

JOURNAL of MILK TECHNOLOGY

Volume 3

July-August, 1940

Number 4

Editorials

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

Contamination of Drinking Containers

Increasing attention is being given to the sanitization of drinking utensils. This requires a great deal of the health officer's time and involves the expenditure of considerable money by the operator. These steps would be warranted if there was evidence that supported their public health significance. Until clear-cut evidence of a directly positive character is forthcoming, the health officer will be handicapped in finding an effective prophylactic measure and will be hindered by those persons who see only an unwarranted interference with business.

This problem is particularly important in the dairy field. Here, avenues of infection have been closed by the clean production and handling of milk, its pasteurization, the proper cleaning and sterilizing of utensils and equipment, and the sanitization of milk bottles and paper containers. Such well-protected milk should be safe-guarded from contamination by serving it in drinking containers which have been sanitized according to methods which scientific research have shown to be necessary. The same considerations obtain in the dispensing of ice cream, especially at fountains and lunch counters. All the great expense and care of sanitary production and processing may be rendered void by insanity dispensing.

The record as to conveyance of disease by infected glass drinking utensils is perhaps no clearer than the record of conveyance of disease by other vectors, and not as clear as some. Dr. Cumming's studies (1) in army cantonments at the time of the World War still remain the most notable epidemiological record in this matter. Even this evidence, like most of that for conveyance of typhoid fever through milk, is largely presumptive. It shows only that in a large group of men whose eating and drinking utensils were poorly washed, the influenza rate was significantly higher than in a comparable group whose drinking utensils were thoroughly sanitized.

Beyond this, the exact evidence of disease transmission is rather fragmentary. Physicians and others in various parts of the country have reported instances where in their opinion certain individuals had been infected by trench mouth, syphilis, and other diseases through the common drinking glass which had not been adequately sanitized. However, these reports cannot be considered to be scientific evidence although they have been made by persons with scientific training.

On the whole, it would seem that this subject is in much the same position that prevailed some years ago with respect to untreated water supplies. As is generally known, it was difficult if not impossible to recover a typhoid organism from the water or to prove that any single case of typhoid fever was the result of drinking from an infected supply. Frequently the best that could be done was to show that the channel was open for organisms from the intestinal tract to reach the water supply and to demonstrate the presence of colon bacilli—which may or may not have come from the intestinal tract.

The situation that obtained then is now analogous with respect to eating and drinking utensils. There is evidence that the channel is open for the transmission of organisms that may be discharged from the respiratory tract. Bacteriological studies of drinking glasses and other utensils have shown the presence of organisms usually found in the respiratory tract, some of which appear to be pathogenic.

There is the greatest need for experimental work in this field. A great service to public health would be rendered by research studies set up with proper controls to secure much-needed evidence. The brilliant success which has attended health workers in reducing intestinal-borne diseases by blocking the channel of infection can be repeated in reduction of respiratory diseases by blocking the channels of infection of those diseases. All evidence seems to point to the fact that these diseases are communicated by secretions of the nose and throat. We need to know a great deal more about the behavior of these infectious agents as they pass from one host to another through various vectors.

J. H. S.

- (1) Eating Utensil Sanitation, by J. G. Cumming and N. E. Yongue. *Amer. J. Pub. Health* 26, 237 (1936). This paper contains references to Cumming's earlier work. See also: The Incidence of *Borrelia Vincenti* and Other Spirochaetaceae on Beverage Glasses, by D. Lyons, *J. Bact.* 31, 523 (1936).

New Federal Transfers

The Federal Food and Drug Administration will become a subsidiary organization in the Federal Security Agency after June 30th, in accordance with authority recently given the President by Congress. This food control unit used to be the Bureau of Chemistry, an important unit in the Department of Agriculture. It has been re-organized several times, the latest name being the Food and Drug Administration. It is somewhat difficult for old-timers in food control work to think of this excellent bureau as no longer a part of the Department of Agriculture. Without doubt, its functions have been expanding far outside of its original agricultural setting. The new change is supposed to be in the interest of increased efficiency. We cannot see how food and drug control can be effectively administered without much investigation and even a degree of research. This means either a new series of laboratories or much lost motion and administrative difficulty in getting another independent unit interested to devote itself to "imported" problems.

The Public Health Service, so long known as a part of the Treasury Department, likewise becomes a part of the Federal Security Agency.

It is doubtless an advisable move to group these two agencies in the same administrative unit because both have been developing along lines that have been increasingly approaching a degree of overlapping, and particularly it is desirable to recognize food control as more a health problem than an agricultural one.

J. H. S.

Possible Errors in the Phosphatase Test Resulting from Bacterial Growth in Milk*

Harold W. Leahy, Leslie A. Sandholzer, and Marian R. Woodside

Rochester Health Bureau Laboratories, Rochester, New York

It has been demonstrated in this laboratory (1) that a wide variety of gram-negative bacilli contain a phosphatase which possesses the ability to hydrolyze disodium phenyl phosphate. Under optimal conditions some organisms were found to liberate as much as 1.12 mg. of phenol per mg. of lyophilized cells in 24 hours at 37° C. In view of these findings, it seemed advisable to investigate the effect of bacterial growth in milk upon the various phosphatase tests employed for the detection of improperly pasteurized milk.

PROCEDURE

The following technic was employed. Cultures belonging to the genera *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Salmonella*, *Escherichia*, *Aerobacter*, *Klebsiella*, and *Pseudomonas* were inoculated in duplicate into tubes of sterile milk. One tube of each culture was incubated for 24 hours and the other for 48 hours at 37° C. Following incubation, 0.5 cc. portions of the 24-hour cultures were added to each of four tubes (A, B, C, and D) containing 10 cc. of Kay-Graham's (2) disodium phenyl phosphate solution buffered with sodium veronal to a pH of 9.6. In like manner, 0.5 cc. portions of the 48-hour cultures were added to a similar series of tubes (E, F, G, and H) containing 10 cc. of Kay-Graham's buffered substrate. To tubes A and E, 4.5 cc. of Folin-Ciocalteu's reagent (3) had been added previously to provide controls. To tubes B and F, 0.1 cc. of basic lead acetate solution (U.S.P.) was added immediately and thoroughly mixed. These

tubes also served as controls. Additional controls were prepared from uninoculated skim milk in buffered substrate solution, one tube for each reagent. A drop of chloroform was added to each of the remaining tubes (C, D, G, and H), and the mixtures were incubated for 24 hours at 37° C. At the end of this period, 4.5 cc. of Folin-Ciocalteu's reagent were added to tubes C and G, while 0.1 cc. of basic lead acetate was added to tubes D and H. The contents of tubes A, C, E, and G were then filtered through Whatman No. 30 filter paper, and color formation was brought about in the filtrates by continuation of the method according to the Kay-Graham technic (2).

The mixtures in the remaining tubes (B, D, F, and H) containing the basic lead acetate solution were also filtered. When necessary, the filtrates from these tubes were clarified with a few drops of M/5 sodium pyrophosphate solution. To each filtrate 0.1 cc. of Gibbs's phenol reagent (4) was added. This reagent is a freshly prepared 0.4 percent solution of 2,6-dibromoquinonechloroimide in 95 percent ethyl alcohol. The colors formed by Folin-Ciocalteu's and Gibbs's reagents were then compared with their corresponding standards containing known amounts of phenol treated in like manner. The color comparisons were made in a Bausch and Lomb colorimeter using appropriate filters for the respective colors formed. The colors obtained with each reagent in the cultures and in the controls were expressed as milligrams of phenol producing an equivalent color intensity. In addition to this measurement of phosphatase activity, direct microscopic counts and electrometric pH determinations were made on most cultures.

*Read before the Laboratory Section, 68th Annual Meeting of the American Public Health Association, Pittsburgh, Pennsylvania, October 17-19, 1939.

RESULTS

The results obtained by subjecting the various cultures to this procedure are shown in table I. In approximately one-fourth of the cases the unincubated controls gave high values with Folin-Ciocalteu's reagent. The culture showing the strongest reaction in this respect was *Streptococcus liquefaciens* (8). Employing the same reagent, the cultures of *Lactobacillus acidophilus* (2), *Lactobacillus helveticus* (6), *Escherichia sp.* (16), *Aerobacter oxytocom* (20 and 22), and *Pseudomonas sp.* (29) also produced values higher than the uninoculated control. On the other hand, only one control among the 28 cultures examined,

Aerobacter oxytocom (22), showed a measurable amount of phenol with Gibbs's reagent.

The results of the incubated phosphatase tests showed that 16 cultures, or more than half of those studied, produced values higher than normal with Folin-Ciocalteu's reagent. Seven of the cultures, *Lactobacillus helveticus* (6), *Streptococcus liquefaciens* (8), *Streptococcus agalacticae* (9), *Salmonella paratyphi* (11), *Escherichia sp.* (16), *Aerobacter hibernicum* (25), and *Pseudomonas sp.* (29), showed no phenol present when tested with Gibbs's reagent. In contrast with these results, the cultures of *Staphylococcus aureus* (10), *Aerobacter*

TABLE I.

The Development of Phosphatase in Milk as the Result of Bacterial Growth

No.	Organism Examined	Bacterial Counts		pH		Equivalent Phenol Values ¹						
		x10 ⁶		24 hr.	48 hr.	Unincubated Control		Phosphatase Tests				
		24 hr.	48 hr.			Folin's	Gibbs's	Folin's	Gibbs's			
	Sterile milk control	—	0	—	6.51	—	—	—	.02	—		
2	<i>L. acidophilus</i>	99	1130	5.78	4.56	0.3	.08	0	0	.04	.08	0
3	<i>L. acidophilus</i>	62	1720	5.40	3.57	.05	.05	0	0	.04	.08	0
4	<i>L. acidophilus</i>	206	1530	5.25	3.86	.03	.06	0	0	.03	.05	0
5	<i>L. acidophilus</i>	48	186	5.80	4.53	.02	.05	0	0	.02	.02	0
6	<i>L. helveticus</i>	64	175	5.98	5.23	.06	.08	0	0	.06	.16	0
7	<i>L. bulgaricus</i>	10	19	5.97	5.77	.02	.05	0	0	.03	.04	0
8	<i>Strep. liquefaciens</i>	314	2700	4.48	4.01	.12	.17	0	0	.28	.32	0
9	<i>Strep. agalacticae</i>	216	1950	5.84	—	.03	.04	0	0	.07	.12	0
10	<i>Staph. aureus</i>	194	1610	5.73	5.70	.05	.06	0	0	.10	.13	3.0
11	<i>Sal. paratyphi</i>	705	1030	—	—	.02	.03	0	0	.07	.09	0
12	<i>Sal. sp. 398-S</i>	236	390	—	—	.02	.02	0	0	.04	.06	0
13	<i>E. communis</i>	179	1200	6.02	5.65	.05	.06	0	0	.06	.08	0
14	<i>E. communior</i>	686	930	4.76	4.31	.02	.03	0	0	.06	.08	0
15	<i>E. sp. 229-1</i>	308	420	5.06	4.72	.03	.04	0	0	.07	.07	0
16	<i>E. sp. 234</i>	226	610	4.96	4.51	.05	.07	0	0	.08	.10	0
17	<i>A. aerogenes</i>	360	950	4.80	4.96	.03	.06	0	0	.28	.38	5.0
18	<i>A. aerogenes</i>	189	734	4.35	4.48	.06	.06	0	0	.20	.47	5.0
19	<i>A. aerogenes</i>	183	812	4.54	4.71	.04	.05	0	0	.16	.50	0
20	<i>A. oxytocom</i>	189	612	4.52	4.61	.02	.07	0	0	.26	.32	7
21	<i>A. oxytocom</i>	144	804	5.77	6.13	.02	.04	0	0	.04	.08	0
22	<i>A. oxytocom</i>	123	756	5.61	5.65	.07	.09	2.5	5.0	.27	.29	7
23	<i>A. cloacae</i>	293	1050	5.08	5.13	.02	.04	0	0	.07	.08	0
24	<i>A. cloacae</i>	226	972	5.31	—	.02	.04	0	0	.06	.09	0
25	<i>A. hibernicum</i>	434	1100	4.81	4.92	.02	.06	0	0	.13	.16	0
26	<i>Klebsiella sp. 901-A</i>	84	380	5.43	6.18	.03	.04	0	0	.10	.10	5.0
27	<i>Klebsiella sp. 901-B</i>	78	420	5.32	5.61	.02	.03	0	0	.08	.11	7.5
28	<i>Klebsiella sp. F.B.</i>	168	1250	5.56	5.71	.05	.06	0	0	.07	.13	1.7
29	<i>Pseudomonas sp. 1367</i>	405	1010	6.01	—	.06	.09	0	0	.14	.16	0

¹ The values for Folin-Ciocalteu's reagent are in terms of mg., while those for Gibbs's reagent are reported in gammas (.001 mg.)
T=trace

aerogenes (17, 18, and 19), *Aerobacter oxytocom* (20 and 22), and *Klebsiella sp.* (27 and 28) produced measurable amounts of phenol with Gibbs's reagent as well as values above normal with Folin-Ciocalteu's reagent. The latter cultures, therefore, demonstrated a definite phosphatase activity under the conditions of the tests. All of the cultures studied which gave positive reactions with Gibbs's reagent also gave high values with Folin-Ciocalteu's reagent.

No marked correlation was noticed between the pH of the various cultures examined and their ability to produce high phenol values with either Gibbs's or Folin-Ciocalteu's reagents. Moreover, little or no relation was observed between the bacterial counts and the intensity of the colors produced. The lowest microscopic count on any culture which produced values higher than normal was 64,000,000 per cc., which was obtained with *Lactobacillus helveticus* (6). This corresponds to a standard plate count of 16,000,000 per cc. (5).

The results obtained with the cultures of *Lactobacillus helveticus* (6), *Streptococcus liquefaciens* (8), *Streptococcus agalacticae* (9), *Escherichia sp.* (16), *Aerobacter hibernicum* (25), and *Pseudomonas sp.* (29) indicated that other substances beside phenol were responsible for the colors given by Folin-Ciocalteu's reagent. To determine, if possible, the nature of these substances, a number of carefully purified compounds were treated in veronal buffer solution with Folin-Ciocalteu's reagent and with Gibbs's phenol reagent. The standardized procedure for examination was to dissolve a weighed amount of each substance in distilled water and dilute until its concentration was from 1 to 10 mg. per cc. Appropriate dilutions of each compound were then prepared in sodium veronal solution at pH 9.6. Following this, duplicate portions of each solution were treated quantitatively, one portion with Folin-Ciocalteu's reagent and the other with Gibbs's reagent. Comparison was made of the colors produced by the

various reagents, and their phenol equivalents were determined as previously described.

The results of this experiment demonstrated that small concentrations of many non-phenolic compounds were able to reduce Folin-Ciocalteu's reagent to give a blue color. In many instances, the intensity of the color given by Folin-Ciocalteu's reagent was greater than that given by an equivalent concentration of phenol. Those compounds producing the strongest reactions with Folin-Ciocalteu's reagent are shown in table II. The amino acids *l*-tyrosine, diiodotyrosine, tryptophane, cystine, and *l*-leucine gave a very strong color with Folin-Ciocalteu's reagent in concentrations of from 0.01 to 0.1 mg. per 10 cc. Moreover, indole and indole derivatives in the same concentrations produced marked color with this reagent. The oxypurines, uric acid, xanthine, and guanine, and the pyrimidine, allantoin, also gave strongly positive tests in low concentrations. The aminopurine, adenine, on the other hand, gave no color with this reagent. The ketones, diacetyl and acetyl acetone, as well as the hydroxy ketone, acetyl methyl carbinol, gave positive tests. It was found, furthermore, that ferrous, manganese, and stannous ions and sulfides of the alkali metals affected Folin-Ciocalteu's reagent. (These are not shown in table II). None of the compounds examined produced the typical blue color with Gibbs's reagent. A few compounds gave slightly yellowish or slightly brownish colors with Gibbs's reagent, but the colors so produced could not be confused with the blue color given by phenol itself.

DISCUSSION

From the data recorded in the present paper, it is obvious that many easily oxidizable substances produce strong colors with Folin-Ciocalteu's reagent. Moreover, these data confirm and extend the work of Gortner and Holm (6) who found that many compounds were easily oxidized by this reagent to form a blue color. It is apparent, therefore, that

TABLE II
Phenol Equivalents Given by Bacterial Metabolites with
Folin-Ciocalteu's and Gibbs's Reagents

Substance Examined	Concentration mg. in 10 cc.	Amount of Phenol giving Equivalent Colors by	
		Folin-Ciocalteu's Reagent mg. in 10 cc.	Gibbs's Reagent mg. in 10 cc.
Diacetyl	0.1	0.12	0
Acetyl acetone	0.1	0.21	0
Acetyl methyl carbinol	1.0	0.16	0
l-Tyrosine	0.1	0.58	0
l-Tyrosine	0.01	0.09	0
Diiodotyrosine	0.1	0.35	0
Diiodotyrosine	0.01	0.06	0
Tryptophane	0.1	0.48	0
Tryptophane	0.01	0.10	0
Cystine	0.1	0.22	0
l-Leucine	0.1	0.05	0
Indole	0.1	0.31	0
3-Methyl indole	0.1	0.27	0
Indole-3-N-propionic acid	0.1	0.28	0
Uric acid (2, 6, 8-Trioxypurine)	0.1	0.29	0
Uric acid	0.01	0.06	0
Xanthine (2, 6-Dioxypurine)	0.1	0.17	0
Allantoin (glyoxal diureide)	0.1	0.05	0
Guanine (2, amino-6-oxypurine)	0.1	0.33	0
Adenine (6 amino purine)	0.1	0.00	0
Vanillin	0.1	0.19	0

Folin-Ciocalteu's reagent cannot be employed to determine phenol (C_6H_5OH) unless it is certain that there is no other substance present which may reduce the reagent. Compared to this reagent, that of Gibbs is far more specific for phenol. Gibbs's reagent, however, forms no indophenol colors with those phenols substituted in the *para* position. This accounts for the absence of color formation when tyrosine and vanillin were treated with Gibbs's reagent.

The experiment comparing Folin-Ciocalteu's reagent with that of Gibbs explains the disagreement between the two when applied to the bacterial phosphatase studies. It was pointed out previously that a number of cultures gave strong colors with Folin-Ciocalteu's reagent, but no color with Gibbs's reagent. Many of the pure compounds which reacted with Folin-Ciocalteu's reagent, however, may be classified as bacterial metabolites. For example, the strain of *Streptococcus liquefaciens* (8) and the unidentified *Pseudomonas* (29) are strongly proteolytic. A slight hydrolysis

of casein by these organisms should form sufficient tyrosine, tryptophane, cystine, and leucine to produce considerable color with Folin-Ciocalteu's reagent. It is known that the hydrolysis of casein yields 10.5 percent leucine, 4.5 percent tyrosine, 1.5 percent tryptophane, and 0.2 percent cystine (7). The production of color by these products of bacterial metabolism, therefore, becomes a more convincing probability. It is suggested that other proteolytic organisms might produce similar results.

It was also pointed out (table I) that the cultures of *Lactobacillus helveticus* (6), *Streptococcus liquefaciens* (8), *Streptococcus agalacticae* (9), *Salmonella paratyphi* (11), *Escherichia sp.* (16), *Aerobacter hibernicum* (25), and *Pseudomonas sp.* (29) gave higher values with Folin-Ciocalteu's reagent in the incubated tests than in the controls. These cultures, however, gave negative results with Gibbs's reagent. It would appear that the cultures possessed an active phosphatase as indicated by Folin-Ciocalteu's reagent, but the fact that no phos-

was indicated by Gibbs's reagent shows that this is not the case. The limit of detection of phenol by either reagent is approximately the same (0.5 p.p.m. for Gibbs's reagent and 1.0 p.p.m. for Folin-Ciocalteu's reagent). If a true phosphatase had been active, phenol would have been detected by Gibbs's reagent. Perhaps the nucleoproteins or the nucleic acids within the bacterial cells which were added to the mixture hydrolyzed by long contact with the alkali (pH 9.0) to yield purine derivatives. It was found, in a separate experiment, that hydrolysis of yeast nucleic acid at pH 9.0 for 24 hours at 37° C. yielded increased colors with Folin-Ciocalteu's reagent. This fact coupled with the fact that various oxypurines give strong reactions with Folin-Ciocalteu's reagent (table II) explains the false phosphatase tests obtained.

That bacterial growth in milk may cause subsequent hydrolysis of the disodium phenyl phosphate in the phosphatase test is quite evident. The results obtained with the cultures of *Staphylococcus aureus* (10), *Aerobacter aerogenes* (17, 18, and 19), *Aerobacter oxytocum* (20), and *Klebsiella sp.* (27 and 28) definitely indicate that certain bacterial phosphatases show some activity at pH 9.0. A heavy growth of organisms, however, is required to interfere with the test when Gibbs's reagent is employed. The least number of organisms determined by microscopic count in any culture showing a positive result with Gibbs's reagent was 78,000,000 per cc. obtained with a strain of *Klebsiella sp.* (27). The color produced in this case was approximately ten times that allowed for properly pasteurized milk. Phosphatase tests made on milks having microscopic counts higher than 7,800,000 per cc., therefore, should be interpreted cautiously. This corresponds to a standard plate count of approximately 1,950,000 per cc. (5).

The least amount of growth in any culture showing values higher than normal with Folin-Ciocalteu's reagent was 64,000,000 per cc. as determined microscopically with *Lactobacillus helveticus*

(6). The intensity of color formed by the culture was eight times as great as that of the uninoculated control. A growth of this organism showing microscopic counts higher than 8,000,000 per cc., therefore, should produce colors above normal. This is equivalent to a standard plate count of 2,000,000 per cc. Thus, it is safe to conclude that bacterial growth in milk greater than 8,000,000 per cc. as determined by the microscopic method, or 2,000,000 per cc. as determined by the standard-plate-count method, may affect the various phosphatase tests. The results of phosphatase tests made on pasteurized milk containing more than this number of bacteria should be interpreted with caution.

