippers contested the seizures in Federal court late in March, 1949. The jury supported the Government seizure. Seven firms and their responsible officers were indicted for criminal violation of the act. The first criminal case coming to trial was vigorously contested; the court reserved decision until fall.

SEA-FOOD INSPECTION SERVICE

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

FOOD STANDARD

. . . No new standards were issued by the Administrator in 1949, but several existing standards were amended. The most significant amendment was the deletion from the list of optional ingredients for flour of an artificial maturing agent, nitrogen trichloride, which had been in use for 25 years and was recognized as an optional ingredient when the flour standards were established. . . . Chlorine dioxide was added to the standard as an optional maturing agent.

The flour standards were also amended to lower the proportion of bromates in bromated flour. . . .

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

The amendment to standards for cream cheese, Neufchatel cheese, and cottage cheese permits the use of concentrated and dried forms of milk and skim milk along with other ingredients in preparation of these soft uncured cheeses. It was established at the hearing that this would result in an acceptable product and that the change would permit a more even production throughout the year. . . .

LEGISLATION AND CHANGES IN REGULATIONS

. . . H. R. 160, amending the import sections of the Act, provides for charging the importer with the costs incurred by the Food and Drug Administration in supervising the relabeling or other operations on imported articles to bring them into compliance with the Act. It also recognizes the long-standing practice of conditionally releasing violative articles for relabeling or other necessary treatment. . . .

SCIENTIFIC INVESTIGATIONS

. . . One new substance studied in 1949 was vitamin B_{12}, which has shown great promise in clinical use. One millionth of a gram is a sufficient daily dose for a pernicious anemia patient. In addition to its value for human use, if the vitamin is fed to poultry and swine the importance of using rations containing protein from animal sources is diminished. Concentrates of vitamin B_{12} are now being sold for use in animal rations. Research is under way to develop microbiological methods of examination of B_{12}. Its production is a striking example of the kinship of foods and drugs. It was first isolated from liver extract; then it was discovered that the same organism which produces streptomycin also produces B_{12}. All of the streptomycin producers plan to market it for use in animal feed. . . .

A new and completely revised publication entitled "Methods of Analysis Applicable to Certifiable Coal-Tar Colors" reflects the development of rapid and accurate coal-tar color testing. . . .

Commercial oystermen are experimenting with treating oysters in their native waters with bichloride of mercury to eliminate certain destructive pests. An extremely sensitive method for detection of mercury was developed to test these oysters. Some treated oysters were found to contain up to 2.5 parts per million of mercury. Quantitative tests were worked out not only for poisonous spray material but also for the deadly new rat poison, 1080, which was mentioned in the section, Actions on Foods. The method for determining its presence in foods was sent to both manufacturers and distributors of the poison. Methods for the quantitative determination of monochloracetic acid in beverages and for its identification have been adopted by the Association of Official Agricultural Chemists.

Previously developed quantitative methods were used to detect quaternary ammonium compounds in imported frozen shrimp, but work is continuing on refinement of these methods, particularly with reference to retention of the chemical by the food itself and possibly by chemical glassware. A 15-minute chemical test was developed to enable inspectors to distinguish butter from oleomargarine without dependence on taste, smell, or color. . . .

	Seizures	*Criminal prosecutions instituted*	*Main complaint*
Beverages and beverage materials	56	2	Monochloracetic acid; filth
Bakery products	27	46	Filth; food poisoning
Cereals and grain products and feeds	283	49	Contaminated in terminal storage, filth; mislabeling
Chocolate and sugar products	99	19	Filth; short weight
Dairy products:			
Butter	94	26	Use of sodium fluoride; filth; decomposition;
Cheese	15	17	low fat; short weight;
Miscellaneous	15	7	mislabeling
Eggs and egg products	13	3	Decomposed
Flavors, spices, condiments	41	4	Filth; insect and rodent infestation
Fruits and fruit products	179	13	Lead arsenate; decomposed berries; wormy and moldy dates
Macaroni and noodle products	23	11	Insanitary operations
Meat products and poultry	9	..	Strong obnoxious feed odors in dressed turkeys
Nuts and nut products	99	5	Contaminated in terminal storage
Oils and fats	14	11	Mislabeling
Sea food	141	11	Decomposed fish; pollution; excess water; poisonous quaternary compound
Vegetables and vegetable products	274	27	Filth; decomposition
Miscellaneous foods	25	3	Deteriorated war surplus rations
Vitamin, minera, and other products of special dietary significance	59	27	Misleading claims; self deterioration
Cosmetics and colors	2	0	Uncertifiable dye and also some that were not recertified.