Moreover, it is felt that a few other precautions suggested by this study should be pointed out. False positive tests and controls of high color intensity may be obtained on butter, cheese, and ice cream with the Kay-Graham method. The variable diacetyl and acetyl methyl carbinol content of butter might be a source of error. Many kinds of cheese contain high concentrations of bacterial metabolic products (indole, tryptophane, tyrosine) and also innumerable bacterial cells which may exert a dephosphorylating action. In the case of ice cream, vanillin would produce controls having colors higher than normal. Furthermore, one of us (H.W.L.) has found in current studies (unpublished) that certain chocolates produced extremely dark colors with Folin-Ciocalteu's reagent. Many chocolates also contain a phosphatase of weak activity.

The results obtained by Gilcreas (8) on gravity-separated cream may be explained by the fact that cream undoubtedly contains a larger proportion of bacterial cells per unit volume than either whole milk or skim milk. It should be pointed out that more time is required for the gravity method of cream separation than for the centrifugal method. In view of this, bacterial growth would be favored in the former process, and this would tend to give increased color in the

Kay-Graham method. An increased phenol value given by properly pasteurized cream over that given by properly pasteurized whole milk from which it was separated, however, has never been experienced in this laboratory when Gibbs's reagent was employed.

It is shown in table II that uric acid gives a strongly positive test with Folin-Ciocalteu's reagent in a concentration of 1 p.p.m. On the average, milk contains this substance in a concentration of 1.5 p.p.m. (7). With these facts in mind, it is indeed surprising that blank determinations having values below 0.09 mg. of phenol equivalent can be obtained on milk with Folin-Ciocalteu's reagent. Experience, however, bears out this contradiction for which there is no adequate explanation at the present time.

SUMMARY

Twenty-eight cultures of organisms belonging to the genera *Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Escherichia*, *Aerobacter*, *Salmonella*, *Klebsiella*, and *Pseudomonas* have been tested for their ability to produce phosphatase when grown in milk. One strain of *Staphylococcus*, five strains of *Aerobacter*, and three of *Klebsiella* were shown to produce a true phosphatase reaction which showed slight activity at pH 9.0. Seven additional strains of various genera (*Lactobacillus*, *Streptococcus*, *Escherichia*, *Salmonella*, *Aerobacter*, and *Pseudomonas*) were found to produce a false phosphatase reaction when Folin-Ciocalteu's reagent was employed.

A comparison of Folin-Ciocalteu's reagent with the phenol reagent of Gibbs

Influence of Sunlight on Flavor and Ascorbic Acid Content of Milk Exposed in Three Different Types of Paper Containers. J. L. Henderson, D. C. Foord, and C. L. Roadhouse, *Food Research*, 5, 153 (1940).

Three types of paper milk-containers of different paper stocks were compared for protective effects against the flavor production and ascorbic acid destruction caused by exposure to sunlight.

All the paper containers were found to exhibit greater protection from the effects of the

demonstrated the superiority of the latter. Folin-Ciocalteu's reagent gave as strong colors with ketones, hydroxy ketones, indole derivatives, oxygenated purine derivatives, amino acids, and several inorganic ions as it gave with phenol itself. It is suggested that false phosphatase reactions obtained with the Kay-Graham method are due to the hydrolysis of the bacterial nucleic acids to oxypurines. Phosphatase tests made upon milks having microscopic counts higher than 8,000,000 per cc., or standard plate counts higher than 2,000,000 per cc. should be interpreted with caution.

NOTE:—The authors wish to express their thanks to Dr. R. S. Breed of the Geneva Agricultural Experiment Station and to Dr. L. H. Burgwald of Ohio State University for supplying some of the cultures used in this study.

REFERENCES

- (1) Leahy, H. W., Sandholzer, L. A., and Woodside, M. R. The Hydrolysis of Disodium Phenyl Phosphate by Gram-negative Bacilli. *J. Bact.*, 38, 117 (1939).
- (2) Kay, H. D., and Graham, W. R., Jr. The Phosphatase Test for Pasteurized Milk. *J. Dairy Res.*, 6, 191 (1935).
- (3) Folin, O., and Ciocalteu, V. On Tyrosine and Tryptophane Determinations in Proteins. *J. Biol. Chem.*, 73, 627 (1927).
- (4) Gibbs, H. D. Phenol Tests. III. The Indophenol Test. *Ibid.*, 72, 649 (1927).
- (5) American Public Health Association. *Standard Methods of Milk Analysis*. A. P. H. A. New York, 1934, p. 34.
- (6) Gortner, R. A., and Holm, G. E. The Colorimetric Estimation of Tyrosine by the Method of Folin and Denis. *J. Am. Chem. Soc.*, 42, 1678 (1920).
- (7) Associates of Lore A. Rogers. *Fundamentals of Dairy Science*, Chemical Catalog Co., Inc., New York, 1935, pp. 3 and 49.
- (8) Gilcreas, F. W. Value of Phosphatase as an Indicator of Pasteurization. *A. J. P. H.*, 29, 158, (1939).

light rays than did clear glass milk bottles.

Paper containers studied varied greatly among themselves in their ability to protect the milk from the development of "sunshine flavor."

The container made of thick paper with bleached or colored inner plies gave complete protection against the flavor defect and complete protection against ascorbic acid destruction.

The effect of sunlight on ascorbic acid destruction was found to be useful as an indicator of the effect of sunlight on milk flavor.

M. E. PARKER

Comment on Licensing of Pasteurizer Operators from Oakland, California

R. L. Griffith

Oakland, Cal.

The Oakland Municipal Code of August 14th, 1933, reads as follows:

"Section 4-2.24—Pasteurizer's Permit

It shall be unlawful for any person to engage in, or for any person to cause or permit an employee to engage in, operating a pasteurizer for the processing of pasteurized market milk, unless the person operating such pasteurizer shall have first obtained and shall hold a valid permit to do so, which permit shall be issued by the Health Department of the City of Oakland. Application for such permit shall be made in writing to the Chief Dairy and Milk Inspector and shall contain such information as he may require. The applicant shall satisfy the Chief Dairy and Milk Inspector that he is fully qualified and that he possesses a thorough knowledge of all provisions of law applicable and pertaining to pasteurization. Such permit shall in no event be transferable.

"Any pasteurizer's permit herein provided for may be revoked by the Chief Dairy and Milk Inspector for violation by the permittee of any of the provisions of the Pure Milk Law or the General Dairy Law of the State of California or of this Article."

FORMER TYPE OF EXAMINATION

The first examinations given were of the question and answer type, listing thirty general questions with answer attached, concerning the law and practice of pasteurization. This had been prepared by the State Department of Agriculture for the San Joaquin Local Health District at Stockton, California, and had been in use by them for a year or two previously. Approximately seventy men appeared for

the first examination from about twenty plants, five of which were outside of Oakland but which held permits for distributing milk in Oakland. Practically all of these men passed upon first examination, since the material was simple and presented in its easiest form. Ten questions were selected at random from this list, and four separate sets were compiled. A different set was then used in each section of the examination and for later individual examinations during the year.

We quote a typical question and answer of this type:

6. Q. Why is agitation necessary during the process of pasteurization?

A. Agitation is necessary during the process of pasteurization to be sure that all of the milk is uniformly heated and maintained at the proper temperature.

The disadvantages of such an arrangement of examination seem to us to be numerous. It would be difficult to cover a field very intensively with so cumbersome a system. We found with the small number of questions that some of the men with little or no experience memorized the entire set of answers and made very high grades, whereas the more experienced and practical men could not make such high grades. Also considerable time is required to read and grade several pages of uncertain written material, and some applicants have difficulty in writing out what they know. To avoid many of these difficulties, we considered it best to prepare our own instructions, since this entailed no purchase

of books or material by employees, and a large amount of ground could be covered as briefly or fully as considered necessary, with as much or little detail and technical material as desired. Also we could thereby make this study course fit the necessities of our men, avoid technical language, and include many things we consider of importance for a pasteurizer operator to know, and which could not be readily found available in books and bulletins. Thus we made our instruction sheet separate from the examination sheet, and made the instruction very brief, concise, and as clear as possible, covering the general topics of history of pasteurization, state and local laws, purpose of pasteurizing, methods, effects of pasteurizing, cleaning of equipment, recording thermometers, and modern machinery. As a beginning, our examination covers only market milk and cream, but as the plan is developed, we expect to include the pasteurizing of all other dairy products, since there is no difference in the protection to public health, regardless of the type of product.

PRESENT TYPE OF EXAMINATION

Our first examinations five years ago were rather general and brief. The written answers were graded and passed on the basis of 75 percent, with the same weight to all questions. The second year, the separate instructions were enlarged considerably, containing about twice the material of the first year's question and answer set. The third year's instructions contained about fifty percent additional material. Further enlargements of these instructions will no doubt cover other dairy products than market milk and additional detail in many places, as for example, bacteriology and scientific points of pasteurization. In the later stages, the examination may also be changed to the true and false type. The more modern types of examination seem to bring out the practical working knowledge of the applicant far better than the older written essay form. The multiple choice, completion, or true and false types have some similarity to actual working condi-

tions where an operator must choose definitely between a right or wrong way of doing the detail of a certain operation. This course, covering three years or more with advancing stages of instruction and examination, prevented throwing older men of long experience out of work on account of failures, and gradually trained these men over the three year period of study and practice. It has proven highly satisfactory in our location. The type of men we have to deal with in the case of many of our pasteurizers, are foreign born or of foreign extraction, and a few do not read and write English, and very few have had training in dairy schools. In the past three years, we have had only two or three failures out of about eighty examinations annually. We feel this speaks well for the progressive system used, for the simply clarity of the instruction sheet, and the examination adapted to the needs and understanding of our men.

This type of examination was chosen after some study because it is about the simplest form of examination paper to place before a person where we do not wish to test the general intelligence but are only seeking to find the practical working knowledge of the applicant. The preparation of this type of paper requires considerable work, since each question must be readily understood and not misinterpreted, and the statement must clearly apply to only one of the words given. Any statement misunderstood by a considerable percentage of applicants is in need of clarifying until the percentage of errors is largely reduced. These are called multiple choice statements, and seem to be adapted best to the less educated class of men.

The completion type, where a word or more of completion must be written at the end of the statement, may be used for better educated classes. The true and false statements, while similar to the multiple choice method, require men of intelligence and training. This in general is the reason why we choose

the form which is simple for the examiner to operate and grade, and is adjusted to the many men who lack school advantages. The section relating to state and local laws should of course be revised to conform to the locality where used, and be certain to include all the laws and regulations relating in any way to pasteurizing of the products to be covered. The rules for grading this examination can be readjusted by anyone to suit their own conditions. A larger percentage can be allowed for schooling and experience if considered advisable. A larger or smaller number of statements may be used, but should be kept between 80 and 120. Other technical information regarding examinations can be supplied no doubt by your local Chief Examiner of Civil Service or some of his technical assistants.

Part of a sample examination is given on page 192. The last ten questions of the examination are of the completion type.

RESULTS OF LICENSING

Our past three years experience in examining pasteurizer operators shows that the men are enthusiastic regarding the study and the examinations. They have taken the work to heart and have been very serious in their study, and judging from the very high percentage that have passed the examinations, they have secured a very great deal of good from it. A few who do not read English relied on their wives or children reading the instructions to them many times over a period of several weeks until all details new to them were thoroughly in mind.

It would be difficult to say as to what the effect has been on the safety of pasteurized milk since the installation of a pasteurizer's permit in this City. Our state has required for many years licenses for weighers and samplers of milk and for all operators of the Babcock test, where these tests are used for payment to farmers. However, no attention has been paid legally to the education, training, dependability, and veracity of the person doing the actual pasteurizing of market

milk and cream. Yet health officials, milk inspection departments, and many others lay great stress upon the necessity for pasteurization of our general run of bulk milk. Several attempts have been made to include pasteurizer operators' licenses among others in California but none have so far succeeded. The state has given great attention to the licensing of men operating the commercial angles of the milk industry, but it has not applied the same reasoning and protection against incompetence, poor training, carelessness, and deceit when it comes to the very important operation of pasteurization.

This practice now gives us a means of control which affects directly the man responsible for safe pasteurizing through the suspension or revocation of his pasteurizer's permit. In this locality, the pasteurizer's wage is \$10 per month above other inside plant workers, and a few receive as high as \$25 above schedule, or \$200 per month. Our creamery operators have been greatly interested in our work of training our pasteurizer men, although so far it has been rather meager.

Out of our entire 80 active men, only a small percentage have attended dairy school for their training. However, this is not always a criterion for a good pasteurizer operator. Along with intelligence and training goes the most important factor of trustworthiness. I am inclined to think that this should bear more weight than training and experience. We have a number of men who have had no schooling in this country and none in their European homeland outside of grammar school, yet I consider them to be among our best pasteurizers simply because they are perfectly reliable, dependable, and honest in all of their work and dealings with the company and the Health Department. On the other hand, some of our old experienced men and some of our college graduates occasionally slip when it comes to the factor of trustworthiness in attention to every detail of pasteurizing. The City of San Jose, California,

Sample Examination
CITY OF OAKLAND
 Health Department
 Dairy and Milk Inspection Division
EXAMINATION FOR PASTEURIZER'S PERMIT

Fill in the following information concerning yourself: Date.....193.....
 Name Age Phone.....
 Address City.....
 Plant Health Certificate Expires.....193.....
 Years experience in pasteurizing: Full Time..... Relief.....
 Years in school: High..... College..... Others.....
 Time in dairy schools (or short courses).....

In each sentence below you have several choices for the ending. Underline the ONE word or phrase making the truest statement. The two samples just beneath are correctly marked.
 SAMPLES: Louis Pasteur was a scientist of.....England.....France.....Italy

After production, market milk and cream for Oakland must be pasteurized within2 days.....24 hours.....30 hours.

The chief source of bovine tuberculosis germs in unpasteurized milk is.....untested cows.....strainers.....dirty cows.

In the following sentences, FILL IN the proper words which have been omitted at the end of each.

For sterilizing the open parts of equipment, use

The temperature in all parts of pasteurizing milk must be.....

I HAVE NEITHER RECEIVED NOR GIVEN HELP DURING THIS EXAMINATION.
 NAME

Rules for Grading
CITY OF OAKLAND
 Health Department

Dairy and Milk Inspection Division

RULES used in grading third grade Pasteurizers' examinations for 1937 permits.

Total grade allowed on perfect paper100%
 Grade allowed on pasteurizing experience and schooling10%
 Grade allowed on 100 points (items) of multiple choice and completion questions.....90%

Grading on Personal Section

For each year pasteurizing experience, not over five.....1%
 For each year pasteurizing experience, relief or part time.....1/2%
 For high school completion, (if not college graduate).....1%
 For college completion or equivalent, (each year .5).....2%
 For agricultural college completion.....5%
 For agricultural college each year less than four.....1%
 For each ten day or weeks short course in dairying.....1/2%
 No credit allowed for high school work, to college graduates.

Grading on Multiple Choice Section

For omission to answer questiondeduct 1 point
 For underlining more than one word....." 1 "
 For underlining wrong word in statement....." 1 1/2 "

Grading on Completion Section

For incorrect or incomplete answer to statement.....deduct 1 point

Computing the Final Grade on Paper

Add the total deductions (cuts) made on the last two sections. Subtract this amount from 100 (the total number of questions) and multiply this remainder by .9 to reduce the credits on a basis of the 90% allowed on these sections. Add this result as per cent to the percent allowed on the personal section. This sum is the final grade on the paper. 70% is a passing grade.

and the San Joaquin Local Health District at Stockton, California, also have had pasteurizers' examinations in use for some time.

The application form used may be varied to suit the needs of the department, and should be used to check against statements made in the examination upon which credit is given, and these may also be verified on the outside. For the past two years, we have given no examinations, but required application for renewal of permits, since we did not desire permits to run indefinitely, or have permits outstanding to men who have left the city or creamery business, in other words we wish all our permit holders to be on the active list. New and enlarged examinations are subject to call at any time around the end of a year. A card index is kept which gives quick reference to any permit at a moment's notice. The following form is used:

No fees for pasteurizers permits have so far been charged, although there is no reason why a small fee should not be collected to cover cost of work required and materials, if so desired by the department. The permit form was approved by the City Attorney's office so that some important legal points would not be overlooked. An up-to-date list of valid permits is kept posted on the office bulletin board and furnished field inspectors.

On May 28, 1940 Oakland's new milk ordinance was passed and went into effect. This provides for the licensing of operators for the pasteurizing of all dairy products. The Instructions to Pasteurizers are under revision and enlargement to include additional information covering all types of dairy products. For the first time, an examination fee, amounting to \$3, is required of the applicant.

DOE, JOHN J.

Yr.	Exam'd	Grade	Pos.	Remarks
1935	11/10/34	92.0%	Rel.	Lucerne
1936	12/13/35	67.9%	Failure	
do	1/9/36	83.2%	Past.	Lucerne
1937	1/29/37	77.9%	Past.	Lucerne
1938	1/1/38	—	Past.	Lucerne Ren.
1939	1/1/39	—	Ext.	Golden Valley Ren.

(This is the common 3 x 5 inch file card. Failures are in red. Position is indicated by Past.—Pasteurizer; Rel.—relief; Ext.—extra. Name of plant is inserted with any changes each year. Ren. is for renewal without examination.)

Influence of Feeds on Lecithin Content of Milk and Possible Relationship of Lecithin Content to Susceptibility of Milk to Copper-Induced Oxidized Flavor. I. A. Gould, W. K. Fox, and G. M. Trout. *Food Research*, 5, 131 (1940).

No relationship was observed between the susceptibility of milk to copper-induced ox-

idized flavor and the lecithin content. The individuality of the cows was apparently the most important factor in this connection, and rather wide variations in the percentage of lecithin in the milk could not be correlated with variations in susceptibility of the milk to oxidation.

M. E. PARKER.

Syllabus on Milk Sanitarians Associations

V. M. Ehlers

Texas State Department of Health, Austin, Texas

VALUE OF ORGANIZATION

The value of organization to society might be enumerated as follows: It unifies effort, it provides standardization, it avoids dictatorship and duplication, it assures mass judgment, it makes available research, it permits of democratic representation, it guards against restraint of trade, it makes available new developments, reduces waste, establishes confidence of the public, and reduces the cost of production.

The health officer or inspector cannot secure any sanitary or other health reforms single-handed. To be successful, he must have moral, financial and public support. Such support is only obtainable through ethical organizations. Political reforms have been the result of activities of our political parties; social reforms through the social agencies or rather organizations; health and sanitary reforms through the American Public Health Association, American Medical Association, the International Association of Milk Sanitarians, and such organizations as yours. The support and assistance that has been given by these organizations to the Surgeon General resulted in the nation-wide Social Security Program, the Venereal Disease Program, the Pneumonia Program, and the like. The American Water Works Association was in part responsible for the public works program. The Isaak Walton League of America and health organizations have made progress on stream pollution abatement. Industries too have taken advantage of prestige and protection that organization offers: for example, the steel industry, Portland Cement Association,

Chlorine Institute, Clay Products Association, Lumbermen's Association, Milk Dealers Association, Ice Cream Manufacturers Association, Manufacturers Association, Retail Merchants Association, etc. The professions in order to maintain ideals and to uphold ethics have seen fit to organize into such groups as the American Bar Association, American Medical Association, American Society of Civil Engineers, American Water Works Association, American Nurses Association, etc. Better working conditions, installation of safety devices, and improvement in hygienic conditions have been brought about by the labor and trade organizations.

The essentials of a good organization are:

- (1) There must be a need.
- (2) It must have definite and constructive objectives.
- (3) It must have honest and intelligent leadership.
- (4) Its activities must not conflict with government.
- (5) It must have high moral standards.
- (6) It must be ethical in its performance.
- (7) It must provide just representation.
- (8) It must be fair in its dealings and tolerant in its attitude.
- (9) It must observe the principle of free speech and support the provisions of state and national constitutions.

The value of an organization to an individual are:

- (1) Educational benefits.
- (2) Social benefits.
- (3) Information bureau.
- (4) Support for meritorious ideas and plans.

- (5) Outlet for discussion of mutual problems.
- (6) Consultation service.
- (7) Security to the members.
- (8) Opportunity for the advancement of one's ideas.

OBJECTIVES

Since there are quite a number of municipal, district, state, and regional and one international milk sanitarians' organizations, it might be well to take inventory and analyze the objectives or the goal that is being reached and what is possible to obtain. Then next we should endeavor to coordinate these activities and evolve a structure which will permit of obtaining the greatest possible benefit for the consuming public, the producers, the distributors, the manufacturers, and the control officials.

First, we will discuss the health officials' objective. They are all interested in seeing a program established which will prevent further milk-borne outbreaks and which will bring about a greater consumption of milk. In these endeavors we need the support of physicians, sanitarians, veterinarians, agricultural agencies, agricultural schools, producers, distributors, manufacturers, law makers, and the public. It involves many angles:

- (1) The solving of unemployment by finding greater uses for milk.
- (2) Making milk more attractive to the consuming public.
- (3) Making milk available to everyone.
- (4) Making milk profitable to produce or to process and distribute.
- (5) Providing more prestige and security for control officials.

This is quite a large order of business. However, much has already been done through the national tuberculosis testing program, through making available the U. S. Public Health Service Standard Code, through fair trade commissions, and through training facilities made available to sanitarians from Social Security funds. The *International Association of Milk Sanitarians* is endeavoring

to approach this goal. It has reduced the price of its membership and changed its constitution so as to permit everyone engaged in the field of milk supervision to become a member. It has established the *JOURNAL OF MILK TECHNOLOGY* as an outlet for new developments in the field of milk sanitation. It is endeavoring to promote research, make awards for meritorious performance, and is encouraging the formation of State associations.

ORGANIZATION PROGRAM

Not being familiar with what your State Association is endeavoring to accomplish, I shall probably be repeating what your organization is already doing but nevertheless, as we see the picture, any state organization might profitably give consideration at least to a program of activities and an organization that will:

- (1) Set up qualifications for its own personnel.
- (2) Set up qualifications for those engaged in the important and more scientific phases of milk handling.
- (3) Provide adequate facilities for carrying on such activities.
- (4) To be as helpful as possible in making the milk business a profitable one.

If given the authority and means with which to carry out the state program, I would first bend every effort to the development of a strong consulting and advisory central milk control authority. The central authority should be well staffed with qualified men, and provided with adequate laboratory facilities and funds with which to promote a state-wide, uniform milk program. Since milk control is one among the important activities that can be carried on by state health departments, the salaries paid to the supervisors should be commensurate with other activities of similar importance. Various educational phases should be woven in to the public health nursing program, the engineering, laboratory, and educational activities of the state health department. With intelligent leadership

* Presented before the Missouri Association of Milk Sanitarians, May 2, 1940, University of Missouri.

in this division, the remainder of the program is comparatively simple.

Next in importance is the development of local milk supervisory units made a part of either city or county health units. Here again minimum qualifications should be set up for the milk supervisors or sanitarians. Progress of the local program will depend largely upon his organizing ability. The goodwill and the cooperation of the producers and the industry must be secured. Consumers must be satisfied. Enforcement of health, sanitation or milk laws and regulations by threats and court procedures will kill even an otherwise meritorious program in the "deep South".

State legislation does not always permit state agencies to carry on real promotion programs or educational programs or might I further say "selling programs" so essential in the business world. Here is where the association can do its most effective work and carry on these activities which the state agencies are legally restrained from doing. Yet as we have found in Texas with the water, sewage, swimming pool, and tourist court organizations, this promotion and education work is the most important part of a successful program.

A strong state organization needs an active secretary. A good start could be made with a part-time secretary who is thoroughly conversant with all phases of the milk business.

The association needs finances for its meetings and to carry on these education programs.

A publication is desirable and until one can be underwritten, space can probably be secured from some other magazine such as the organ of the mayors, county judges, school teachers, and the like.

Timely leaflets and bulletins always help promote the objectives of the association. If funds are not available for printing bulletins, they may be secured through Federal and state departments, dairymen's leagues, insurance companies, and the like.

A speakers bureau is a valuable adjunct to the association. It should be organized and even speakers can be selected outside of the milk field by drawing upon various public officers including the governor and state educational institutions.

PROFESSIONAL ADVANCEMENT

Short schools for inspectors should be provided as you have done here and which you may care to expand into three or even six months terms for the training of your assistants that you will need as this program progresses. I do not anticipate that any state will have difficulty in financing such a short school.

On the other hand, there are many men engaged in milk inspection and milk supervision work on a part, supplemental basis. We assume that every state is like Texas where sometimes the city marshal, city health officer, city plumbing inspector, or the city water plant operator in a small town spends one day a month inspecting the three small dairies in his community, collecting samples and the like. This man, of course, cannot afford to attend short schools and for him we have endeavored to supply teaching facilities through the medium of itinerant vocational instructors. There are several ways in finding such an instructor but perhaps the easiest way would be through vocational educational facilities. Such itinerant schools last usually one month in a community, two evenings a week for the inspector and possibly two evenings a week for the dairymen. The school is held in different sections of the state so as to permit the inspectors from the adjoining towns to attend. Upon the completion of the course, an examination is given which is one of the requirements in our water operators licensing system that could also be applied to the milk inspectors.

The state organization by recommending standards for milk laboratories to the state health department will further the milk program.

State organizations should promote research, promote pasteurization plant op-

NATIONAL ASPECTS

While state associations can accomplish wonders in the development of a state milk program, there are certain national aspects as well as international aspects to the milk and milk products industry. These call for a national program—the clearing house and research laboratory for the various states. Much hard feeling has been developed in the past because of conflicting milk ordinances and state milk regulations. One of the things which helped bring on the World War was the customs and trade regulations of the foreign countries. We here in the United States should be able to organize the milk products business in such a manner that the products of one state could be sold in any other state without restraint instead of having two or even three inspectors as now occurs.

The state should become affiliated with the national association and thus secure representation and have a voice in the conduct of the national organization, and by this means develop such a strong national organization as will bring about more rapidly newer methods, newer equipment, newer by-products, and legislation as needed. Nationally we need a larger laboratory, more funds for the promotion of a program on a national scale, a national program on the eradication of contagious abortion, a national program to expedite cheaper and better transportation facilities for dairy products and many other things. All of this will come but the speed with which it is to be accomplished and the influence which we will be able to exert in a constructive direction will depend upon the quality and quantity of public sentiment that can be developed by the various district and state organizations.

erators schools, a laboratory for the testing of equipment, cooling stations, and electrical power for farmers through REA and other agencies.

Perhaps one of the most beneficial results of the Texas organization is the formation of district associations. We have seventeen waterworks and sewage associations which meet each month to hold an educational and social program. These have passed the experimental stage. Men are able to talk with their neighbors in the same language. Since these meetings are held in the various towns in the district, they have been quite successful in having mayors, commissioners, city health officers, and even legislators to attend, all of which has been helpful in getting across their objectives to governing bodies. These district meetings have brought about friendly rivalry and competition, one town vying with another on some new, novel, unique way of bringing about improvement of some kind. Awards are made for attendance and gadgets. At the annual meeting honor cities are announced, and silver cups are presented with great formality in a rather auspicious setting.

An inspector may get in a rut and stay there or he may go forward with his organization. It is the inspectors who make the organizations and the organizations that propel the program. District organizations come into being following the itinerant short schools. The itinerant teacher prepares the operator for the examination required for a license. Here again it is the licensed man who desires to progress to higher grades that make the district meetings interesting and help to expand the educational program. One hundred and twenty hours of a school attendance or attendance at district meetings are now required for the highest grade license.

High Temperature-Short-time Pasteurization of Milk *

F. C. Button

Rutgers University, New Brunswick, N. J.

The earliest of milk pasteurizers employed a process known as the continuous or flash principle of heating. In this process milk was momentarily subjected to a rather high but not very definite heat treatment which was under the manual control of the operator. These early flash machines not only failed to yield with certainty properly pasteurized milk but also definitely destroyed the cream layer and imparted to the milk a cooked flavor. Thus arose, some fifty years ago, the two problems which have always complicated the choice of an acceptable temperature and time of exposure in milk pasteurization. The public health official demands a heat treatment that definitely will destroy pathogenic bacteria. The processor and distributor are concerned, in addition to safety, with the preservation of the desirable physical and chemical properties of milk—factors that have come to play an important role in maintaining consumer acceptance of the product.

EARLY STUDIES

In an attempt, therefore, to find a "middle ground" that would insure a safe product as well as one with minimum creaming destruction, scientists were led to the use of lower temperatures for a longer time of exposure. Outstanding in their contributions to the thermal death points of pathogenic bacteria were Theobald Smith (1) in 1899, Russell and Hastings (2) in 1900 and Roseneau (3) in 1906. Their findings, showing that heating at 140° F. for 20 minutes was sufficient for the destruction of tubercle bacilli and other pathogenic bacteria, were responsible for the de-

velopment of the holding process of pasteurization in this country.

North (4) in 1911 published his classic chart which showed the effects of temperature and time on pathogenic bacteria and on the physical and chemical properties of milk. Since this work and a later contribution by North and Park (5) (1929), it has been generally accepted that tubercle bacilli are among the most heat-resistant of any disease bacteria that may infect milk and that preservation of the creaming property of milk is the most important physical property to protect against heat deterioration.

Using the data of North and Park (5) on thermal death points for tubercle bacilli and the data of Marquardt and Dahlberg (6) relative to temperature and time for the smallest reduction in cream layer, Dahlberg (7) stated that "a definite correlation was shown to exist between the periods of time and various temperatures that were required to destroy the tubercle bacillus in milk. This relationship could be represented as a straight line or a semi-logarithmic graph, and the same relationship held for the maximum temperature to which milk could be exposed for varying periods of time without affecting creaming." Dahlberg's conclusion in part further adds: "While the total time variation became less with increased temperatures of pasteurization, the time required to destroy the tubercle bacillus was exceeded at each temperature by the same percentage of the total variation in time. On this basis milk should be pasteurized at 145° F. for 13 minutes and at 160° F. for 15 seconds. Pasteurization at 142° permitted a maximum time variation of 10 minutes while 160° gave a safety period of 5 seconds."

LATER FINDINGS

In the period between 1920 and 1927 there was developed a method of pasteurization which properly was termed high-temperature—short-time pasteurization. "It is not strictly flash pasteurization", a U. S. Public Health Report says (8), "since it is designed to hold the milk at a lethal temperature for a definite though short period of time. Unlike the early flash machines these short-time-hold pasteurizers make use of automatic devices which are designed: (a) to control thermostatically the temperature to which the milk is raised by the apparatus; (b) to control the rate of milk flow so as to insure that every particle of milk is held at the required killing temperature for a minimum period of time; and (c) to stop the flow of milk instantly if its temperature drops to a predetermined minimum." This report contains abstracts of several studies of the effect of short-time pasteurization on milk infected with *M. tuberculosis* as well as other pathogens. These studies were made in 1927 by the New York State Health Department, the Pennsylvania State Health Department, the New York City Health Department, and the U. S. Public Health Service. On the basis of the above studies, high-temperature—short-time pasteurization has been specifically approved in the 1933 and all subsequent editions of the Public Health Service Milk Ordinance and Code. The present definition provides that every particle of milk shall be heated to at least 160° F., and held at not less than that temperature for at least 15 seconds in approved and properly operated equipment.

The fundamental essential of any modern process of pasteurization is to render milk free of pathogens. We must be certain that every particle of milk attains the specified temperature and is held at that temperature for the specified time.

Marked progress has been made along these lines in the nature of technical requirements, particularly in connection with the short-time pasteurizers. This

has resulted from continued research with the process by health laboratories, experiment stations, and research divisions of the dairy machinery manufacturers.

PRESENT REQUIREMENTS

How high these standards have been raised and how well the manufacturers have endeavored to attain them readily can be gleaned from a study of the revised (1938) requirements (9) of the U. S. Public Health Service. High-temperature—short-time pasteurizers are now available that will meet these specifications which are as follows:

(1) The apparatus must be equipped with a dependable automatic thermostatic control of the milk temperature.

(2) A milk-flow diversion valve is now required at the holder outlet. This has superseded the earlier devices which caused a milk-pump stop.

(3) Daily tests are required to determine the cut-out and cut-in responses of the flow diversion device. New rapid-acting recording thermometers automatically record these responses.

(4) These flow-diversion valves are sealed to prevent their adjustment for operation at lower temperatures without the authorization of control agencies involved.

(5) All flow-diversion devices are operated automatically and are set so that they act as safety devices and the routine temperature of operation is far enough above the setting of the valve so that they are not brought into frequent operation during a run.

(6) When the diversion valve is in operation all of the milk flow must pass through the device and be completely diverted away from the forward flow side. Provisions for the removal of all leakage are also required.

(7) Installation tests are required so that the interval between the time of power cut-out during descending temperatures and the moment when the forward flow of milk ceases shall not exceed one second.

* Presented at New York Conference of Health Officers and Food and Drug Officials on December 6, 1939. Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

(8) The usual requirements prevail for the indicating and recording thermometers. A specific requirement is that the indicating and recording thermometers shall read not less than 160° F. at all times when the milk is not being diverted. In Chicago, for instance, 161° F. for 16 seconds is the requirement.

(9) Specific details are given for checking the holding time controls.

(10) Many systems of pasteurization make use of regenerators or heat exchangers. To insure against the possible contamination of the pasteurized milk by the raw milk due to structural defects in the metal or in the joints, a study of this problem was made by Fuchs (10). His report "Contamination of Pasteurized Milk by Improper Relative Pressures in Regenerators" led to the adoption of the safety factor developed by his research in the present standard requirements. Fuchs concludes, "To combat (the aforementioned) danger, regenerator heater coolers, if used, shall be so constructed and operated as to maintain the pasteurized milk constantly under higher pressure than the raw milk or the heat-transfer water."

CONTROL CHECKING

Progress has likewise been made in the methods employed in checking the holding time of short-time—high-temperature pasteurizers. Roger (11) reported the difficulties usually encountered in the standard method which involves the use of dye injection. In this procedure, the length of time taken by dye to traverse the holding chamber is measured from the time dye is injected into the entrance, up to the time it appears at the outlet. In place of the dye he used a saturated solution of sodium chloride. With electrodes connected at the inlet and outlet of the holding chamber and with microammeters he could readily note the deflection of the ammeters as the salt solution passing the electrodes increased the conductivity and allowed more current to pass through the circuit. He reports, "It is much easier to watch for and time the deflection of a galvanometer than

it is to catch and time the first appearance of dye."

Enough experience from commercial operation and enough data from research studies have been accumulated in recent years to give us a good picture of the status of high-temperature—short-time pasteurization and to indicate what further work should be done to round out and extend its usefulness in the field of dairy manufactures.

It has been noted that it is possible to design equipment and set up accurate controls to assure the proper operation as to temperature and time. The more important question, undoubtedly, is to ascertain whether milk pasteurized in such equipment will be safe. A review of the literature indicates that all pathogens including those which have thermal death points as high or higher than the tubercle bacillus are destroyed by this process. Of still greater interest is the fact that the phosphatase test with slight modifications can be used as an index of proper pasteurization by this method. In this connection, permit me to quote from a letter by Dr. M. D. Kay (12) of the National Institute for Research in Dairying, University of Reading, England. Under date of March 21, 1939 he says, "I think you can take it as definitely established that the phosphatase test is applicable to high-temperature—short-time pasteurization as it is to the holder process. Our own results show that in the high-temperature—short-time treatment the tubercle organism is destroyed at a lower temperature and a lesser time of exposure than the phosphatase test in all the ranges examined." "This work has been done," he continues, "by actually checking phosphatase findings against the presence of *M. tuberculosis* which is generally understood to be the most resistant of the pathogens commonly occurring in milk."

BACTERIOLOGICAL RESULTS

Studies (13) on the total reduction in the bacterial count on short-time pasteurization have shown favorable results. Much of this early work, however,

was suffered from lack of proper temperature controls and the failure to make comparisons on the same milk treated by the holder method. Much interest will attach to the publication of reports on the bacteriological studies performed by Dr. E. H. Parfitt (14) in the Chicago area and by Dr. T. W. Workman in the New York City area.

A more significant aspect of the bacteriological efficiency of the short-time pasteurizing process is to be found in the effects of the process on the thermophilic and thermoduric groups of organisms. Krueger (15) and Dotterer (16) in reports on tests in Chicago have stated that there is a marked reduction in thermophiles when milk is pasteurized in the short-time process. This is of particular interest because Krueger states that one of the prime motives for studying this method was trouble with high thermophile counts when the holding process was used in continuous operation for periods of five or six hours or more. Yale and Kelly (17) in studying thermophilic bacteria in milk pasteurized by the short-time process had earlier found that "thermophile counts in plants using this method of pasteurization were not as high as have been reported at plants using the holder process of pasteurization of 142° to 145° F. for 30 minutes."

It is to be noted that the operation of high-temperature—short-time equipment does not expose the milk to temperatures suited to the growth of thermophilic organisms for the long periods characteristic of the holder process. High temperature processing eliminates the contamination of unpasteurized milk by cream seeded with thermophiles, remaining so frequently in batch holders. Incomplete emptying of batch holders, another method for inoculating raw milk with thermophilic organisms, is also avoided. The development of these heat-loving organisms through prolonged holding periods caused by shut-downs, has long been known. In the high temperature process, milk heating can be stopped immediately in case of shut-

Yale and Kelly's and other studies, indicate that, at times, higher counts of thermoduric organisms may be obtained in short-time pasteurized milk than those found in milk held for thirty minutes. Thermoduric organisms on first thought would seem to be more resistant to short-time pasteurization than to the holding process. Extensive commercial experience as well as laboratory studies, however, show that the survival of thermoduric organisms may be common to both methods of pasteurization. In other words, conditions of production at the source of the milk or the cleanliness of equipment with which the milk comes in contact affect the types of organisms in the initial flora. This will have a great bearing upon the number of thermoduric organisms present in the finished pasteurized milk.

The natural habitat of these organisms is in the soil. It readily can be understood how they may become localized in unclean or poorly sterilized utensils on the farm, at the receiving plant or even in the processing equipment itself.

For some time this problem of high initial raw milk quality and its relation to thermoduric counts has been a problem in our New Jersey plants where only the holding process of pasteurization is officially recognized. The problem here has been to determine whether thermodurics are being cultured in the plant, or are coming in only from the producers. Laboratory pasteurization of the milk stored in holding tanks, and of composite samples of producers' milks before they touch any plant equipment, and then the analyzing of these pasteurized samples bacteriologically, show the origin of the contaminating group. Contamination originating in plants, once located, readily may be eliminated. When contamination is traced to producers, laboratory pasteurization of each producer's milk and vigilant follow-up methods in the field have been required to eliminate the farm sources of thermoduric organisms. The same laboratory procedure and field work have been required to meet this problem in those areas where

short-time pasteurization has been adopted. One might say, indeed, that the difficulty with thermophilic bacteria in the short-time process has been a boon to the improvement of raw milk quality. The methods formulated to meet this problem can be applied with profit to the whole field of milk production and handling.

ECONOMIC FEATURES

Irwin (18) Rishoi (19) and others have presented some of the reasons for the economic soundness of short-time pasteurization. These may be listed as follows:

(a) It is readily practical for large plants where a continuous system is desirable.

(b) The cost of machinery is about one-half as compared to similar thirty-minute holding equipment.

(c) On a 4-hour run there is a considerable (about one-third) saving in steam consumption.

(d) Less floor space is required. In the case of plate machines, the floor space is 20 percent of that required for holders. Capacity can be readily increased by the addition of plates or pipes without using additional floor space.

(e) Plate apparatus gives more regeneration than tubular holders.

(f) The cleaning time for plate machines is about 40 to 50 percent less than that required for holder installations.

(g) The process can be interrupted and quickly restarted.

(h) All of the equipment is available for cleaning at the same time. The closed system with a pump makes an ideal arrangement for sterilizing by the use of hot water.

More studies and commercial experience may be required to determine how flexible the short-hold process is in relation to handling cream and other fluid products such as chocolate milk and skim milk for use in manufactured products. Studies of these problems are now under way at several experiment stations.

CREAM

There is no appreciable difference in creaming efficiency between the two processes of pasteurization. Some unpublished results with one particular make of short-time pasteurizers show cream layer formation in favor of the high temperature method.

The efficiency of high temperature cooling equipment will, of course, have a definite bearing on cream layer formation, if the equipment is used in the manner proposed for this purpose. The studies of Hening and Dahlberg (20) have shown that the creaming efficiency of milks and the viscosity of creams may be improved by manipulating the cooling procedure in a manner which develops rigid fat clusters. The plate regenerators and coolers used in high-temperature—short-time installations can be adapted with no trouble to such processing. The practice has not been adopted where standard holding pasteurization is used because of the extensive softening of butterfat held above its melting point for a long period, and the expense of cooling equipment needed to process such soft fat globules.

FLAVOR

The literature has definitely established that the flavor of short-time pasteurized milk is markedly better than that of milk processed by thirty-minute holding. In some areas this flavor has been favorably compared with that of highest quality raw milk. Of special significance are the conclusions noted by Josephson (21) and Doan in the November 1939 issue of *The Milk Dealer* in an article titled "Observations on Cooked Flavor in Milk." They show that when milk is heated to high temperatures or held insufficiently long at lower temperatures, sulfhydryl compounds are formed from certain milk proteins. These sulfhydryl compounds, in turn, impart to milk a cooked flavor. These compounds, likewise, were found to be active reducing substances. They are active antioxidants and appear to inhibit the development of cardboard

tallowy flavor in milk heated to temperatures over 170° F. They noted further that these sulfhydryl substances actually act as anti-oxidants toward ascorbic acid. This latter substance itself protects milk against tallowy flavor. It seems that the high-temperature short-time method of pasteurizing milk does protect the vitamin C (ascorbic acid) content of the milk and it, in turn, protects milk against the development of oxidized or tallowy flavor.

It has been the purpose of this paper to report the present status of the high-temperature short-time process of milk

REFERENCES

- (1) Smith, Theobald. *J. of Exp. Med.*, 4, 233 (1899).
- (2) Russell, H. L. and Hastings, E. G. *Wis. Ag. Exp. Sta.*, 17th Ann. Rep. 170 (1900).
- (3) Rosenau, M. J. "The Milk Question." Houghton-Mifflin Co. New York, p. 95 (1912).
- (4) North, Chas. E. Chart Time and Temp. for Milk Past. (1912).
- (5) North, Chas. E. and Park, H. *Am. J. Hyg.* 7, 147 (1927).
- (6) Marquardt, J. C. and Dahlberg, A. C. N. Y. (*Geneva*) *Ag. Exp. Sta. Tech. Bull.* 180 (1931).
- (7) Dahlberg, A. C., N. Y. (*Geneva*) *Ibid. Tech. Bull.* 203 (1932).
- (8) The (1931) joint progress report of the Committee on Milk Supply of the Engineering Section of the American Public Health Association and the Committee on Milk Sanitation of the Conference of State Sanitary Engineers.
- (9) U. S. Public Health Service, High Temperature Short-Time Pasteurization Report (revised 1938).

pasteurization. The coming years will see further advances. It must be remembered that despite a number of years of using the holding method, improvements are always being made, and are always possible. Short-time equipment has been engineered with all automatic features. The elimination of the human element in the controlling operations is a step in the right direction, and one which could be adopted with profit in the holding method. From the rate at which research work on the high temperature system is being pursued, we soon shall have as complete data on this method as we have on the holding process.