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(*including* MILK AND FOOD SANITATION)

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At the May meeting, a Publicity Committee was appointed by President A. J. Powers, consisting of the following members:
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ASSOCIATION NEWS

1950 Meeting of Florida Association of Milk Sanitarians

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

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

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

H. H. WILKOWSKE
Secretary-Treasurer

Connecticut Association of Dairy and Food Sanitarians

More than one hundred persons were in attendance at the annual meeting. Major interest was shown in the subject of trends in the dairy industry. There is increasing responsibility being placed on the milk dealers and processors for the quality control of the product which he handles from the point of production right through to the consumer, under the supervision of state and municipal health agencies. This procedure of Connecticut follows similar ones in the Boston and New York City markets.

H. C. GOSLEE
Secretary

Iowa Association of Milk Sanitarians

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

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

The second day meeting included the following program:

"The Ring Test for Detecting Brucellosis"

"The Application and Use of Pen Barns"

"Milk Plant Cleaning Aids"

"Cost of Producing Grade 'A' Milk"

"Timing Short Time High Temperature Pasteurizers"

MILTON E. HELD
Secretary-Treasurer

Metropolitan Dairy Technology Society

Mr. Charles A. Herrmann, Chief, New York District, Federal Food and Drug Administration, spoke at the May meeting on the subject, "The Application of the Federal Food Law to Dairy Products." He said milk may be found to contain DDT if the dairy cows are fed ensilage bearing DDT, if the cows are sprayed with DDT, or if DDT is used in dairy barns. The use of methoxychlor obviates much contamination.

With regard to the quaternary ammonium compounds, no regulations dealing specifically with these substances have been promulgated under the Act but the general requirements of the statute apply. These compounds are classed as poisonous or deleterious substances, and any food containing a quaternary ammonium compound is deemed by the Food and Drug Administration to be adulterated and thus illegal for interstate shipment.

A Publicity Committee was appointed by President A. J. Powers, consisting of the following members:
Gene Maccni, *Chairman;* D. X. Clarin, Dr. S. Lear, A. L. Moon, A. Quencer.

The third annual outing of the Society was held May 25th on the campus of the Long Island Agricultural and Technical Institute.

GENE MACCNI
Chairman, Publicity Committee

Minnesota Milk Sanitarians Association

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

9:15 a.m., the same day. The program is as follows:

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

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

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

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

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

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

J. C. OLSON
Secretary-Treasurer

New York State Association of Milk Sanitarians

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

Tentative Program

1. World Health Organization—Mr. Sol Pincus
2. Resazurin Test in Improving City Milk Supplies—Mr. M. Cohn, Schenectady
3. National Sanitation Foundation Report—Mr. W. D. Tiedman

3A Standards—Report—Mr. C. W. Weber

International Association of Milk and Food Sanitarians — Report — Mr. Geo. West

4. Effect of Penicillin in Milk—Mr. F. W. Gilcreas
5. Education of Food Handlers—Dr. M. H. Thompson, Rensselaer County
6. Construction and Operation of Pen Stables—Dr. George Hopson
7. High - Temperature Short - Time Pasteurization Above 160° F. in Vacuum—Dr. F. W. Barber, National Dairy Research Lab.
8. Use of Glass Piping in the Dairy Industry—Dr. Robert Holland, Cornell
9. Use of the Ring Test in Detecting Brucellosis—Dr. Gilman, Cornell
10. Well Water and Blue Babies—Mr. Charles Cox
11. Vending Machines — Mr. J. Trichter
12. Cooperation between County Agents and Health Departments—Messrs. Grunge—Crowe—Pratt
13. Food Plant Sanitation—Robert Taggart, National Biscuit Company, New York
14. Topic to be Announced—C. J. Babcock—U.S.D.A.
15. Breeding of Dairy Cows—Mr. Gregory Pincus
16. Economic Aspects of Public Health Regulations—Mr. Ed Vial
17. Business Meeting
18. Question Hour
19. How Fluid Milk Consumption can be Increased—Mr. Wesley McCune

C. S. LEETE
Secretary-Treasurer

Oklahoma Association of Milk and Food Sanitarians

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

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

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

Dr. O. K. Gregg, Director, Cleveland County Health Department.