- (10) Fuchs, A. W., *U. S. Public Health Reports*, 53, 495 (1938).
- (11) Roger, D. M. *J. Milk Technol.* 2, 191 (1939).
- (12) Private correspondence.
- (13) Sommer, H. H. "Market Milk and Related Products" p. 327 (1938).
- (14) Parfitt, E. H. *Proc. 17th Ann. Conf. N. Y. State Assoc. Dairy and Milk Inspect.* (1939).
- (15) Krueger, P. F. *J. Milk Technol.* 1, No. 7, 29 (1938).
- (16) Dotterer, W. D., *Ibid.* 2, 197 (1939).
- (17) Yale, M. W. and Kelley, C. D. *N. Y. (Geneva) Ag. Exp. Sta. Bull.* 630 (1933).
- (18) Irwin, R. E. *Milk Plant Monthly.* 24, No. 3, 24 (1935).
- (19) Rishoi, A. H., *J. Milk Technol.* 2, 200 (1939).
- (20) Hening, J. C. and Dahlberg, A. C. N. Y. (*Geneva*) *Ag. Exp. Sta., Tech. Bull.* 197 (1932).
- (21) Josephson, D. V. and Doan, F. J. *The Milk Dealer*, 29, No. 2, p. 35 (1939).

Twenty-ninth Annual Meeting
International Association of Milk Sanitarians
 in joint sessions with the
New York State Association of Dairy and Milk Inspectors
Hotel Pennsylvania, New York, October 17, 18, 19
Make your reservations NOW

Obtaining Good Results with Broadhurst-Paley Stain for Milk Smears

Isaac Cohen

Certified Laboratories, Inc., New York City

Experience of two years with the Broadhurst-Paley stain (1) has led to the following improvement in technique.

It is essential, of course, to go back to the fundamental principles of making good milk smears, to work with clean slides and clean lenses, and to use a very gentle heat in drying the milk smear, so that the smear is smooth with no granulation. In washing and drying the slide, it is advisable to flame the slide to remove any moisture or dirt particles. Then cool the slide before making smears. This will help to eliminate some of the possibility of losing the smear or part of smear in the staining and washing process.

After drying the milk smears on a slide, apply the Broadhurst-Paley stain for about one minute with a dropper. Drain the stain off the slide, and dry in a

gentle heat. Cool the slide for a few minutes, and immerse in a glass of cold water, holding the slide vertically for a minute without shaking. Lift the slide a few times out of the water and the excess stain will be drawn off the smears smoothly. Empty the glass of water, and repeat the procedure in a fresh glass of water. Then dry the slide. This has an advantage over the methylene blue stain in that the technician knows when all the excess blue stain has been removed by there being scarcely any blue stain left in the 2nd glass.

It will be observed that the smears will be free of excess particles of stain, and the stained smear will make a more substantial background under the microscope.

- (1) Broadhurst, J. and Paley C. A Single-dip Stain for the Direct Examination of Milk. *J. Milk Technol.* 2, 226 (1939).

"Oxidase Reaction" of Bacteria in Relation to Dairy Products. C. H. Castell and E. H. Garrard. *Food Research*, 5, 215 (1940).

In the past the oxidase test has been used mainly for the differentiation of certain pathogenic bacteria. As it is based on the oxidizing capacity of the organisms, an important activity not included in other common tests, it appears reasonable that it should have a wider application, especially in the study of microbial spoilage of foods.

The test is easily made and has a great advantage in that it can be employed on plates prepared for ordinary counts or other purposes, and provides a very convenient method for identifying bacteria bringing about the oxidation changes.

From the observations described in this paper, organisms of the *Pseudomonas* and *Achromobacter* genera are the most strongly ox-

idizing types; those belonging to *Alcaligenes* and *Brucella* are somewhat less, although strongly positive; members of the *Aerobacter*, *Escherichia*, and *Proteus*, are weakly positive, variable, or negative; the *Bacilli* also vary weakly positive to negative; while the *Corni* and the only anaerobe represented are definitely oxidase-negative. It is interesting to note that all organisms which are strong oxidizers are gram-negative and those which are definitely not strong oxidizers are gram-positive.

That the *Pseudomonas* and *Achromobacter* strong oxidizers should be of interest to those working with butter, lard, chilled beef, and other fatty products, both because of the numerous types of rancidity and off-flavors produced by these organisms and because they frequently are able to grow actively at storage and refrigeration temperatures.

M. E. PARKEE

Nutritional Aspects of Milk II*

W. E. Krauss

Ohio Experiment Station, Wooster, Ohio

For a long time the value of milk as a food was taken for granted. Consequently, emphasis was placed on improving the sanitary conditions surrounding the various phases of milk production. In this phase of development, certified milk has, of course, been outstanding. As the science of nutrition was developed and new hitherto unknown factors that contribute to the value of foods were discovered, the value of milk as a food was reinvestigated. As a result of our newer knowledge of nutrition many of the things formerly taken for granted have been shown to have sound scientific basis, and other favorable attributes not previously known have been brought to light. At the same time one or two weaknesses or deficiencies were encountered, thus making it possible better to understand the limitations of milk, as well as its virtues. Knowledge such as this regarding any food product results in more intelligent use of it.

As examples of deficiencies of milk might be mentioned the anemia-producing effect of an exclusive milk diet and the presence of insufficient vitamin D in milk to prevent rickets in infants receiving milk entirely and not having access to some vitamin D source. As yet the first of these two deficiencies has not been overcome by improved dairy cow feeding methods, although the deficiencies of milk (iron and copper) are well known and can be corrected by adding traces of these mineral elements to milk after it is produced. The possibility of producing milk which will permit mammals to live indefinitely on it exclusively has

not been eliminated. The growing of proper plants on soil enriched in iron and copper is suggested as a possible avenue of approach toward the solution of this problem.

The lack of vitamin D in milk as it is ordinarily produced no longer presents the problem it once did. The feeding of irradiated yeast to the cows raises the vitamin D potency of milk to the point where such milk becomes a preventive and therapeutic agent.

The virtues of milk are many. Fortunately the cow has been endowed with an inheritance that makes it possible for her to produce a product which so far as its usual constituents are concerned is quite uniform. It is true that certain variations in fat, casein, albumin, globulin, lactose, and ash occur, particularly when some extreme feeding condition is present, and to a much less degree on the same feed, but by and large the chemical composition of milk is dependable. Two notable exceptions exist, namely, the depressing effect exerted on the fat percentage of milk when cod liver oil is fed to cows, and the variations in iodine content that can be made to fluctuate at will, depending upon how much iodine is fed.

With the introduction of permissive pasteurization, some questions have probably arisen as to the effect such heat treatment may have on the nutritive value of milk. In an extensive study of this question made in our department, we were unable to demonstrate any significant difference between raw and pasteurized milk so far as total nutritive effect (i. e. rate of growth on mineralized milk exclusively) and calcifying properties were concerned. About 25 percent of the vitamin B₁ was destroyed by such treatment and varying amounts of vita-

* Paper read before the joint annual meeting of the Metropolitan Certified Milk Producers and the Certified Milk Producers' Association of America, Monday, January 29, 1940 at the Hotel Roosevelt, New York City.

min C, ranging from 25 to 75 percent, depending upon how the milk was handled before pasteurization. These results have been substantiated at the National Institute for Research in Dairying, England. In fact the final report of the Milk Nutrition Committee of England comes to this conclusion: "There are no differences between the nutritive value of raw and pasteurized milk of any practical importance for school children who receive milk as part of their ordinary diet." In the case of infants receiving only milk this committee thinks it would be unjustifiable not to supplement cow's milk, either raw or pasteurized, with some vitamin C source.

In this connection it is interesting to know that the destruction of vitamin C in milk by pasteurization depends largely upon how the milk is handled before the heat treatment is applied. If milk is collected in such a manner as to prevent light from striking it, very little vitamin C is lost upon pasteurization. This emphasizes the importance of proper handling of milk at all times because even after pasteurization, losses of ascorbic acid (vitamin C) are known to depend upon age and condition of storing.

We can probably give some consideration now to comparisons between milk as it is ordinarily produced and certified milk as it should be produced. After having established the premise that so far as usual constituents are concerned no significant differences in chemical composition can be anticipated, we must look to some other possible differences. The vitamin content of milk suggests itself as such a possibility.

Milk as it is ordinarily produced for

the fluid market comes from cows that are fed various combinations of pasture, legumes, legume and grass mixtures, corn silage, corn fodder, corn stover, and grain containing numerous combinations of home-grown feeds and purchased concentrates. To emphasize any differences that might exist let us confine our discussion to winter feeding. Let us assume a feeding program that uses the following combination of feeds:

- Mixed hay
- Corn silage
- Corn and cob meal
- Ground oats
- Wheat bran
- Soybean oil meal
- Salt

Compare it with one that makes maximum use of our present knowledge regarding the relationship between good feed and good milk:

- Grass or legume silage
- Alfalfa hay
- Corn silage
- Corn and cob meal
- Ground oats
- Wheat bran
- Soybean oil meal
- Iodized salt
- Irradiated yeast.

What could one expect the vitamin content of the milk produced under each of these systems of feeding to be? Data are presented in Table 1.

The values used above are not actual ones obtained by a comparison of milk produced on the two types of rations given but are based upon experience with other combinations that approach these and upon knowledge provided by the scientific literature.

TABLE 1.
Vitamin Content of Milk (Per Quart)

	Ordinary Dairy Ration		Ideal Dairy Ration	
	Raw	Past.	Raw	Past.
Vitamin A (I.U.)	1000	1000	3000	3000
B ₁ (I.U.)	125	100	125	100
C (mg.)	25	7.5	25	150
D (I.U.)	10	10	400	400
G (S.B.)	500	500	650	650
P—P	?	?	?	?

The vitamin content of milk is important, especially during the winter when, because of unavailability and seasonal variation, certain foods normally rich in certain vitamins cannot be depended upon. This difference between milk as it is ordinarily produced and as produced under an ideal feeding program would seem to be of sufficient magnitude and importance to claim superiority of one over the other from the standpoint of the nutritive value of the milk capable of being produced on each type of ration. In all fairness to the milk industry at large, however, it must be pointed out that even milk produced under the poorest feeding conditions is still our most nearly perfect food and that considerable milk going into ordinary market milk channels is produced on rations approaching the ideal one used as an example.

Emphasis has been placed upon the importance of producing a high-vitamin milk during the winter because it is then that milk as a vitamin source receives its most severe competition. Should milk producers be able to point out that the consumption of a quart of milk a day by people of all ages would, together with the food they normally eat, meet their daily requirements for practically all the known vitamins, some of the millions of dollars that are now spent each year for special vitamin preparations would be directed toward that group which produces the nation's food supply and of which the milk producer is so important a member.

As our knowledge of preserving the nutritive properties possessed by green plants increases, so will our ability to produce milk of greater nutritive value increase. The most recent contribution

to such progress comes in the form of a yet unidentified substance known as the "grass juice factor" which may be responsible for the difference between the growth-promoting properties of winter and summer milk and which has recently been found to be preserved in grass and legume silage. Further stimulation for attempting to approach summer feeding conditions during the winter comes from the University of Wisconsin where it has been demonstrated that milk produced under poor winter feeding conditions loses some of its growth-promoting properties when pasteurized.

What is the relationship between certified milk production and all this? Because of the regulations that must be met in the production of Certified Milk, we can expect those engaged in this business to be of such type as to be concerned not only in a source of income but in the production of milk with superior merits of all possible kinds. Certified milk producers have been pioneers in sanitation; they are now pioneers in the movement to make milk a better food than it already is. As such they are definitely concerned with the public welfare which is so directly dependent upon good nutrition. The stages of development of the certified milk business are aptly represented in a passage from the book "Nutrition and the Future of Man" by James S. McLester.

"In the past, science has conferred on those people who availed themselves of the newer knowledge of infectious disease, better health and a greater average length of life. In the future it promises to those races who will take advantage of the newer knowledge of nutrition, a larger stature, greater vigor, increased longevity, and a higher level of cultural attainment. To a measurable degree, man is now master of his own destiny, where once he was subject only to the grim hand of Fate."

Plan NOW to attend the ANNUAL MEETING
in New York, October 17, 18 and 19
Headquarters: Hotel Pennsylvania

Symposium on Tryptone-Glucose-Extract-Milk Agar*

Howard E. Lind

St. Louis County Health Department, Clayton, Missouri

The value of any new procedure such as the T-G-E-M Agar introduced for routine plate counting is evidenced by its practicability in a routine setup. If it is not practical, easy to handle, and comparable in results to previous procedure, a change is not warranted. It was with this in mind that directors of state health laboratories and in some instances the departments of agriculture were contacted and asked to comment favorably or unfavorably on the use of T-G-E-M Agar in their daily routine. The response was surprisingly great and in most instances comments were given on one or many of the phases of its preparation, use, results, and interpretation.

Representative of the replies received are those from the following: Michigan State Department of Agriculture, Michigan State College, New York State Department of Health, Kansas State Health Department, Massachusetts State Health Department, Connecticut Department of Health, California State Health Department, Indiana State Health Department, Ohio State Health Department, Pennsylvania State Department of Agriculture, Ann Arbor Health Department, Detroit Health Department, and our own St. Louis County Health Department.

Among the various items of importance commented on with regard to the T-G-E-M Agar were colony size, pin point colonies, thermophylic and thermoduric organisms, pasteurized counts, raw counts, dilution variations, split sampling variation, pH, precipitates and flocculates, and skim milk.

All the above investigators with no exceptions reported that the use of this

medium increased the size of the colony, thereby making it easier to see and easier to count.

EFFECT ON COLONY SIZE

The general consensus of opinion was that this medium had a tendency to decrease the incidence of pin-point colonies and the California State Health Department went so far as to say it practically eliminated pin-point colonies. There is no doubt that these pin-point colonies become colonies of demonstrable size. New York Department of Health and we in the county noticed an increase in the occurrence of pin-point colonies. This can probably be explained by the fact that prior to the use of this medium we had little occasion to come across pin-point colonies, and by noticing the frequent occurrence on the T-G-E-M Agar lead us to believe that we seldom picked up such organisms at all on the old medium. Pennsylvania State Department of Agriculture felt unable to comment on the occurrence of pin-points because the new medium had a tendency to obscure the pin-points by their blending with the medium.

As far as the detection of thermophylic and thermoduric organisms was concerned, it was generally agreed that this new medium increased the presence of these types of organisms. New York reported that it was very satisfactory in locating evidence of mastitis.

EFFECT ON COUNTS

With regard to counts on pasteurized milk, New York and Kansas felt that the difference in counts was so negligible as to be unworthy of comment. Some counts were higher and some were lower. Michigan State College and we in the county experienced the greatest increase on the low count milk. As the

PRECIPITATES IN MEDIA

With no exception the investigators reported that they had had more or less trouble at one time or another with the occurrence of precipitates and flocculates, the former occurring immediately after sterilization and the flocculates occurring after remelting, just prior to pouring. This is naturally a disadvantage for standard methods. California attempted to filter and resterilize and even freeze the precipitated media but the final result was most unsatisfactory (there was always the chance of losing certain nutrients). In Pennsylvania, similar difficulties were encountered in trying to re-use the media.

Closely associated with the occurrence of precipitates and flocculates was the addition and temperature application of skim milk. In most instances individuals preferred the use of fresh skim milk. Kansas reported that there seemed to be little variation between the use of fresh and dehydrated skim milk. We in the county have had excellent results with the use of Difco's dehydrated skim milk. On the other hand California reported that they preferred fresh skim milk but that dehydrated forms were satisfactory with the exception of Difco's product.

SUMMARY

A summary of the advantages and disadvantages, as expressed by laboratories in other parts of the country, in part expresses our own viewpoint in Missouri.

1. The size of the colony is increased, consequently, the plates are easier to read, reducing the incidence of pin points.
2. There is an easier detection of thermophylic and thermoduric organisms, that is, its use allows one to control plant problems more easily.
3. Those who did comment on the dilution factor and split sampling concluded:

- a. The total count showed close agreement between dilutions.
- b. Split samples between laboratories showed a close agreement. In

range increased the variability between the two media decreased, although in many instances there was actually a decrease.

The remaining investigators in general reported that the greatest increase was on the higher count milk. In other words, the poorer the quality of milk the greater the increase in count.

On raw count milk we had a similar parallelism with that of the pasteurized milk. In general there seemed to be a slight increase in all the count ranges on raw milk.

Those who previously encountered difficulties with decided variation between dilutions were glad to report that with this new medium the ratio between dilutions (specifically 1-100 and 1-1,000) was usually less than 2:1.

SPLIT SAMPLING

Similarly with split sampling within the laboratory and with other laboratories, there was less variation. In our own experience where two large metropolitan districts such as St. Louis County and the City of St. Louis (where a milk supply bacteriologically is tested by both laboratories), naturally it is quite imperative that there be close correlation between the two laboratories so as to avoid any possible embarrassment from the administrative standpoint. Our last split sampling with the City of St. Louis revealed that we were in very close agreement. New York State Department of Health reported that as the quality of milk decreased there was a decided variation in split samples.

Very few commented on the hydrogen ion concentration (pH). The Massachusetts State Department of Health indicated that if the pH varied from 6.8-7.0, there would be a decided effect on the final count. The New York State Department of Health reported that if standard methods were followed, there would be no cause for trouble if a pH between 6.2-7 were maintained. This had been our experience in the county likewise.

* Presented before the Missouri Association of Milk Sanitarians, May 2, 1940, University of Missouri, Columbia, Missouri.

our own series of check split samples with the City of St. Louis there was remarkably close agreement.

4. With regard to actual counts on pasteurized and raw samples, there seemed to be quite a variation in experience. Some reported that there was very little change from the old to the new medium, whether in the low count range or in the high count range; a few reported that the greatest increase occurred in the low count range; more investigators concluded that the greatest increase occurred in the high count range, that is, the poorer the quality of milk the higher the count. Amusingly, one state reported that there seemed to be too many high counts on too many good samples. Just what they mean by good samples is open for question. This confession speaks well for this medium in its ability to detect supposedly good samples.

5. Although the factor of temperature does not come into our discussion, there was one laboratory which commented on this phase. New York State reported favorably on a suggested change on temperature to 32° C.—to give more consistently higher counts. This laboratory also commented on the pH of the medium and reported that if standard methods were followed and a pH of 6.2 - 7.0 were maintained there would be no trouble from this angle. Massachusetts State Health Department reported that if the pH varied from 6.8 - 7.0 there would be considerable variation in plate counts.

6. Very consistent and inclusive in all instances was the unfavorable comment on the formation of precipitates and flocculates. All the above mentioned laboratories reported trouble at one time or another with precipitates. Naturally, this would affect counts mainly because of the decrease in transparency in medium and the formation of spreaders, due to the shrinking of the media as the precipitates formed. Dr. Breed and his co-workers suggested a means of preventing this difficulty by controlling the temperature and holding the re-melted agar at a temperature of 47 - 55° C. In our own experience, sterilizing at 15 pounds pressure for 20 minutes and holding the melted agar at 50° eliminated the problem of the formation of precipitates.

7. In most instances where the factor of the quality of skim milk was brought up, it was advocated that the use of good fresh skim milk was superior to any form of dehydrated skim milk. In our own experience we find Difco's dehydrated skim milk most satisfactory.

Several laboratories reported that the medium was tricky to prepare and one laboratory bluntly stated that if Dr. Breed would admit that it was tricky to prepare, it should not be inserted in *Standard Methods* and could not be relied upon for standard practice.

8. No laboratory advocated the return to the former Standard Nutrient Agar. All agreed that it was a step in the right direction and would not upset any operation under the Sanitary Code.

Discussion

Dorothy Dixon

Health Department, Kansas City, Missouri

In no matter what field of endeavor, a feeling of skepticism, mistrust, and even doubt accompanies a proposed change of methods. In the scientific world, even more is this true.

It was with a great deal of interest, therefore, from a laboratory as well as

a milk dealer's standpoint, that the use of the new tryptone-glucose-extract-milk agar has been substituted for the standard plate counts by the American Public Health Association in July 1939.

With the characteristic thoroughness of the Association, research stations

set up throughout the country and also individual or small groups of workers carried on investigations as to the applicability and properties of the new medium.

Bowers and Hucker (1, 2) first introduced a new tryptone-carbohydrate enriched medium, and Safford and Stark (3) confirmed the earlier workers' investigation that the addition of skim milk to milk plating media increased the colony counts. These workers state that the higher counts on the new medium would not affect seriously the producers of high grade milk. Phelan (4), in working with raw milk from scattered portions of New England, and with samples pasteurized in the laboratory in test tubes, showed clearly by charts that the better grade products show the smallest percentage increase.

Dennis and Weiser (5) showed by litmus milk transfers from individual colonies that tryptone-glucose medium supported a larger variety of organisms than any other medium. Surely if the milk is to be examined from a sanitary consideration, the many sources of contamination would be indicated by the greater number of organisms possible to grow.

Foltz and Martin (6) and Yale and Hickey (7) separately studying the tryptone-glucose skim milk media for use in counting ice cream, state that the percentage increase was greater in the higher ranges of standard plate counts than in the lower ranges. These facts would indicate that the new medium is superior in recognizing good quality products and prevents poor quality from being easily overlooked. Also, they suggest that the manufacturers will be required to exercise even greater sanitary precautions.

Yale (8) summarizes the data obtained from 56 laboratories that were requested to do research work on this problem by the Committee on Standard Methods for the Examination of Dairy Products. He states that if it is desirable to use a procedure that will grow bacteria associated with unclean utensils and inadequate cooling, the modified methods are advantageous. They further conclude from

this research program that the modified methods produce a greater spread between the counts of truly good quality and truly poor quality milk than the old standard method. Also, the numerical increase in count is slight in cases of truly high grade products.

Abele (9) foresees that the use of the new medium may be expected to increase beyond legal limit such counts that on old standard agar, were close to the legal limit. This will affect those milk supplies that have not been presented a true bacterial content by the old agar.

Obviously, it is true, that the conclusion drawn from the research sections were striking and correlated consistently. And the question immediately arises as to how the old and new media compare over a period of time in practical, daily use as, for instance, in a municipal health department, upon which a city is relying for the safety of its milk supply. Also, are there any significant changes in the bacteriological plate counts and, most important of all, can they be interpreted with an advantage to the inspectors and health officers? Will the new medium help the inspectors to know which milk supplies are questionable, which equipment is not always clean, and which handlers are lax in detail? In short, will the new method show greater efficiency in weeding out the potentially undesirable milk? If the answer to this question is affirmative, then surely the change is both feasible and practical for routine work.

The records of the bacteriological plate counts for milk for the Kansas City, Missouri, Department of Health have been studied and the results recorded as follows. All standard plate counts were made according to the American Public Health Association regulations by the same group of workers and under the same laboratory conditions. The only change was in the type of medium employed. From the period of July 1, 1938 to July 1, 1939, the old standard extract agar (Difco's) was used. From July 1, 1939 to April 1, 1940, the tryptone-glucose-extract-milk agar was used.

TABLE 1
Raw Milk of Border-line Dairies

Dairy	1938-39			1939-40		
	No. of Samples	>30,000	Excessive Counts %	No. of Samples	>30,000	Excessive Counts %
B. F.	80	6	7.5	81	40	50
H. A.	22	5	22	17	7	41
I. O.	30	3	10	17	5	30
J. D.	29	8	27	30	10	33
T. E.	31	2	6.5	43	11	26
G. B.	14	3	21	15	6	40
G. A.	20	1	5	28	9	32
G. R.	28	5	17	18	5	27
K. L.	30	1	3	19	4	21
S.	30	15	50	30	15	50
J. T.	50	7	14	26	10	38
TOTALS	334	41	12	294	107	36

tone-glucose-extract-skim-milk agar (Difco's) was used.

From eleven dealers during 1938, 334 samples showed 41 or 12 percent above 30,000 count, as shown in Table I. During 1939 from the same 11 dealers 294 samples showed 107 or 36 percent above 30,000. This gives a percentage increase of 175 percent of the excessive counts in 1939 over those of 1938. These dealers are what we call "borderline dairies". They meet all grade "A" requirements as to physical inspection. As a whole they have approved milking methods, the finest in refrigeration, good boiler supplies for steam, approved bottling equipment, satisfactory storage and cooling, with intelligent, healthy, and steady employees. Some exceptions as to conditions are noted. Dairy H.A. has temporary and changing personnel with consequently less efficient handling, and not as large steam and chlorine capacity. Dairy G.A. and G.B., although showing

good mechanical equipment, have buildings which are worn and harder to keep clean. Dairy J.T. shows a prevalence of mastitis by animal examination.

In spite of the fact that these dairies passed inspection, it has been felt for a long time that there was something lacking in efficiency, although this could not be proved by visible means, such as lax handling technique and undependable cleaning methods. It was not until the new plate counts showed a more frequent occurrence of excessive counts and a higher percentage that there was concrete proof of this negligence. Therefore, the inspector had a basis for his demand for improved and constantly supervised techniques. So the laboratory test per se assumed more value and significance.

Out of a group of superior dairies which have maintained their standard for three years as far as gross inspection is concerned and also have run low counts, we find (as shown in Table 2) that the

TABLE 2
Raw Milk from Superior Dairies

Dairy	1938-39			1939-40		
	No. of Samples	>30,000	Excessive Counts %	No. of Samples	>30,000	Excessive Counts %
B. L.	30	0	0	32	2	6
B. G.	43	5	11	42	2	5
C. R.	55	7	12	83	15	18
E. T.	33	2	6	34	1	3
H. H.	42	2	4.5	73	4	5
TOTALS	203	16	6.7	264	24	7.1

TABLE 3
Milk of Pasteurizing Plants

	1938-39			1939-40		
	No. of Samples	>30,000	Excessive Counts %	No. of Samples	>30,000	Excessive Counts %
C. S.	116	6	5	90	24	26
S. U.	150	12	8	64	28	43
	266			154		
A. N.	238	14	6	182	3	1.5
C. H.	184	6	3	197	14	7
TOTALS	422			379		
	266			154		
TOTAL NO. Samples	688			533		

percentage of excessive counts for 1938 and 1939 remain quite similar.

We feel that the new medium does not produce high counts indiscriminately, but when the quality of the milk and the handling is kept constantly good, the plate counts will run at a low percentage level, whether tested on old or new standard medium.

In studying the pasteurizing plants, we have four large representative plants with a total number of 688 samples tested in 1938, and 533 in 1939, as shown in Table 3. The first two plants, C.S. and S.U., do not have careful supervision or laboratory control within the plant itself. However, the equipment is good and on a par with the last two plants shown. C.S. shows 116 samples with 5 percent or 6 excessive counts for 1938, and 90 with 24 or 26 percent excessive counts for 1939. Plant S.U. with 150 samples in 1938 and 64 samples in 1939, show an 8 percent and 43 percent excessive counts respectively. On the other hand, plant A.N. with 238 samples and 182 samples respectively for 1938 and 1939, show 6 percent and 1½ percent excessive counts for those two years. Plant C.H. with 184 and 197 samples in 1938 and 1939 give a 3 percent and 7 percent excessive count percentage respectively. These latter two plants are particular with details and have excellent laboratory control methods themselves. It seems evident that when the control methods are good, there is no great difference in the results of the two media.

Pre-pasteurized milk, brought in from farms and used for pasteurizing, must comply with a maximum tolerance limit of 200,000 organisms by the standard plate count. During 1938 and 1939 there were 1,594 samples tested with 88 or 5½ percent above the 200,000 mark. Out of 1,925 samples of 1939-40, there were 138 or 7 percent above 200,000. With the new medium, the increased percentage is apparent also in the milk of the higher count quality.

DISCUSSION

In three types or grades of milk, raw, pasteurized, and pre-pasteurized, whose records we have investigated, there has been percentage increase of counts with the tryptone-glucose-extract-milk agar in all three. The increases are not found from all milk sources irrespective of the possibility of it being poor quality. They seem to be correlated with those sources which have questionable consistency of methods, or where the supervision is not constant. Negligence of the production is never underrated by the tryptone-glucose-extract-milk medium: the counts are either parallel or higher than with the old agar. Although an inspector knows where laxity is possible, he is often unable to see where the plant is cutting corners, but with the aid of the new bacteriological tests the count is an indication. In earlier papers (5) it has been shown that the new medium allows a larger variety of organisms to develop. Hence, if there is a careless handling technique, there will be an introduction

of more kinds of bacteria, which will be more evident with this media-plating.

SUMMARY

From the standpoint of a city health department, we feel that the tryptone-glucose-extract-milk medium, designated as the standard in the 7th edition of the *Standard Methods for the Examination of Dairy Products*, is a distinct improvement in a practical way.

As a consequence, the laboratory is of more service than before to the milk control authorities by indicating poor quality and technique which formerly was ascertained for the most part by visible means only.

Furthermore, the milk dealer himself is convinced by the more sensitive laboratory tests that his plant care, control, and supervision must be of a constantly high calibre.

BIBLIOGRAPHY

- (1) Bowers, C. S. and Hucker, G. J. The Composition of Media for the Bacteriological Analysis of Milk. *New York Agric. Exper. Sta. Tech. Bul. No. 228, 1935.*
- (2) Bowers, C. S. and Hucker, G. J. Further Studies of the Composition of Media for the Bacteriological Analysis of Milk. *Amer. J. Pub. Health 26, 350 (1936).*
- (3) Safford, C. E. and Stark, C. N. The Advantages of Skim Milk Agar for the Determination of the Sanitary Quality of Milk. *J. Dairy Science 18, 539 (1935).*
- (4) Phelan, J. T. Some Effects of the Proposed New Bacteriological Techniques. *Ibid. 19, 385 (1936).*
- (5) Dennis, J. D. and Weiser, H. H. Comparative Studies on Bacterial Milk Counts in Various Types of Media Incubated at 20°-30° and 37°C. *Ibid. 20, 445 (1937).*
- (6) Foltz, V. D. and Martin, W. H. Comparison of Tryptone-Glucose-Skim-Milk and Standard Nutrient Agars as Media for Determining the Bacterial Count in Ice Cream. *Ibid. 21, 289 (1938).*
- (7) Yale, M. W. and Hickey, R. C. The Effect of Using Tryptone-Glucose-Skim-Milk Agar and 32°C Incubation on the Bacterial Count of Ice Cream. *Ibid. 20, 755 (1937).*
- (8) Yale, M. W. Standard Agar Counts as Compared with Counts on Improved Agars at 32°C. *Amer. J. Pub. Health 28, 148 (1938).*
- (9) Abele, C. A. Results of Bacterial Plate Counts of Milk on Three Media and at Two Temperatures of Incubation. *Ibid. 29, 821 (1939).*

Notice to the Butter Industry

(Editor—The following notice was issued by the U. S. Department of Agriculture on June 3, 1940.)

For a number of years the Food and Drug Administration has studied the significance of mold mycelia in butter in relation to the quality of cream from which butter is manufactured. These studies, completed in the summer of 1939, showed conclusively that a high mold mycelia count in the finished butter never occurs unless some of the cream was to a material degree filthy, putrid, or decomposed. Butter made from such cream is considered to be adulterated within the meaning of the Food, Drug, and Cosmetic Act.

The Administration has instituted actions in the past against interstate shipments of unfit cream, and butter made from such cream. The butter industry as a whole has welcomed these actions

as encouraging the production of more wholesome food. The Administration expects to continue legal actions against unfit cream and butter. In addition to other recognized methods it will use the Wildman mold mycelia count method (*J. Assoc. Official Agr. Chemists 22, 76-77 (1939)*; this Journal p. 239) to determine whether unfit cream has been used in manufacturing butter.

Experiments and observations under commercial conditions indicate that where good sour cream, produced and handled with reasonable care, is used for churning, the mold mycelia count of the butter will be well under 60 percent. The goal of all manufacturers should be elimination of all unfit cream. There is evidence that careful manufacturers may, without serious difficulty, produce butter showing mold mycelia counts in the neighborhood of 20 percent.

Progress Report on Grade A Milk Control Laboratories (Bacteriological) *

C. E. Safford

State Department of Agriculture and Markets, Albany, N. Y.

The statutory provision for licensing workers to make bacterial counts on milk and cream to be used as a basis for premium payments to producers, was added to the Agriculture and Markets Law in 1926. The provision for the state branding of accurate bacteriological pipettes was included in 1927. During 1929, a survey was made of the conditions in the commercial laboratories to determine what improvements could be made to establish more accurate and uniform practices. The paying of premiums for low count milk simulates the practice of paying for milk on the basis of its fat content. The first premiums for low bacterial counts were paid in 1915.

The progress made since 1930 to unify and improve sampling methods and laboratory technic will be reviewed in this report. The control exercised by the Department is essentially to assure uniform and fair treatment to milk producers. This involves setting up minimum standards for the qualifications of the laboratory personnel, for the equipment and technic used in the laboratories and for representative sampling.

Because the payment of a premium may depend on the presence or absence

of a single colony on any plate, it is positively essential that the utmost care be exercised at all times. Periodically, directions to supplement *Standard Methods for the Examination of Dairy Products* have been issued by the Department in order to satisfy the peculiar needs conditioned by this special application of bacteriological technic. Tolerances above the accepted premium limits are untenable because no one knows what such tolerances should be. The acceptance of any tolerance merely means an increased premium limit and is therefore unjust. For these reasons, both the count and, where averages of counts are made, the averages are reported to the producer exactly as determined from the laboratory's original records.

At the present time there are 66 laboratories and 125 licensees operating under the supervision of the Department of Agriculture and Markets, as compared with 62 laboratories and 125 licensees in 1933, and 54 laboratories with 100 licensees in 1928, respectively. So far as available records would permit, the present laboratory personnel has been classified according to the types of analysis made as follows:

Milk Control Laboratories Supervised by Department of Agriculture

Type of Analysis	Laboratories No.	Licensees No.	Plants No.
Standard agar plate counts (premiums)	24	58	65a
Direct microscopic examinations	16	34	88a
Local control	64	121	100b

a Milk receiving stations.

b Agencies for which local control work is done, including milk supplies, plant operations, retail delivery samples, etc.

* Condensed from 13th Annual Report of New York State Association of Dairy and Milk Inspectors, 1939.

The relatively recent degrading of Grade A plants is an inevitable result of economic conditions forced on the industry in part by the consumption of an increasingly larger proportion of Grade B instead of Grade A products. Although laboratory work is necessarily influenced by economic conditions, effective control of milk quality can be accomplished only by means of adequate equipment and properly trained personnel.

PERSONNEL

The licensees referred to in the foregoing table include only those actually employed in bacteriological laboratories. Although the Department examines all properly qualified candidates, no licenses are issued until the applicants become employed in laboratories having adequate equipment and facilities.

Two types of licenses are issued, namely "technician" and "supervisory"; these terms replace the former "limited" and "unlimited" licenses, respectively. The qualifications recommended for the supervisory license, which is necessary for every laboratory where the nature or volume of work so demand, include graduation from a recognized college or university with specialization in dairy bacteriology (or the equivalent) and sufficient practical laboratory experience. In view of the increasing demand for the sanitary control of milk quality, the examination for this type of license covers essentials in the sanitary production of milk, all of the commonly employed laboratory methods for determining the quality of milk and the control of milk-borne diseases under practical conditions. The minimum qualifications for a "technician" license are graduation from a standard senior high school (or the equivalent) and at least six months' satisfactory laboratory experience. The candidate must also pass an examination demonstrating his ability to make bac-

terial counts while under the direction of a supervisory licensee.

Unemployed individuals who pass the license examination satisfactorily are issued certificates stating they have qualified for licenses which will be issued on full compliance with the necessary requirements. This policy was adopted because it is impossible to control the activities of a licensee not specifically assigned to any laboratory. The Department maintains a list of successful candidates from which an employer can select a laboratory technician or bacteriologist if he so desires. In the interests of establishing better qualified laboratory personnel, selection of employees from this list is welcomed.

Laboratory workers are now licensed by the Department to make direct microscopic as well as standard agar plate counts. In some cases licenses are desired for direct microscopic examinations only, even though no premiums are involved. For these reasons licenses are now being issued according to the type of work done in the particular laboratory, that is, for standard agar plate counts, direct microscopic examinations, or both.

Although less training is ordinarily required for the microscopic method than for the plate count procedure, there is good reason to believe that many persons making direct microscopic counts do not have sufficient training and experience.

In addition to greater general efficiency, there are several other very good reasons for placing special emphasis upon the qualifications of laboratory personnel. A well-trained individual is capable of doing other types of analyses not specifically connected with the license, for example: laboratory control on mastitis, the phosphatase, colon and methylene blue tests, as well as miscellaneous plant control work which might include routine examinations on equipment, maintenance of starters and in emergencies, prompt checking of ropy milk, thermophilic, and milkborne disease outbreaks.

SAMPLING MILK AND CREAM FOR BACTERIAL CONTENT AT RECEIVING STATIONS

Perhaps the most important change in sampling methods was made in 1935 when the weigh vat sample replaced the single can sample as a basis for premium payments. An investigation to determine the merits of the weigh vat sampling method was conducted by Doctors Yale and Breed (1) of the Geneva Agricultural Experiment Station with the cooperation of Sheffield Farms, Borden's Farm Products Company and the Dairy-men's League Cooperative Association. Although certain precautions must be strictly observed in weigh vat sampling, the results of the investigation showed this to be a practical method of securing a more completely representative sample of the producer's milk. The special precautions include:

1. Complete drainage of the milk from the vat (total residual milk not to exceed one pound).
2. Use of a shallow strainer which will not interfere with proper mixing of the milk.
3. Maintenance of a record of the patrons in the order of delivery. This record provides a means of determining whether or not a high count (or failure to earn a premium) may be attributed to contamination with the small amount of the preceding producer's supply left in the weigh tank.

A few complaints were submitted soon after weigh vat sampling was introduced. As soon as the advantages were understood, there seemed to be no further protests. Contamination from deliveries of milk previously dumped into the weigh vat has been shown to be so slight, if any, and so infrequently positively demonstrable, that no changes or tolerances in the premium limits were considered feasible. In case of doubt regarding a sample, the practice is to replace the questionable sample at a later date with one preferably secured by preparing a composite from all the single cans, each of which must be thoroughly agitated. Considering the normal bacterial content of Grade A milk, re-sampling will be seldom necessary if the

above precautions are observed. (Samples taken for certain public health purposes obviously must not be secured from the weigh vat.)

Considerable emphasis has been placed on the technic of sampling, because the results of analysis on a sample improperly taken or handled are unreliable. The plant managers in the respective receiving stations are held responsible for the removal of representative milk samples. Sampling instructions are supplied to plant managers or other interested persons. The regular inspection service rendered to Grade A milk plants has resulted in considerable improvement of sampling methods.

The milk or cream shall be thoroughly and vigorously agitated or poured into the weigh vat in such a manner as to be thoroughly mixed immediately before sampling. A satisfactory arrangement for sampling consists of having two milk cans filled with water in the milk receiving room. After each use, the sampling instrument (dipper or milk thief) is rinsed in the first can which is kept full by means of a stream of cold water continually flowing through it while the samples are being collected. The rinsed instrument is then scalded in the second can which is full of hot water. The temperature of the water in the second can shall be kept at not less than 180° F. by continually bubbling steam through it. This is most satisfactorily done by having a removable steam pipe leading into the bottom of the can so that the latter may be washed when not in use.

The usual procedure is to remove the sample with a suitable instrument through an opening in the cover of the weigh vat. The samples are usually placed in screw cap glass specimen vials which are kept cool in trays containing fine cracked ice and water at about 40° F. or less.

For protecting the vials during transit, covered metallic water-tight containers surrounded by cracked ice and just deep enough to hold one tier of vials, will offer much greater protection during transportation than when the sample vials

are in direct contact with the ice and water. One or more of these containers, each for a single tier of vials, may be placed in a large metallic watertight container, the latter being surrounded by cracked ice in a wooden shipping box.

Samples are unfit for analysis if the temperature has, at any time, exceeded 45° F. during the interval between sampling and plating. Temperatures lower than 40° F. are preferred particularly when samples are held more than eight hours.

Just so far as is practical and possible, the samples shall be examined on the same day that they are taken. When establishing new laboratories, it is recommended that they be located at points which will reduce the interval between taking the samples and the subsequent laboratory examination to a minimum.

EQUIPMENT AND FACILITIES

Since the payment of practically all bacterial premiums is confined to low count milk, the analysis must be made by the standard agar plate procedure. Therefore emphasis is placed on certain requirements pertaining to this method.

1. Working space

A satisfactory working space including tables for plating samples, storage space for equipment, and washing and sterilizing facilities, shall be provided in each laboratory. The laboratory should be reasonably free from currents of air, free from dust, and not regularly employed for the performance of miscellaneous chemical work. Such conditions as high temperatures and excessive humidity in the plating and incubating rooms probably enhance the formation of spreaders. Washing and sterilizing rooms therefore should be separated from the plating room.

2. Plate-counting apparatus

The Quebec colony counter (obtainable from Spencer Lens Company, Buffalo, New York), or its equivalent, is the only apparatus approved by the Department for counting agar plates, because

it seems to combine all of the desirable features of a satisfactory plate-counting device. The lens is supported at a fixed elevation and is sufficiently large to permit examination of the entire plate in a single position. Satisfactory illumination is provided by means of a 60 watt frosted electric light bulb and a mirror, so arranged that both direct and reflected light rays are uniformly focused on all portions of the agar plate. Perhaps this is the most important advantage of the Quebec colony counter because pinpoint colonies, especially those near the bottom or edges of the Petri dish, are easily identified. Other desirable features include the Wolffheugel glass guide plate, a clamp for holding the petri dish, which can be adjusted to fit any plate of ordinary size, and the inclined position of the device which increases ease in counting. Questionable objects and doubtful colonies should always be examined under higher magnification to determine their identity.

3. Incubators

Whereas a water-jacketed incubator is usually preferable, many of the anhydric incubators which are often characterized by exceedingly high-temperature, small-area heating units, have been satisfactorily rewired according to directions secured from Professor C. N. Stark of Cornell University. Unless rewired, certain types of these anhydric incubators do not provide adequate protection from excessive spot-heating or proper direction of convection currents. Moreover, attention is called to the probable relation between the development of spreaders and the excessive too-rapid heating of plates near high-temperature heating units.

4. Bacteriological Pipettes

Although detailed specifications for pipettes are given in the seventh edition of *Standard Methods*, it is well to note that the 1 cc. pipette is now calibrated to deliver (not contain) 1 cc. under the special conditions of use. Such pipettes shall be drained within a 2 to 3 second interval for proper delivery.

5. Stoppers for dilution bottles

Rubber stoppers, one-hole type fitted with short glass rods, are recommended for closing dilution bottles. Special stoppers made completely of rubber, resembling the above in design, are satisfactory also. Such stoppers permit more vigorous shaking. They do not absorb water; they limit evaporation; and they protect the water from contamination during storage. Furthermore, they are relatively inexpensive and practically indestructible if made from high grade rubber. Cotton plugs as stoppers for dilution bottles are unsatisfactory for several reasons.

6. Autoclave

The autoclave must be equipped with both a thermometer and a pressure gauge each of which shall be accurate.

TECHNIC

Unquestionably the most significant change in the bacteriological examination of milk in recent years was made on July 1 of this year when the new tryptone-glucose-skim milk agar was officially recommended by the American Public Health Association. Whereas the major increases in plate counts occur with pasteurized milk samples, appreciably higher counts than were formerly secured may be expected on the new medium with raw milk, including some of the Grade A premium samples. The new agar permits the growth of mastitis organisms and other fastidious types and in substance, gives a count which more accurately represents the number of bacteria present. Special care must be taken to avoid re-heating milk agar or prolonged holding of the same after melting to avoid precipitates.