Dr. Binn, Veterinarian in Charge of Communicable Disease Control of Cattle in the State of Oklahoma.

Mr. W. H. Hudson, President, Oklahoma Retail Grocers Association.

R. K. MATTHEWS
Secretary-Treasurer

Pennsylvania State Short Courses

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

1. Testing Milk, Cream, and Dairy Products, fee $10.25 *
   a. July 24 to 29, 1950.
   b. February 12 to 17, 1951.
2. Ice Cream Course for Dairy Equipment and Supply Men, fee $12.25
   December 4 to 9, 1950.
3. Ice Cream Course for Plant Men, fee $18.75
   January 15 to 27, 1951.
4. Market Milk and Milk Supervision, fee $18.75
   January 29 to February 10, 1951.

Full information and application blanks can be secured from Mr. A. Leland Beam, Director of Short Courses, School of Agriculture, State College, Pennsylvania.

A short course in dairy bacteriology will be held August 14 to 30, 1950, fee $21.00. The daily work will consist of both lecture and laboratory work on the techniques essential to the functioning of a dairy laboratory. A written and practical examination will be given at the end of the course which will be the basis of certification to the State Department of Health for those wishing to be licensed as Dairy Laboratory Directors. This examination will cost $10 per person, and is scheduled for August 31, September 1, and September 2. For further information about this course, write Mr. J. Frank Cone, Associate Professor of Bacteriology. Application for admission should be sent to Mr. Beam, as above directed.

* Non-residents of Pennsylvania must pay an additional fee of $5 per week for each course.

Wisconsin Milk Sanitarians Association

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

L. W. BROWN
Secretary-Treasurer

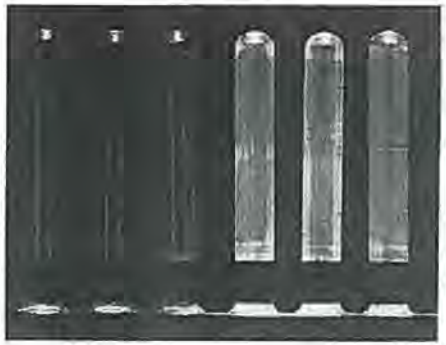
Veterinarians for the U. S. Public Health Service

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

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

Industrial Notes

Two New Wyandotte Products



The six test tubes shown above were immersed for eighteen hours at 210° F. in 0.30 percent solutions of glass washing detergents. The trio of badly etched tubes at the left were exposed to a standard detergent solution; the new-looking tubes at the right were immersed in Wyandotte Dural H—an inhibited detergent for safely washing laboratory glassware by hand.

Cherry-Burrell Corporation Issues New Bulletin

The Cherry-Burrell Corporation has just issued a new Bulletin G-460 describing their line of factory type weigh cans and receiving vats. It is fully illustrated with photographs and line drawings of equipment with dimensions.

Food Sanitation Supervision Spreads to Germany

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

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

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

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

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

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

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

Mr. Raphael's professional affiliations include membership in the New York Academy of Sciences, the New York Microscopical Society, the American Association of Candy Technologists, and the INTERNATIONAL ASSOCIATION OF MILK AND FOOD SANITARIANS.

NEW MEMBERS

ACTIVE

K. E. Cox, Director, W. Virginia State Hygienic Lab., Charleston, 5, West Virginia
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Obituaries

Dr. Wm. A. Evans, Aberdeen, Miss., d. Nov. 8, 1948. Health Commissioner of the City of Chicago, 1907–1911, and health editor of the *Chicago Daily Tribune*, 1911–1934. Honorary Member of this Association.

Dr. Wm. C. Woodward, Washington, D.C., d. Dec. 22, 1949. Health Officer of the District of Columbia 1894 to 1918; health commissioner of the City of Boston, 1918 to 1922. Honorary member of this Association.

A. J. Reppen, Monroe, Wis., d. Feb. 2, 1950. Member of the Wisconsin Milk Sanitarians' Association, and of this Association since 1945.

Thomas L. McPeak, d. Feb. 28, 1950. Member of this Association since 1947.

H. E. Breedlove, Mobile, Ala. Active member since 1942.

Alan J. Turnbull, Racine, Wis. Member of this Association since 1941.