Less important changes dealing with the agar plate count technic include:

1. Maintenance of a daily temperature record for each incubator. Temperature readings (both A. M. and P. M.) are recorded for the top and bottom shelves, and a copy of these readings submitted to the Department.
2. A slight change in the accepted incubator temperature range to conform with

the new edition of *Standard Methods for the Examination of Dairy Products* as published by the American Public Health Association. The preferred range is from 35° to 37° C.

3. Increased use of the 1:200 dilution which eliminates the necessity for averaging many bacterial counts, especially in the Grade A premium class.

* * * *

Special efforts have been made to maintain uniformity with *Standard Methods* in recommending analytical procedures for milk control laboratories in this State. Since this special application of bacteriological analysis demands a higher degree of accuracy, and at the same time, rapid and less expensive practical methods, it has been necessary to prepare a supplement to *Standard Methods* specifically designed for Grade A premium samples. The supplement takes the form of Department Bulletin No. 323, copies of which will be distributed to interested agencies on request.

DISCUSSION

Mr. Powers: I need not discuss before this group the actual laboratory procedure which was very adequately covered by Dr. Safford. I would like to take this occasion, however, to express the appreciation of the industry, particularly those of us who have been associated with Grade A milk control laboratory work, for the activities of the division of the Department of Agriculture and Markets represented by Dr. Robertson, Dr. Safford and formerly Dr. Schacht.

These men have prepared a code of standard practice for making bacterial counts which serve as the basis for Grade A premium payments. This code follows closely the *Standard Methods for Milk Analysis* of the APHA but its authors have seen fit to modify these standard methods the better to meet the practical conditions encountered in Grade A milk control laboratories. To illustrate, a neighboring state following strictly the letter of standard methods found it necessary to require that two plates, each of different dilution, be made on every

Grade A premium sample. The New York State regulatory body wisely provided for one plate per sample at a dilution that would yield a significant count within the 10,000 and 25,000/cc premium limits thus avoiding a more costly, greater time-consuming, and quite unnecessary procedure. Such reasonable and understanding regulation together with periodical checking of technicians and laboratory procedure under the licensing provision has been effective in promoting confidence in this work among Grade A dairymen and has helped the industry to keep this work on a high plane.

The possibility of licenses for direct microscopic counting was mentioned and this might be a good thing. Even though the direct microscopic method is not generally used for premium counts it is being employed in many cases as the basis for rejecting milk before pasteurization.

A few years ago the industry suggested that Grade A premium samples taken from the weigh can would yield more representative counts than samples from a single can of each dairy delivery to the raw milk receiving station. The Department of Agriculture and Markets directed the work that proved the feasibility of this procedure and then made provision for weigh can sampling in the Code.

A few years ago this department, real-

izing the excellence of the Quebec colony counter for bacterial plate counting, provided several of these counters for trial by the industry, and after convincing the industry of the value of the counters proceeded to make the use of such counters mandatory in the Code.

The American Public Health Association decreed that effective as of July 1, 1939, standard nutrient agar be replaced with tryptone-glucose-skim-milk agar for making bacterial plate counts. Appreciating the possibility of somewhat higher bacterial counts due to the abrupt change in media during warm weather and desiring to cushion the effect on the dairymen, this department ruled that adoption of the new medium would be held in abeyance until January 1, 1940. This action is calculated to provide the dairymen an opportunity to adjust sanitary methods to the new medium at a time when prevailing temperature conditions will be in his favor.

The reasonable and understanding regulatory requirements of this department are deeply appreciated by dairymen and milk dealer alike.

REFERENCE

- (1) Yale, M. W. and Breed, R. S. Comparative Fairness of Single Can and Weigh Vat Samples for Bacterial Counts Used as Basis of Premium Payments to Grade A Dairymen. *Proceedings Twenty-Eighth Annual Convention International Association of Milk Dealers, 1935.*

A Comparative Study of Standard Lactose Broth and Modified Eijkman Medium for Isolation of Escherichia Coli from Nut Meats. Morris Ostrolenk and Albert C. Hunter. *Food Research, 5, 141 (1940).*

Use of the Eijkman method for the examination of pecans failed to enhance the recovery of ESCHERICHIA COLI. Although the Eijkman medium surpassed standard lactose broth in limiting the development of Aerobacter and Citrobacter, it reduced the development of Escherichia. Three times as many standard lactose broth tubes developed presumptive tests

as did a corresponding number of inoculated Eijkman broth tubes. In determining the incidence of fecal pollution on pecan meats standard lactose broth yielded Escherichia coli in 28.6 percent more plants, 15.7 percent more samples, and six per cent more inoculated tubes than did the Eijkman medium.

In addition to the less favorable bacteriological results obtained by the Eijkman method its limiting temperature requirements greatly influence its practicability in routine laboratory practice.

M. E. PARKER

The Application of Wetter Water to Dairy and Milk Plant Use

F. M. Scales and Muriel Kemp
Sheffield Farms Co., New York City

INTRODUCTION

A report of the application of wetter water to dairy and milk plant use must really be in the nature of a prophecy of the possibilities of its application to this work for, at the present time, so little is used that it is negligible. Even where it is employed in mixes, the purpose is to correct the faults of the present cleaning materials and is not, in any way, along the lines to be considered in this report which will show the efficiency of some of these products as detergents when used alone.

In any cleaning operation, the most important function of the solution used for that purpose is its wetting quality* (1). This enables it to squeeze in between the surface to be cleaned and the film that soils it. The condition governing this power depends on the interfacial tension between the liquid and the solid. When the solution has completely wet the surface, other physical and chemical factors become active in removing the residue. These factors are deflocculating, emulsifying, and dissolving qualities. When they function properly, the solution will so loosen the soiling film that it may be removed by a thorough rinsing.

REVIEW OF LITERATURE

For centuries soap was the wetting agent universally employed. In soft water it showed good wetting properties but it combined readily with some forms of organic matter and left a residue. If

the water were hard then insoluble metallic soaps were formed. These were curdy in character and apt to adhere to any surface.

The examination of soap solutions (2) in 1873 started the study of the properties of liquid surfaces. Lord Rayleigh studied the application of surface chemistry which is involved when oil is poured on troubled water. He determined the quantity required to accomplish the result. No great progress was made, however, until Langmuir (3), the American physicist, in 1917 discovered that many of the surface films were only one molecule thick. In addition, he studied the kind of molecules forming them. The studies of Adam (4), Harkins (5) and others have shown that in the control of liquid surfaces, the shape of the molecule, whether it is long and thin or short and thick, is an important factor. Some of the best wetting agents have long oil soluble chains of carbon atoms attached to a short water soluble group. The length of the oil soluble chain, whether straight or helical in structure, whether the water soluble group is attached to the end or side of the chain, whether the most active water soluble head is used and whether it can be compressed, are all factors that must be considered in developing a product that will have the oil soluble portion of the molecule properly balanced against the water soluble portion to produce a good surface active product.

Before all this information on liquid surfaces was available, Cochenhausen (6), in 1898, reported the characteristics of his sulfonated higher alcohols. Later the development of high pressure hydrogenation made these alcohols commercially available. Within several years

*The term "wetting," as employed here, is considered as vertical spreading while horizontal spreading is indicated by readings in surface tension. Some instances with vertical wetting or penetrating qualities may also show deflocculating properties. This latter quality is being investigated further as in some cases it appears to be the result of physical action and, in others, of chemical activity.

the right of marketing a further modification of his discovery was granted to two American concerns. The author (1) reported on the use of one of them before this Association in 1938. These were the first simple compounds of the surface active group.

By taking advantage of all the information made available by American and English physicists and chemists, a number of new products have been developed which in some ways are much superior to the sulfonated alcohols. There is much activity in this field so that new products may be expected on the market from time-to-time.

Wetting agents, as has been described, have as a group certain characteristic molecular structures. Such a molecule is composed of both hydrophobic and hydrophilic groups. The former may be either a straight or branched hydrocarbon chain or chains and may be an alkyl, aryl, or alkylaryl group. The latter or hydrophilic group, which is attached to the former, will most likely be a SO_4H (sulphate), SO_3H (sulfonate) or COOH (carboxylate) radical (7). The radicals which show this hydrophilic property may be divided into four groups.

1. Oxygen or sulphur with or without hydrogen
—C=O, —OH, —SH
2. Groups containing nitrogen
—CN, —CNS, —NO₂, —NH₂
3. Groups containing sulphur and oxygen or phosphorus and oxygen
—SO₂H, —SO₃H, —SO₂H, —PO₄H₂, —PO₃H₂, —OS₂O₂H
4. Groups containing halogen
Cl, Br, and I.

Langmuir and others proved that if a small quantity of a fatty acid is placed on water it spreads as a monomolecular layer. In the case of homologous fatty acids, the thickness of the oil film increases as the number of carbon atoms in the chain while the area per molecule on the surface remains the same. As the molecular cross section remained the same regardless of the length of the hydrophobic chain it was concluded that the molecules were standing on end. The hydrophobic group extend above the sur-

face of the water while the hydrophilic group are pointed down into the water. The molecule of the wetting agent thus oriented at an interface reduces the surface tension by changing the balance of molecular attraction on the surface of the solution.

Caryl, C. R. and Ericks, W. P. (8) in their report on Esters of Sodium Sulfosuccinic Acid have given the following as the properties which a wetting agent should possess:

1. High wetting power even in very low concentration.
2. Stability in dry form and in solutions, especially in acid solutions where soap cannot be used.
3. Solubility in water and in organic solvents especially in nonpolar solvents.
4. Resistance to hard water.

They further give a revision of the classification of wetting agents as given by Wilkes and Wickert (9) to include later discoveries of members of this group. Since this is the most complete classification, it is reproduced in Table 1.

EXPERIMENTAL WORK COMPARISON OF

WETTING TIME AND SURFACE TENSION

With the discovery of groups of new products with certain qualities superior to any other in use in the industries, there naturally comes a need for a method of grading or classifying them according to the strength of these qualities. This need was met by Doctors Carl Z. Draves and R. G. Clarkson in their report "A New Method for the Evaluation of Wetting Agents." The apparatus and procedure which they describe has become known as the Draves Method and has been officially adopted for the evaluation of wetting agents by the American Association of Textile Chemists and Colorists (10). They were the first investigators to present a test that showed accurately that equivalent quantities of wetting agents produced wetting at the same rate. These data furnish a basis

TABLE 1
Classification of Wetting Agents

Group	Type No.	Formula*	Description	Percentage relative wetting power of best of each type as marketed
A	1	R—COONa	Soaps	
	2	R—SO ₂ Na	Fatty alcohol sulfates	23
	3	R—CONHC ₂ H ₄ SO ₂ Na	Sulfated fatty acid amides	5
	4	R—COOC ₂ H ₄ SO ₂ Na	Sulfated fatty acid esters	6
B	1	R ¹ —CHSO ₂ Na	Secondary alcohol sulfates	25
	2	R ¹ —OOC—CH ₂ — C—O—SO ₂ Na H	Sulfated esters of higher alcohols and dibasic acids	Not marketed
	3	R ¹ —Ar—SO ₂ Na	Alkylaryl sulfonates	6
	4	R ¹ —OOC—CH ₂ — C—SO ₂ Na H	Sulfonated esters of alcohols and dibasic acid	100
C	—	—	Miscellaneous	24

* R=fatty alkyl group R¹=primary or secondary nonfatty alkyl group;
Ar=aryl or aromatic group.

for comparing the efficiency of products according to cost

The du Nouÿ tensiometer has long been recognized as a precision instrument for determining the surface tension of solution, but in a cleaning operation other properties besides this one are important. Wetting, deflocculating, emulsifying, and dissolving factors complete a detergent action that cannot be determined by surface tension data. The Draves method supplies data of more importance to the investigator than those obtained from readings with the du Nouÿ

TABLE 2
Surface Tension Measurements*

Percent concentration	du Nouÿ tensiometer dynes per centimeter	Draves method penetrating time in seconds
0.5	32.4	6.2
0.25	32.6	12.9
0.125	32.5	34.9
0.0625	31.8	180.0+

* Reference: Monsanto Chemical Co., Bulletin No. 14.

tensiometer. The results obtained with the former depend not only on surface tension but also on wetting or penetration. The difference between the value of the two methods is made clear in Table 2 by a comparison of the results obtained when different percentages of solutions prepared with the same wetting agent are examined.

FAULTS OF PRESENT CLEANING AGENTS

Anyone who has had experience with the various cleaning powders on the market knows that in many cases the alkali is so strong that in hot solution it darkens any metal surface with which it comes in contact, with the possible exception of stainless steel. This is due to the fact that detergency with these products depends on the chemical reaction which they produce. In addition to this, considerable labor is required in brushing metal surfaces on which milk has been heated. In other equipment, the alkali used creates a precipitate of lime which, unless precautions are taken, becomes cooked on and very hard to remove. Continued use of some of the

hot alkali solutions even corrodes the metal surfaces. Where it is desired to have a clean, bright surface before the milk is started through the equipment, it is common practice to give the equipment a light treatment with a scouring powder to get rid of the film formed by the cleaning powder. All of these operations require considerable time.

The ideal to be sought in detergency with these new products is to obtain some that will give a satisfactory cleaning so that at the end of the operation the metal surface will be left in its normal, bright condition. The wetting agents will have no harmful effect on the hands as is frequently the case with the alkali cleaners. Lime salts will be held in a dispersed condition so that there will be no build-up of milk stone on the equipment. It will not be necessary to have the solutions at such a high temperature, thereby causing less harm to the metal surfaces that they come in contact with.

Just what the saving of time will be cannot be prophesied at present but these agents will most certainly lighten the labor of the cleaning operation, do it in a more efficient manner, and reduce or make unnecessary any follow-up polishing operation.

THE WETTING AGENTS

The wetting agents are in general high priced, ranging from 15c to \$1.50 per pound. When the demand has been increased so that the companies producing them can step up the production, the price will undoubtedly be reduced. Very small quantities of these agents will reduce the surface tension and the wetting time considerably, but in this work, since the relative detergency of the products was to be compared, it was necessary to adopt a standard time and then use the quantity of each product that gave a wetting time within the limits. The time selected was from 10 to 15 seconds as determined by the Draves method.

Since these materials have not yet been used in plant tests, the work described here is considered as preliminary and the

products are designated by letters. Names may be given later when their qualities have been established.

These products do not all act in a uniformly progressive way in wetting as a few examples of results with the Draves method will show.

TABLE 3
Results Obtained With Draves Test
On Different Concentrations of Wetting Agents

Percent	Wetting Time in Seconds Temperature 77°F. (25°C.)				
	A	B	C	D	M
0.02	-----	37.7	-----	-----	-----
0.025	-----	15.0	-----	-----	-----
0.050	-----	6.0	-----	-----	-----
0.100	80.0	Instantaneous	772.0	130.4	1800+
0.200	36.6	-----	21.4	-----	-----
0.250	-----	-----	16.0	-----	-----
0.300	-----	-----	7.6	-----	-----
0.350	18.6	-----	-----	-----	-----
0.400	-----	-----	-----	31.4	-----
0.450	12.3	-----	-----	-----	-----
0.500	-----	-----	4.0	24.0	213.2
0.600	-----	-----	-----	22.0	-----
0.700	-----	-----	-----	20.0	-----
0.800	-----	-----	-----	18.0	-----
1.000	-----	-----	-----	16.6	61.8
1.500	-----	-----	-----	12.6	28.8

In column A the product examined had a wetting time of 80 seconds in 0.1 percent concentration. In 0.2 percent concentration the time was 36.6 seconds and at 0.45 percent the time was 12.3 seconds. This reagent was tested at 0.4 percent concentration.

In column B the product at 0.1 percent concentration gave instant wetting. By starting wetting tests at 0.02 percent concentration, it was found that the time at 0.025 percent concentration was 15 seconds.

The next product (column C) at a concentration of 0.1 percent had a wetting time of 772 seconds. At a 0.2 percent concentration, the wetting time is reduced to 21.4 seconds, a reduction in time of 97.2 percent. A solution of 0.25 percent concentration brings the wetting time to that which includes it in the group for further tests. The additional figures show that a half percent concentration brings the wetting time to 4 seconds.

In column D the wetting time of the product in 0.1 percent concentration was 130 seconds. The wetting time in this case is reduced very slowly so that at ten times this concentration or 1.0 percent it is 16.6 seconds which brings it in the group for further tests provided the cost of solution is not excessive.

In column M it is found that the wetting time of a solution of 0.1 percent concentration is over 1800 seconds and that increasing the concentration 5 times reduces the time to 213.2 seconds while an increase of 15 times reduces it to 28.8 seconds. This latter is almost twice the upper limit (10 to 15 seconds) which we adopted as a good wetting range.

The wetting activity of these five products will give an idea of the characteristic action of the wetting agents when examined by the Draves method.

When figures similar to those shown in Table 3 had been obtained for all the

TABLE 4
Comparative Costs of Wetting Solutions
Figured on Basis of 100 Gallons of Solution
with ** Selected Wetting Times

Product	Percent	Wetting Time in tap water at this same temperature was 10,800 seconds	
		Wetting Time	Cost 100 Gal.
A	0.40	11.5	\$0.835
B	0.025	15.0	0.312
C	0.25	16.0	0.624
D	1.50	12.6	3.125
E	1.00	14.4	1.251
F	1.45	12.4	6.050
H	1.50	21.0*	2.127
I	0.70	11.3	1.752
J	0.70	10.5	1.051
M	0.50	213.2	1.000
N	0.10	11.3	0.375
O	0.50	15.0	0.625
P	0.30	12.4	0.375
Q	0.50	10.0	0.626
R	0.50	12.6	1.000
T	0.40	11.3	1.168
W	1.00	15.0	0.719

* Higher concentrations were of such a milky consistency as to make it impossible to see test.

** Two of the products that showed a longer wetting time than the limit selected were included in the tests because they were different in composition.

wetting agents available, another table was prepared to present the percentage strength of solution required to give a wetting time within the range desired for this work. The corresponding wetting time is shown in Table 4 and also the cost of preparing a large volume of solution—in this case 100 gallons.

As previous experience had proved that a number of the wetting agents would readily clean equipment that had been in contact with cold milk, an effort was made to obtain some information on their value against a deposit on metal formed by hot milk. This was obtained by means of a laboratory test.

CLASSIFICATION OF PRODUCTS
ACCORDING TO DETERGENT QUALITY

The metal with an adhesive milk film was obtained by exposing 3 inch squares of tinned copper to milk just below the boiling point for 2½ hours. During the exposure the squares were removed from the milk three times for ten minutes each to give the milk an opportunity to dry on. After the 2½ hours in the milk, the films were allowed to dry for four days at room temperature before the test was made. The metal squares were dipped in a solution of the particular wetting agent of the strength shown in Table 4 at a temperature of 120° F. for one minute without agitation, and then moved back and forth for one minute to create a circulation of the solution across their surface. They were rinsed by dipping in tap water at a temperature of 120° F. When the squares, after this treatment, were completely free of milk

TABLE 5
Classification According to Detergent Quality

Designation	Good	Medium	Fair	Poor
A	-----	++	-----	-----
B	-----	+	-----	-----
C	+	-----	-----	-----
E	++	-----	-----	-----
F	+	-----	-----	-----
H	-----	-----	++	-----
I	+	-----	-----	-----
M	+++	-----	-----	-----
N	-----	-----	++	-----
S	-----	+	-----	-----
T	-----	-----	-----	+
W	++	-----	-----	-----

film and appeared bright and clean without any trace of grease on the surface, the detergent quality of the wetting agent was considered perfectly satisfactory and was graded as "Good" in Table 5. When this treatment had to be followed by a very light brushing it was considered "Medium," when followed by hard brushing it was classified as "Fair," and if it showed little or no detergent quality it was placed in the "Poor" column.

The results presented in Table 5 show that 50 percent of the products examined can be classified as good detergents. One of these was excellent, two very good, and three good. The high percentage serves to indicate the possibilities in this field since this is only the beginning, and these products can be modified so that their action will be greatly improved. In this connection it should be pointed out that an increase in length of the carbon chain in the wetting agent (see M in Tables 4 and 5) improves its detergency at the expense of wetting. The reverse holds true with shortening of the chain. Therefore, with the wetting agents available at present, the best results will, in general, be obtained by choosing a wetting agent with an intermediate wetting time and a fairly long carbon chain or else mixing two such reagents, one with a short carbon chain for wetting and the other with a long chain to promote detergent action.

For comparison with these results, four of the common alkalis were prepared in one-half percent concentration and

were subjected to the same tests. Table 6 shows the results.

In the wetting test these solutions were allowed to stand for 40 minutes but the skein gave no sign of sinking in this time.

The cost for the alkalis is very much less than that for the wetting agents but with improvements in the latter and the development of the proper procedures for them, the difference in cost will be more than balanced by the saving in labor and equipment.

It is apparent that in cleaning the effectiveness of the alkalis depends on a different function from that of the wetting agents.

Since the work of the alkalis in the plant has not been uniformly satisfactory, it seems consideration should be given to new standards for judging detergents.

DISCUSSION

It is known that certain wetting agents may be mixed with alkali cleansers and a great improvement obtained in the work performed. For this reason no examination was made of mixtures of alkalis and wetting agents. Such mixtures would still have the objectionable qualities that have been described previously. The investigation described here is an effort to find a way of cleaning without alkalis but with a new group of products that will give better results with less labor and no damaging effect like spangling and corrosion.

The cleaning of surfaces that have been exposed to cold milk is no problem

with solutions of the wetting agents, provided the right ones are used in sufficient concentration. Cleaning of this kind requires wetting, penetrating (deflocculating), and emulsifying qualities. These are fairly common characteristics of the new agents.

When the metal surface is exposed to hot milk which becomes more or less cooked onto it, the cleaning solution, in order to give satisfactory results, must possess, in addition to the three qualities required for removing deposits left by cold milk, the dissolving one. Thus as the harder films are wet and penetrated deeper and deeper, the emulsifying action removes the fat in them and the solvent action softens and dissolves them right to the metal surface. The first three qualities are physical reactions, the last one is chemical. It seems likely that penetration and emulsification are just different functions of wetting quality. If experience proves that this is true, then cleaning agents may be classified according to wetting and dissolving qualities.

In all food industry, and in none more perhaps than the dairy industry, sanitation is one of the most important factors in obtaining a satisfactory product. Since sanitation is a result of cleaning and sterilizing, it is apparent that detergency is one of the primary factors in the proper preparation of all food for market. The food industry as a whole should long ago have organized and supported an Institute of Sanitation where the many problems connected with proper cleaning could be worked out—an Institute that Boards of Health as well as manufacturers could go to for information. Perhaps this has not been done before because there has not been a full realization of the amount of labor, time, and equipment that may be saved by the application of the best cleaning methods. With the expanding use of wetting agents, some of which may be used for cleaning and sterilizing at the same time, a new day will be dawning for the food industry if it takes advantage of the

opportunity by creating such an organization. With the proper application of these products, there will be no corrosion of equipment. Research is needed to solve these problems.

More data will be presented on this problem in the near future.

CONCLUSION

1. The important qualities at work in detergency are wetting, penetrating, emulsifying, and dissolving.
2. The alkalis tested were deficient in wetting and penetrating (deflocculating) qualities under the conditions of this test.
3. The examinations of the products that make wetter water have shown that there is available for dairy and milk plant cleaning a new group of reagents that will yield better results without the harmful effects produced at present. There will also be a saving in time and labor by their use.

BIBLIOGRAPHY

- (1) Scales, F. M., "Hit-or-Miss" Methods of Controlling Washing and Sterilizing. New York State Assoc. of Dairy and Milk Inspectors, 11th Annual Report, pp. 57-76 (1937). See also *J. Milk Technol.* 1, No. 5, 39 (1938).
- (2) Plateau. *Statique experimentale et theorique des Liquides* 2, 47 (1873), 2nd edition 1920.
- (3) Langmuir, Irving, *Proc. Nat'l. Acad. Sci., U. S.*, 3, 251-7 (1917).
- (4) Adam, N. K., *J. Soc. Dyers & Colourists*, 53, 122 (1937).
- (5) Harkins, W. D., *J. Am. Chem. Soc.*, 2665 (1922), p. 354 (1917), p. 541 (1917).
- (6) Cochenhausen, E. von, (Patent 1898).
- (7) Dean, H. K., *Wetting and Detergency Symposium*, London, p. 25 (1937).
- (8) Caryl, C. R. and Ericks, W. P., *Ind. Eng. Chem.* 31, 44 (1939).
- (9) Wilkes, B. G. and Wickert, J. N., *Ibid.* 29, 1234 (1937).
- (10) Draves, C. Z. and Clarkson, R. G., *Draves Method: 1938 Year Book of the Am. Assoc. of Textile Chemists and Colorists*, Vol. XV Evaluation of Wetting Agents.

TABLE 6
Comparative Cost and Detergent Qualities
of
Common Alkali Cleaners

Present	Percent	Wetting time in seconds	Cost 100 Gal.	Detergent Classification
Caustic Soda	0.5	2400++	\$0.16	Fair+
*Sodium Metasilicate	0.5	"	0.14	Fair+
Soda Ash	0.5	"	0.09	Fair+
Trisodium Phosphate	0.5	"	0.13	Medium

* Darker surface on metal.

Supplementary Notes on the Deaeration of Milk *

Paul F. Sharp, David B. Hand, and E. S. Guthrie
Cornell University, Ithaca, New York

It seems logical to assume that one way to prevent the development of the oxidized flavors in dairy products is to eliminate the oxygen. That is the purpose of deaerating milk.

This raw product in the udder of the cow is almost devoid of oxygen. As it passes through the handling process, it absorbs oxygen. Just in milking alone, approximately 6 or 7 milligrams of oxygen may be absorbed in a liter of milk. This amounts to 6 or 7 parts per million.

The range of the absorbed oxygen content of milk in 40 different cans was from 4 to 7.1 milligrams per liter. This milk was strained into the cans and then cooled in a tank. It was not passed over a surface cooler.

* This paper supplements the one published on page 137 (May-June issue)—Editor.

TABLE 1*

Oxygen Content of Milk as Influenced by Holder Pasteurization and Passage over a Surface Cooler

Samples taken for examination	Experiment No. 1		Experiment No. 2	
	Oxygen content mg/liter	Third day oxidized flavor score†	Oxygen content mg/liter	Third day oxidized flavor score†
Raw milk in past. vat	6.03	1
Heated to 143° F. Start pasteurization	6.03	1	4.94	2
Held at 143° F. End pasteurization	5.42	1	4.94	2
Cooled in vat to 120° F.	5.25	1	5.42	2
Distributor over surface cooler	5.55	2	5.42	2
Collecting pan under surface cooler	8.94	3	9.43	4
After bottling	9.26	3	9.43	4
Held in vat at 120° F. for 30 minutes	5.89	2	5.55	2

* This table is similar to Table 4, page 139, except that the "Oxygen content" is reported higher, as determined by improvement in the analytical methods.

† The flavor score is that used by several investigators who have studied oxidized flavor—no oxidized flavor, ?=oxidized flavor may be present, 1=oxidized flavor is present, 2=a slight increase in oxidized flavor over 1, 3 is a stage more of this flavor, and 4=almost tallowy it is so oxidized.

One of the important steps in the development and testing of this equipment and the deaerating procedure is that of perfecting a method that is both simple and accurate for the determination of dissolved oxygen. A method which already has yielded results of great value has been developed. Suffice it to say now that the time requirement is less than an hour, it is not expensive, it is easy to operate, and many samples can be run at the same time.

Use of this new method has shown that the oxygen content of the milk as recorded in Table 4, page 139, of the previously published paper was greater than there indicated. The figures given in the columns of "Oxygen content" should be replaced with higher values, as shown in Table 1.

It should be noted, in Table 1, that as the temperature is raised, oxygen is eliminated and that when the milk is agitated at the lower temperatures, oxygen is absorbed. The most significant feature of these figures is the correlation that exists between the high oxygen content of the milk and the intensity of the oxidized flavors.

The effect of deaerating milk with the experimental equipment is more correctly shown by substituting the following figures, namely, 6.35, 0.17, 0.44, 0.61, 1.36, 0.66, and 6.58, in the column "oxygen

content" of Table 6, page 141, of the former paper.

The new figures of the oxygen content do not appreciably change the relative values of the oxygen but only the absolute values.

Attention is drawn to the maintenance of vitamin C in the deaerated milk as well as to the correlation that exists in the pasteurized product between the amount of oxygen in the milk and the oxidized flavors.

It is said that "the proof of the pudding is the eating", so in conclusion we offer the data in Table 2.

TABLE 2
Demonstration of the Effect of Deaeration of Milk on the Oxidized Flavors
Flavor Scores

Exhibited at Vermont Dairy Plant Operators and Managers' Convention October 25-26, 1939.

Set I (12 days)			
	O. E. Herreid	J. A. Newlander	E. S. Guthrie
Check sample	18 oxidized	18 oxidized	16 oxidized
Deaerated sample	22½ slightly "off"	22½ slightly "off"	22 slightly coppery

Set II (9 days)			
Check sample	19 oxidized	19 oxidized	18 oxidized
Deaerated sample	23 no criticism	23 no criticism	23 no criticism

Exhibited at the Metropolitan Dairy Technology Society, April 16, 1940.

Set I (8 days)			
	H. F. Judkins	James Bohn	E. S. Guthrie
Check sample	16 or 17 oxidized	16 or 17 oxidized	16 oxidized
Deaerated sample	23 no criticism	23 no criticism	23 no criticism

Exhibited at Massachusetts State College, May 9, 1940

Set I (6 days)			
	H. G. Lindquist	M. J. Mack	E. S. Guthrie
Check sample	18 oxidized	18 oxidized	17 oxidized
Deaerated sample	23.25 no criticism	23.25 no criticism	23 no criticism

Set II (7 days)			
Check sample	18 oxidized	18 oxidized	17 oxidized
Deaerated sample	23 no criticism	23 no criticism	23 no criticism

Set III (29 days)			
Check sample	12 oxidized	16 oxidized	14 oxidized
Deaerated sample	22.50 old	22.5 unclean	22.5 lacked freshness

How to Overcome Defective Babcock Cream Tests

T. J. Grenier

The Fairmont Creamery Company, Buffalo, New York

It is not uncommon in laboratories where cream is tested on a large scale by the Babcock method, to find that at least 10 out of 100 finished tests have fat columns which are cloudy and that have uneven or indistinct feathery lower ends. Many times the tests although not perfect are hardly bad enough to be repeated.

The usual methods of testing cream by the Babcock method as described in textbooks on the dairy industry, and as described in circulars of state departments of agriculture and markets were followed in our laboratory in Buffalo. Hundreds of Babcock cream tests are run daily by several New York State licensed testers. All kinds of cream from the best grades of sweet raw and pasteurized cream to the lower grades of sour cream have been tested. Experience shows that most everyone, when examination is made, has a few tests that are defective.

Many variations of the Babcock test were tried with a view of developing a method that would give all perfect tests. In our laboratory, 32 samples are tested at one time. The method developed and described below has given perfect tests. Since developing this modification, we learn that this very method has been used for many years, and although never before printed is taught in some colleges at the present time. The method gives a means of control on each test, avoiding burning or charring of the cream when treated with sulfuric acid as well as controlling insufficient acid treatment. The method thus allows the sulfuric acid to act on the cream only until it is digested or burned enough to give a perfect fat column in the finished test. When this burning action on the butterfat should stop, it is checked.

METHOD

Weigh accurately 9 grams of well mixed cream into a 9 gram 50 percent

Babcock cream test bottle. Do not add water to the cream. Add 9 cc. sulfuric acid of the usual strength (i. e. sp. gr. 1.82 to 1.83 at 60° F.) directly to the 9 grams of cream. The acid and cream should be about 60° F. before being mixed. Usually having cream and acid at room temperature (about 68° F.) before mixing gives good results. As the acid is being added, rotate the bottle so as to wash into the body any cream adhering in the neck of the bottle. Mix with a gentle rotary motion until all curd has disappeared. Continue shaking for 1/2 minute longer. The cream and acid should be a chocolate brown or pale port wine color. If not dark enough, add about 3 cc. more sulfuric acid and shake. Do this until the above described color is attained.

If several samples are being tested together and a few become dark before the shaking is completed, set the dark samples aside. Immediately treat them with hot water as described below. The rest of the samples are to be shaken and treated with more sulfuric acid if needed until all are dark enough.

Here is the important step in this method. As soon as samples are dark enough add 2 - 4 cc. of hot soft water at a temperature of 160° to 200° F. to each sample. Let the hot water run down the neck of each bottle. Do not shake. This is important as the hot water lifts the butterfat out of the acid mixture and stops the burning action of the acid on the fat. The hot water itself has no burning action on the fat. The hot water rests on top of the cream mixture, because of its lighter weight. It is heavier than the butterfat and forces the fat up out of the acid serving as a protective layer between the acid and fat.

The addition of hot water at just the right time gives the operator a means of controlling the burning action of the acid mixture on the fat. If one is careful to have the cream acid mixture, after shaking, at the correct chocolate brown or pale port wine color, the test will be perfect. The adding of hot water after acid treatment gives a control agent that makes possible the elimination of defective and doubtful tests. If one uses 18 grams of cream in 18 gram bottles, he must double the amount of acid and follow the same instructions as for 9 grams of cream.

From here on, the test may be completed following any of the various authentic methods. In our work the test was finished as follows:

To test bottles containing weighed cream, acid and hot water are placed in the heated centrifuge evenly in opposite cups, and whirled for 5 minutes. Hot

soft water at about 200° F. is added to the bottles, filling them to the bottom of the necks. The bottles are whirled 2 minutes. Hot soft water (about 200° F.) is added to each bottle, bringing the fat column well within the graduated portion of the neck. The bottles are whirled 1 minute and placed in water bath at 135° to 140° F. for 5 minutes. Red reader or meniscus remover are added for reading the tests.

CHECK AGAINST OFFICIAL METHOD

This procedure has been carefully checked against official government methods and Babcock methods described in dairy industry textbooks. In these check tests, some of the work was done in one laboratory and check tests made in another. Most of the tests made in other laboratories were done according to regulations governing the testing of cream by the New York State Department of Agriculture and Markets. Hundreds of these check tests were made.

In all this work, at least 10 percent of the tests following regulation methods had to be discarded because they were defective. Only perfect tests were used. Practically all tests run by the method here described were perfect and were recorded.

Most of the tests by either method were the same. Variations on 100 tests were such that the average for one method gave the same results as for the other. In no case did we find a tendency for this method to give either a higher or lower test than present accepted official Babcock methods.

It is believed the method warrants careful consideration of anyone interested in the testing of cream by the Babcock method.

Whey Solids in Milk. B. H. Weber and C. F. Hufnagel. Food Research, 5, 185 (1940).

An investigation of various whey products that were found suitable for the manufacture of whey candy indicated that sweetened, condensed, Cheddar-cheese or Swiss-cheese whey was, in general, the most satisfactory. When condensed without sugar, this whey was suitable for use in candy but its perishable nature was a serious disadvantage. Condensed, acid, cottage-cheese whey possessed good keeping qualities, but it required neutralization and it sometimes produced candy of inferior flavor.

It was demonstrated that excellent candy containing up to 40 percent whey solids could be made, the whey replacing, in part, sugar, skim milk, and corn sirup. Adjustments were made in handling technique and in formulas to allow for the effect of whey upon sucrose inversion and for the development of proper body through control of the physical state of the lactose.

M. E. PARKER.

Methods for the Determination of Vitamin A in Butter*

FOREWORD

Statements occasionally appear in the dairy press, textbooks and other sources of information, calling attention to possible variations in the vitamin A content of butter, with the implication that its nutrition value is or might be, thereby adversely affected. The Research Committee of the American Butter Institute accordingly has investigated the advisability of defining methods of analysis whereby this particular nutritive property of butter might be authoritatively determined. As a result of such a study, it is indicated that the practical value of applying such nutritional assays as a quality control procedure would prove of doubtful significance. In the bibliography of this bulletin are included the references found in the scientific literature on the subject, for the information of the student interested in further pursuing the impartial opinions therein cited.

DISCUSSION

The historic reaction for vitamin A (arsenic trichloride) has many limitations and is not now in general use (40). Of the methods that are in common use for the determination of vitamin A in various substances, the three principal ones are: antimony trichloride reaction, spectrophotometric determinations, and biological assays. Quite recently an iodometric titration has been suggested, but its application has been very limited (41).

The determination of vitamin A in butter is a rather difficult one. It is pretty generally agreed (17) (18), that the vitamin A activity is almost wholly

in the fat, and the determinations on the fat are taken as a measure of the vitamin A content. Vitamin A exists in butter in two forms: vitamin A per se and carotene the pro-vitamin. There is normally a correlation between the carotene content and the vitamin A value in the milk of a given cow (43) (18) (2) (44). This, however, is not always true (28). In addition, different breeds and different animals within a breed vary considerably in the proportion of carotene to vitamin A in their milk. Therefore, determinations of vitamin A value on butter must take both products into consideration.

As carotene is the principal pigment in normal butterfat, a method of direct colorimetric estimation of the carotene was early proposed (45) (33). However, carotene dissolved in butterfat gives a high and variable color when compared with known solutions of alpha carotene or color standards (37) (39).

A more accurate determination of carotene may be made by means of the absorption spectra, using a spectrophotometer.

This can be carried out by the method of Peterson (20) (35) or the method of Shertz (37). Absorption is usually measured at 4800, 4700, and 4550 Angstrom units.

The antimony trichloride reaction is also used for the determination of carotene. There are many modifications of this method, but the ones in use are primarily modifications of the method of Car and Price (5) (46) (36). The blue color produced is either measured in a tintometer or more accurately measured in a spectrophotometer measuring the absorption at 5900 Angstrom units.

For vitamin A per se the antimony trichloride reaction is also used. Absorption is measured at 6100 to 6300 Angstrom units in this case. Since carotene and vitamin A both give this reaction, for accurate determinations of either it is necessary to remove the other. In addition, it has been shown that the extracted unsaponifiable residue should be used for these determinations (1).

A more accurate determination seems to be by measuring spectrophotometrically the absorption of 3280 Angstrom units which is specific for vitamin A itself. Carotene does not have an absorption band in this position. (32) (11) (7).

In addition to the chemical tests of vitamin A there are, of course, the biological assays which have been considered standard for years. The method of Sherman and Munsell (38) as modified by other workers (44) is accurate for the determination of vitamin A in butter. This procedure is, however, time consuming and necessitates the maintenance of a rather large rat colony. Shorter methods have been proposed, as the single does method, but their exact accuracy is still doubted by some authors.

In measuring the vitamin A value of butter a combination of several of the above methods seems to be necessary: The vitamin A per se can be determined by means of the spectrophotometer and likewise the carotene (1). Since beta carotene is the normal occurring form in butterfat and is, weight by weight, equivalent to vitamin A (31), no great error would be encountered in determining the total vitamin activity by simple addition of these two determinations. These determinations have been shown to agree with the biological test (27). Such a procedure would, however, involve the use of an expensive, delicate piece of apparatus and a skilled technician and would hardly be suitable for routine work.

In this connection the Hilger Vitameter may be mentioned. This is an expensive, modified spectrophotometer

in which readings may be taken at only one wave length—that of the copper arc (3247 & 3274A)—which is close enough to the absorption band of vitamin A to permit of transmitting the desired beam, eliminating the rest through filters (21). This machine is, however, necessarily limited to the determination of vitamin A.

Colorimetric estimations of carotene could probably best be done on a petroleum ether solution of the extracted carotene freed from other carotinoids and vitamin A. Since the proportion of xanthophyll and other carotinoids in butter is small in relation to the carotene content for routine work, they might probably be disregarded (17). Likewise, it ought to be possible to estimate the vitamin A from the extracted unsaponifiable matter of butter freed from carotinoids using antimony trichloride. These procedures may be carried out by means of the method of Peterson et al (35).

CONCLUSION

Closely connected with the problem of methods for the analysis of vitamin A in butter is the problem of the value of such analyses. Many investigators have shown that the vitamin A content of butter is dependent primarily upon the feed of the cow. (30) (13) (29) (35) (10) (12) (15) (25) (34) (14) (23) (24) (19) (43) (18) (22) (44) (26) (3). In addition, this effect is a rapid one as has been recently proved (25) (34) (22) (28) (26) (31) (3) (9) (42). Therefore, any single analysis concerning the vitamin A content of butter would mean very little, as a new sample taken a few days later might display an entirely different vitamin A content due to changes in the character of the feed given the cows. In this connection, it may be mentioned that butter has been reported as containing anywhere between 2½ to 50 Sherman units per gram. These figures were obtained by the same investigators (16). Many other workers have given similar ranges in vitamin A content in butter (22) (44) (28) (6).

* Reprinted by courtesy of The American Butter Institute, Chicago, Ill., Research Committee Bulletin.

ACKNOWLEDGMENT

The Research Committee is indebted to Mr. W. C. Loy for his review of the scientific literature on this subject.

BIBLIOGRAPHY

- (1) Annual Review of Biochemistry 5:382 (1936).
- (2) Bauman & Steenbock, Jour. Biol. Chem. 101:547 (1933).
- (3) Baumann, Steenbock, Beeson & Rupel, Jour. Biol. Chem. 105:167 (1934).
- (4) Booth, Kon, Dann & Moore, Biochem. Jour. 27:1189 (1933).
- (5) Carr & Price, Biochem. Jour. 20:497 (1926).
- (6) Converse, Wiseman, & Meigs. Paper before 27th annual meeting, American Soc. of Animal Prod. (1934).
- (7) Coward, Dyer, Morton & Gaddum, Biochem. Jour. 25:1102 (1931).
- (8) Crawford, Golding, Perry & Zilva, Biochem. Jour. 24:682 (1930).
- (9) Doan, Jour. Dairy Science. 147 (1924).
- (10) Drummond, Lancet 2:482 (1918).
- (11) Drummond & Morton, Biochem. Jour. 23:785 (1929).
- (12) Drummond, Coward, Golding, Mackintosh & Zilva, Jour. Agr. Sci. 13:144 (1923).
- (13) Drummond, Coward & Watson, Jour. Agr. Sci. 14:531 (1924).
- (14) Dutcher, Proceedings, World's Dairy Congress 2:1060 (1923). Pa. State College Agr. Exp. Sta. Bulletin 275 (1932).
- (15) Dutcher, Prieson & Biester, Science 50:184 (1919).
- (16) Fraps & Treichler, Industrial & Engineering Chem. 24:1079 (1932).
- (17) Fundamentals of Dairy Science, 106:491 (1935).
- (18) Gillam, Neilbron, Morton, Bishop & Drummond. Biochem. Jour. 27:878 (1933).
- (19) Golding, Proceedings, World's Dairy Congress 2:164 (1923).
- (20) Guilbert, Industrial & Engineering Chem. (Anal. Ed.) 6:452 (1934).
- (21) Hilger, Publication No. 191/8 (1935).
- (22) Hilton, Hauge & Wilbur, Jour. Dairy Science 16:335 (1933).
- (23) Hughes, Fitch & Cave, Jour. Biol. Chem. 46:1 (1921).
- (24) Kennedy & Dutcher, Jour. Biol. Chem. 50:339 (1922).
- (25) Kennedy, Dutcher & Eckles, Jour. Biol. Chem. 55:345 (1923).
- (26) Krause, Ohio Agr. Exp. Sta., Bulletin 470 (1930).
- (27) Kunerth & Riddell, Jour. Dairy Science 21:41 (1938).
- (28) Loy, Hilton, Wilbur & Hauge, Jour. Dairy Science 20:31 (1937).
- (29) McCollum & Simmonds, Am. Jour. Phys. 46:274 (1918).
- (30) McCollum, Simmonds & Pitts, Jour. Biol. Chem. 27:33 (1916).
- (31) Moore, Lancet, 11:380 (1929); Biochem. Jour. 26:1 (1932); Biochem. Jour. 27:898 (1933).
- (32) Morton & Heilbron, Nature, 122:10 (1928); Biochem. Jour. 22:987 (1928); Biochem. Jour. 24:870 (1930).
- (33) Palmer, Carotinoids and Related Pigments (1922).
- (34) Palmer & Eckles, Jour. Biol. Chem. 17:19 (1914); Mo. Res. Bul. 9, 10, 11, 12, (1914).
- (35) Peterson, Hughes & Freeman, Industrial & Eng. Chem. (Anal. Ed.) 9:71 (1937).
- (36) Rosenheim, Biochem. Jour. 21:1329 (1927).
- (37) Schertz, Jour. Agricultural Research 26:383 (1923).
- (38) Sherman & Smith, "The Vitamins" 256 (1931).
- (39) Shrewsbury & Kraybill, Jour. Biol. Chem. 101:701 (1933).
- (40) Snell, Colorimetric Methods of Analysis 2:617 (1937).
- (41) Solyanikova-Nikolskaya, 2. Vitaminforsch 6:117 (1937).
- (42) Watson, Bishop & Drummond, Biochem. Jour. 28:1076 (1934).
- (43) Watson, Drummond, Heilbron & Morton, Empire Jour. Exp. Agri. 1:32 (1933).
- (44) Wilbur, Hilton & Hauge, Jour. Dairy Science 18:661 (1935).
- (45) Willstatter & Stoll, Untersuchungen über Chlorophyll Methoden und Ergebnisse (1913).
- (46) Wokes & Willimot, Analyst 52:13 (1927).

Temperature Correction in Dr. Rudig's Paper on page 165.

In the last issue of this Journal, the temperatures given at the bottom of the first column of page 165, reading 140° - 145° C. for organisms growing on 240 plates, should correctly read 40° - 45° C. (104° - 113° F.).—Editor.

New Books and Other Publications

Index of the Twenty-five Annual Reports of the International Association of Dairy and Milk Inspectors, 1912-1936. Compiled by Horatio N. Parker, Jacksonville, Fla. Published by the International Association of Milk Sanitarians, Albany, N. Y., 1940, 62 pages. \$1.00.

Ever since the International Association of Dairy and Milk Inspectors was organized in 1912, they have published the papers that were presented at their annual meetings in volumes of "reports". These papers reveal all the steps that have been taken in this quarter of a century of progress in developing the present high standards of milk sanitation—the highest of any other food-stuff and a model for all. A wealth of data of scientific and historic value, heretofore largely buried in these volumes, is brought to light.

Horatio N. Parker volunteered to index this great mass of material, covering about 8,000 pages of text. He had cross referenced the many items and grouped them into title-author, author-title, and discussion indices. The student of the development of dairy sanitation will welcome this splendid addition to a literature that is unique in its practical and experimental value.

J. H. S.

Dairy Cattle and Milk Production, by C. H. Eckles, 3rd edition revised by E. L. Anthony and L. S. Palmer. The Macmillan Co., New York, 1939. 520 pages. \$3.60.

Although this book was written originally by the late Dr. Eckles for the student of agriculture, the text is an excellent presentation for practical dairy-men.

The introductory chapter presents the economic reasons for the growth of the dairy industry, together with many important facts in its historical development.

The subject of breeds is covered in eight chapters. Then follow four chapters on selection, two on breeding, four

on calf-raising, four on management, one on common ailments of cattle, three on feeding, four on feeds, one on stables, one on manure-handling, and two appendices of tables and breed score cards.

The limitation of discussion of common ailments of dairy cattle to one chapter, with no text on milk handling, may restrict the use of this book to those who are more concerned in the care and handling of the animals themselves than to the production of the milk.

The book is well-printed (as usual with Macmillan publications), conveniently arranged, and moderately illustrated with 101 figures. The index might be improved, as for example, the word "colostrum" never appears, although given proper emphasis in the text. The newly discovered cattle disease, trichomoniosis, is given brief discussion.

In its field, the subjects are presented in an unusually clear as well as readable style. The treatment is not intensive but is very practical so that the dairy husbandman and milk inspector will be well informed on matters in this part of the milk business.

J. H. S.

The Cow Tester's Manual, by J. F. Kendrick. Miscellaneous Publication No. 359, U. S. Department of Agriculture, Washington, D. C., 1940. 17 figures as illustrations and charts. 36 pages. For sale by the Superintendent of Documents, Washington, D. C. Price 15 cents. This publication supersedes Miscellaneous Circular 26, The Cow Tester's Handbook.

The present publication is a practical treatise on directions for conducting and recording the procedure followed by the testers of dairy herd-improvement associations. Illustrations show equipment and technique. Reproductions of typical records, and examples of several actual calculations make the manual very practical and useful. A good case is made for the use of "The Centering Day" as the approximate center of the testing period so as to avoid the deflation or inflation that results when production is calculated on a calendar-month basis.

JOURNAL OF MILK TECHNOLOGY

Official Publication of the

International Association of Milk Sanitarians

(Association Organized 1911)

Editors

W. B. PALMER, *Managing Editor*
Orange, N. J.

J. H. SHRADER, *Editor*
Wollaston, Mass.

Associate Editors

C. A. ABELE
Chicago, Ill.

M. A. HEINZMAN
Ventura, Cal.

ERNEST KELLY
Washington, D. C.

M. E. PARKER
Chicago, Ill.

SARAH V. DUGAN
Louisville, Ky.

J. A. KEENAN
Boston, Mass.

P. F. KRUEGER
Chicago, Ill.

G. W. PUTNAM
Chicago, Ill.

J. G. HARDENBERGH
Plainsboro, N. J.

C. K. JOHNS
Ottawa, Canada

H. N. PARKER
Jacksonville, Fla.

F. M. SCALES
New York, N. Y.

H. R. THORNTON
Edmonton, Alberta, Can.

THE JOURNAL OF MILK TECHNOLOGY is issued bimonthly beginning with the January number. Each volume comprises six numbers. It is published by the International Association of Milk Sanitarians, and is printed by The Chronicle Press, Inc., Orange, N. J., U. S. A.

Subscriptions: The subscription rate is \$2.00 per volume. Single copy, 50 cents.

Advertising: All correspondence concerning advertising, reprints, subscriptions, and all other business matters should be addressed to the Managing Editor, W. B. Palmer, 29 North Day Street, Orange, N. J.

Manuscripts: All correspondence regarding manuscripts, editorials, news items, announcements, and

other reading material should be addressed to the Editor, J. H. Shrader, 59 Winthrop Ave., Wollaston, Mass.

Membership and Dues: Active membership in the Association is \$3.00 per year, and Associate membership is \$2.00 per year, including respectively all issues of the JOURNAL OF MILK TECHNOLOGY. All correspondence concerning membership in the INTERNATIONAL ASSOCIATION OF MILK SANITARIANS, including applications for membership, remittances for dues, failure to receive copies of the JOURNAL OF MILK TECHNOLOGY, and other such matters should be addressed to the Secretary of the Association, C. Sidney Leete, State Department of Health, Albany, N. Y.

INTERNATIONAL ASSOCIATION OF MILK SANITARIANS

President, P. B. Brooks.....Albany, N. Y.
First Vice-President, L. C. FrankWashington, D. C.
Second Vice-President, F. W. FabianEast Lansing, Mich.
Third Vice-President, C. A. AbeleChicago, Ill.
Secretary-Treasurer, C. S. Leete,.....State Office Building, Albany, N. Y.

ASSOCIATIONS WHICH HAVE DESIGNATED THE

JOURNAL OF MILK TECHNOLOGY

AS THEIR OFFICIAL ORGAN

CALIFORNIA ASSOCIATION OF DAIRY AND MILK INSPECTORS

President, J. J. Garland.....Redwood City, Cal.
Vice-President, L. E. Holt.....Pasadena, Cal.
Secretary-Treasurer, L. E. Nesson, 2707 L. Street, Eureka, Cal.

CENTRAL STATES MILK SANITARIANS

President, William Dotterer,.....Barrington, Ill.
1st Vice-President, F. M. Keller, Oak Park, Ill.
2nd Vice-President, J. C. Krueger, Chicago, Ill.
3rd Vice-President, Oliver C. Hutter, Lake Geneva, Wis.
Secretary-Treasurer, Donald V. Fitzgerald, Elgin, Ill., P. O. Box 295.

CHICAGO DAIRY TECHNOLOGY SOCIETY

President, O. H. Aulse.....Chicago, Ill.
Vice-President, G. B. Ulvin.....Chicago, Ill.
Treasurer, D. M. Hemb.....Chicago, Ill.
Sergeant-at-Arms, G. B. Ashton, Chicago, Ill.
Secretary, P. H. Tracy, Dairy Department, University of Illinois, Urbana, Ill.

CONNECTICUT ASSOCIATION OF DAIRY AND MILK INSPECTORS

President, I. R. Vail.....Bristol
1st Vice-President, B. E. Bowen.....Waterbury
2nd Vice-President, Harold Clark.....Colchester
Secretary-Treasurer, H. Clifford Goslee, State Office Building, Hartford, Conn.

INDIANAPOLIS DAIRY TECHNOLOGY CLUB

President, W. Roberts.....Indianapolis, Ind.
Secretary, E. H. Parfit, Purdue University, Lafayette, Ind.

MASSACHUSETTS MILK INSPECTORS' ASSOCIATION

President, John B. Enright.....Fitchburg, Mass.
Vice-President, John H. Buckley.....Lynn, Mass.
Secretary-Treasurer, Robert E. Bemis, Cambridge, Mass.

METROPOLITAN DAIRY TECHNOLOGY SOCIETY

President, C. H. Kimberly.....New York, N. Y.
Secretary-Treasurer, O. F. Garrett, Rutgers University, New Brunswick, N. J.

MICHIGAN ASSOCIATION OF DAIRY AND MILK INSPECTORS

President, Grey J. TurneyLansing, Mich.
1st Vice-President, John Vogt, Mt. Pleasant, Mich.
2nd Vice-President, F. E. Holiday, Detroit, Mich.
Secretary-Treasurer, Harold J. Barnum, Ann Arbor Health Department, Ann Arbor, Mich.

MISSOURI ASSOCIATION OF MILK SANITARIANS

President, J. M. Burns.....Nevada, Mo.
Vice-President, C. P. Brandle.....Clayton, Mo.
Secretary-Treasurer, G. M. Young, State Board of Health, Jefferson City, Mo.

NEW YORK STATE ASSOCIATION OF DAIRY AND MILK INSPECTORS

President, E. E. Brosnan.....Binghamton, N. Y.
Vice-President, J. F. Jansen.....Oneonta, N. Y.
Secretary-Treasurer, W. D. Tiedeman, State Office Building, Albany, N. Y.

PACIFIC NORTHWEST ASSOCIATION OF DAIRY AND MILK INSPECTORS

President, F. L. Herrington.....Vancouver, Wash.
1st Vice-President, R. M. Wiley, Seattle, Wash.
2nd Vice-President, J. C. Austell, Spokane, Wash.
Secretary-Treasurer, F. W. Kehrl, Bureau of Health, Portland, Ore.

PENNSYLVANIA ASSOCIATION OF DAIRY SANITARIANS

President, M. E. Dauer.....St. Marys, Pa.
1st Vice-President, R. G. Vogel.....Bradford, Pa.
2nd Vice-President, Maurice Farkes, McKeesport, Pa.
Secretary-Treasurer, G. C. Morris, P. O. Box 141, Troy, Pa.

TEXAS PUBLIC HEALTH ASSOCIATION—MILK SECTION

President, C. B. Kennington, Corpus Christi, Texas.
1st Vice-President, M. B. Starnes, Dallas, Texas
2nd Vice-President, Taylor Hicks, San Antonio, Texas.
Secretary-Treasurer, E. A. Grist, Austin, Texas

WEST VIRGINIA ASSOCIATION OF MILK SANITARIANS

President, W. P. Gainor.....Fairmont, W. Va.
1st Vice-President, J. E. Weber, Charleston, W. Va.
2nd Vice-President, S. W. Frame, Martinsburg, W. Va.
Secretary-Treasurer, (Acting) J. B. Baker, Charleston, W. Va.

Association News

Missouri Association of Milk Sanitarians

The eighth annual convention of the Missouri Association of Milk Sanitarians was held at the Dairy Department of the University of Missouri on May 1 - 3. It was the most successful and the best attended meeting that has yet been held. Former President V. M. Ehlers of the International Association of Milk Sanitarians addressed the meeting in the interest of increased co-operation between the State and the International associations. The membership expressed themselves as highly pleased with the JOURNAL OF MILK TECHNOLOGY, and sixteen members made application for associate membership in the International, and one member for active membership. The new officers are listed on the Association page, and several of the interesting papers are being printed in this and succeeding issues.

GLENN M. YOUNG,
Secretary-Treasurer.

Metropolitan Dairy Technology Society

The next meeting of the Metropolitan Dairy Technology Society is scheduled for Tuesday, September 17.

O. F. GARRETT,
Secretary-Treasurer.

Chicago Dairy Technology Society

The June 11th meeting concluded the series of meetings of the year, and was designated as Ladies' Night. A dinner-dance was held at the Bavarian Hofbrau in Chicago. To maintain interest in the Society over the summer months, a golf tournament is being arranged for July 27.

J. T. THORNE.

Central States Milk Sanitarians

Seventy-five members of the Central States Milk Sanitarians met in the Assembly Room of the Board of Health Building on June 10th to hear Dr. E. H. Parfitt give an interesting and practical talk on heat resistant bacteria.

D. V. FITZGERALD,
Secretary-Treasurer.

International Association of Milk Sanitarians

Announcement of the committee organizations of the International Association of Milk Sanitarians has been sent to the membership. The names of the respective chairmen are as follows:

Committee Chairmen

Communicable Diseases Affecting Man—
Their Relation to Public Milk Supplies. Horatio N. Parker.

Sanitary Procedure. W. D. Tiedeman.

Dairy Farm Methods. F. D. Holford.

Sanitary Control of Ice Cream. F. W. Fabian.

Applied Laboratory Methods. T. H. Butterworth.

Education and Training. H. E. Miller.

Resolutions. Ernest Kelly.

Affiliation. V. M. Ehlers.

Chocolate Milk. Sarah Vance Dugan.

Local Arrangements (Joint Committee

New York State Association of Dairy and Milk Inspectors). C. L. Kern.

The Association's Committee on Sanitary Procedure met at the Hotel Morrison in Chicago on Thursday, June 20th, following the meeting of the Institute of Food Technologists. Part of the day was devoted to consideration of standard specifications for a sanitary type of motor for use in milk and food plants. A committee on motors of the National Electric Manufacturer's Association and a committee on sanitary motors of the Institute of Food Technologists collaborated in this work. Items receiving committee consideration were flow diversion valves, a new interchangeable fitting for installing recorders in vats and pipe lines, the size of holes in strainers, the position of the handle in 3 way valves and finish for stainless steel. It is expected that action taken by the committee will be reported at the coming Annual Meeting in New York City.

C. SIDNEY LEEBE,
Secretary-Treasurer.

Annual Meeting in October

Attention is called to the annual meeting, to be held in New York City on October 17th, 18th, and 19th. The headquarters will be located at the Hotel Pennsylvania. This is a joint meeting of the New York State Association of Dairy and Milk Inspectors with the International Association of Milk Sanitarians. We are expecting an attendance of six hundred or more.

The opportunity afforded by this joint convention to meet many of the leading milk sanitarians will make this an outstanding occasion. Many important papers will be presented. One of the outstanding features of the conventions of these organizations is the interesting and profitable discussions which follow the presentations of the papers.

The Committee on Local Arrangements is planning to offer visits to several of our larger pasteurizing and bottling plants in the New York City Metropolitan area. They are also planning to have a banquet and an entertainment of such nature at the banquet that it will be pleasing to everyone attending.

Public Health Engineering Course at M. I. T. *Municipal Sanitation*, Vol. 10, No. 8, August, 1939, p. 418. *Pub. Health Eng. Abs.* xx, PHA., 4.

New course will consider such subjects as water supplies, water purification, sewerage, industrial waste disposal, stream pollution and collection and disposal of refuse, and sanitation of swimming pools, rural communities, camps, food supplies, stores, and restaurants. Students will also receive instruction in the relationship of insects and rodents to disease, the atmosphere in relation to health and comfort, housing and health, school sanitation, industrial hygiene, organization and activities of health departments, the collection, analysis, and interpretation of vital statistics, epidemiology, and conduct of health surveys, and the use of standard health appraisal forms.

Course leads to degree of master in science one year and is open to men of outstanding scholastic attainment and professional experience who have completed undergraduate work in a recognized engineering school. Candidates must have had one year's experience in a city, county, or State health department or the U. S. Public Health Service, or equal.

All the proposed plans have not been worked out in detail. Complete arrangements will be made to give our visiting members and their guests an opportunity to see as many of the sights of New York City as possible. Of course, they will have the opportunity to visit the New York World's Fair which will still be operating.

A Ladies' Committee has been appointed by the secretaries of the two Associations (see last issue page 176). Miss Vera McCream is Chairman. They plan a tea and social on the first afternoon of the meeting with trips through the hotel and the various department stores in the nearby vicinity of the Hotel Pennsylvania. A sight-seeing trip or boat ride around Manhattan Island (on which New York City stands) is planned for the following day.

This will be an outstanding convention in the history of this work, and no one who can possibly attend can afford to miss it. Plan to come for the whole meeting and bring your wife.

Submission of a thesis dealing with some original investigation is required.
PAUL D. HANEY.

Wildman Mold Mycelia Method

This method of determining mold in butter may be briefly described as follows:

A gum solution of 0.75 percent strength is made by dissolving carob bean gum or gum tragacanth in water, and then adding 2 percent formaldehyde as a preservative.

A 1 gram sample of butter is weighed out by means of a 1/4 teaspoon measure. Then 7 cc. of the hot gum solution is poured over the spoon so that all of the sample is washed into a small beaker. The mixture is well stirred until the fat globules are 0.1—0.2 m.m. in diameter. A portion is then mounted on a mold-counting slide (Howard cell) for estimating the amount of mold under a microscope. No field is reported positive unless the combined length of the two longest filaments exceeds 1/6 of the diameter of the field. The details for making the count are described in *Methods of Analysis*, 1935, xxxv, 2728.

"Doctor Jones" Says—

by

Paul B. Brooks, M. D.

"A man that was well acquainted with Dr. Freud—the other day I was reading something he wrote about him and he says: 'Each person who knew him well . . . received from him just what he was equipped to receive.' I figure that's a comment that'll stand considerable cogitating.

"It makes me think of this radio business. Back in the early days I had a crystal set—and it was a pretty good one for that time. I'd spend half an hour trying one spot after another on the crystal and if I managed to hear anything at all it was pretty near a miracle. The radio waves—the air wasn't so full of 'em as it is now but they were there. The trouble was most of 'em didn't register on the equipment I had.

"Not long ago I was talking to a doctor friend of mine and I mentioned somebody that'd been to see a psychoanalyst. 'Oh,' the doctor says, 'one of those goofs!' Well, sir—I didn't spend any time trying to change his opinion. So far as that sort of thing was concerned he was still working with a crystal set.

"It reminds me of Pat Purdy's watch. Pat was an old fellow that used to be around here and he had a big hunting-case watch somebody gave him when he came over from the old country. About

once a week he'd go up and compare it with the town clock. He never set it. He just wanted to see how far off the town clock was. That's the way it is with a lot of us: we're so well satisfied with some of our own ideas it never occurs to us that anything that don't agree with 'em could be right.

"And, you know, that remark about Dr. Freud—somehow it makes me think of the story of 'the loaves and the fishes.' That hungry 'multitude' there and only five loaves of bread—most anybody would've said: 'Five little loaves! They're no good. They wouldn't be a drop in the bucket.' But one man, because he was equipped to give as well as receive—he saw the value in 'em that the others didn't recognize and he made enough out of 'em to feed the whole outfit.

"The same way, whether it's coming in contact with a great scientist or going to medical college or what not: the fellow that's there just for what he can get out of it, he ain't equipped to receive much. It's the one that's there because he wants to help others—he's liable to be the best receptacle. And it's funny how it sort of works both ways: the more of what he receives he passes on to others, the more he has left for himself."

PAUL B. BROOKS, M.D.

Difficulties in the Diagnosis of Chronic Brucellosis. Alice C. Evans. *Amer. J. Trop. Med.* 1939, v. 19, 319-25. *Bulletin of Hygiene*, Vol. 14, No. 11, November 1939, pp. 813-14. Abstracted by R. Lovell. *Pub. Health Eng. Abs.* xx, Mi: 29.

Because of the difficulties of diagnosing chronic Brucella infection, no cases were recognized in America till recently. A survey has been conducted in Charlotte, N. Carolina, with a population of 100,000. Brucella infection was present in herds supplying the milk of which 81 percent was sold raw. The general results show that in chronic Brucella infection the clinical picture is vague; the chief symptoms are weakness, nervousness, exhaustion on slight effort, insomnia, depression, and irritability. Both the history and the symptoms are of im-

portance in reaching diagnosis. Amongst the diagnostic aids which may be used are the skin test, the opsono-cytophagic reaction and the agglutination reaction. The opsono-cytophagic reaction is considered to be the least reliable whilst a positive agglutination reaction is the most significant evidence of Brucella infection. A negative agglutination reaction however must not be regarded as evidence against Brucella infection, for almost half of the cases regarded as chronic Brucellosis in the survey gave a negative reaction. No one of the specific tests or a combination of them can be relied upon to diagnose Brucella infection though positive reactions may confirm the diagnosis. Brucella infection should, however, be suspected in cases of mild ill health of etiology.