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Contents

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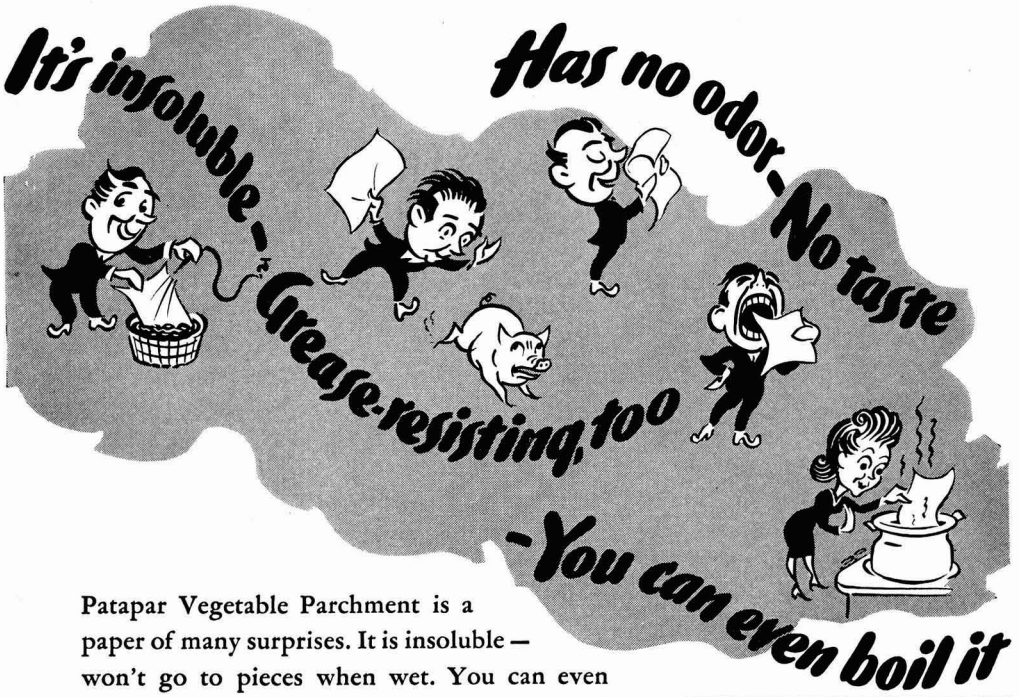

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PURIFICATION OF RENNIN FROM COMMERCIAL RENNIN EXTRACT: PROPERTIES OF PURIFIED PRODUCT*

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The purity of the enzyme, especially with respect to its contamination with pepsin, is of essential importance in understanding the biochemistry of the coagulation of milk by true rennin. Many investigations have been conducted on this general problem from which erroneous conclusions may have been drawn because of the mixed nature of the enzymes in the rennin preparations used. Several attempts to purify this enzyme have been reported but the properties of the purified products do not show much agreement except a marked increase in milk clotting activity in comparison with the crude starting material.

Fenger (1), starting with dried, defatted, powdered calves' stomach mucosa, obtained a product containing 14.00 per cent N, which precipitated from dilute NaCl solution at pH about 3.5–4.0 and which readily dialyzed through parchment. He regarded the purified rennin as a decomposition product of an acid albumin. Its milk clotting activity at 40° C. in 10 minutes, using sweet, certified milk (1:2,310,000) was 770 times that of the original dried mucosa and its peptic activity (1:600 by the U.S.P. method) only 1.7 times that of the starting material. Lüers and Bader (2), starting with sodium phosphate solution extracts of the lead proteinate obtained from sodium acetate extracts of calves' stomach mucosa, purified the rennin by a series of adsorptions on alumina and kaolin and elutions by phosphate buffer. Milk clotting activity was calculated from viscosity determination made at 35° C. for 40 minute activity time (actual time two minutes) and peptic activity was measured by a turbidometric procedure which is stated to estimate about 0.2 mg. pepsin. The most active rennin preparation contained only 0.687 per cent N and the milk clotting activity of the dry substance was calculated to be 1:16,440,000 parts of a boiled, reconstituted

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milk substrate. This was only 39 times that of the dry lead proteinate starting material. Its peptic activity was still 21 times that of the same starting proteinate complex.

More recently Tauber and Kleiner (3) reported the purification of rennin from fresh calves' stomach mucosa. Their purest product, obtained by precipitation with 50 per cent ethanol at pH 5.4, clotted 4,550,000 parts of a reconstituted skim milk containing added $CaCl_2$ in 10 minutes at 40° C. This represented a 2000 fold concentration compared with the original extract. The product contained 14.4 per cent N, 1.19 per cent S, but no P. It is stated that 25 mg. of the rennin did not produce any increase in formal titration of an aqueous suspension of 250 mg. of coagulated egg albumin in three hours at 40° C. and only a negligible amount of N not precipitable by trichloracetic acid. No data are actually given on the peptic activity either of the starting material or the purified rennin. Tauber and Kleiner (4) later found that their purified rennin could be rendered inactive by crystalline pepsin. However, Holter (5) has pointed out that the sensitivity of the methods used by these workers for measuring peptic activity is not sufficiently great to justify their conclusion that their purest rennin was devoid of pepsin.

Recently Rao, Rao *et al.* (6) reported that nitrogen is not an integral part of the rennin molecule, seemingly in support of the earlier work of Lüers and Bader. Like in the work of the latter, adsorption on alumina and kaolin were used; also extraction, centrifugation and filtration procedures. However, Schöberl and Rambacher (7) found that rennin eluted from alumina, gives the biuret and diazo reactions.

The purified rennin obtained both by Fenger and by Tauber and Kleiner had the properties of protein. This is in keeping with the widely accepted view that the protein attacking enzymes of the digestive tract are themselves either proteins or perform their function as a part of protein structures. The products obtained by the workers mentioned may also be regarded as somewhat comparable in milk coagulating activity under like conditions. What seems contradictory is the fact that the Tauber-Kleiner rennin, although much less contaminated with pepsin, nevertheless cannot be regarded as showing as great an increase in coagulating activity as obtained by Fenger, on the basis of the probable dry substance contained in the original extract (which unfortunately is not given); it is the extract, not its dry substance, with which Tauber and Kleiner compare their pure rennin.

The recent successful attempts to bring the digestive enzymes into a highly purified state as crystalline proteins prompted us to examine the possibility of applying similar procedures to rennet extract. Although we were not successful in doing this in the present study, nevertheless a high degree of purity was attained. Since rennin and pepsin are the only known milk clotting enzymes in these stomach tissue extracts it seemed justified to

employ the relative milk clotting and peptic activity as a measure of purity. However, we used a much more sensitive, as well as more accurate measure of peptic activity than was used in previous studies of this problem. The method devised is a technical modification of that reported by Anson (8) and is described below. Moreover, we were fortunate in having for starting material a commercial rennet extract,[1] the dry, salt-free material of which apparently already possessed about 3.5 times the milk coagulating activity of the pure rennin of Tauber and Kleiner, as judged by comparison of our data with theirs.

RENNIN PURIFICATION PROCEDURE

Considerable preliminary work was first carried out in which determinations were made of the activity of the precipitates and the supernatant liquids, at varying pH, salt concentrations, kinds of salt and after electrodialysis. The most active rennin was prepared by the following procedure:

Two liters of the rennet extract, a reddish brown liquid, were adjusted to pH 4.5 with 7.0 ml. of concentrated HCl solution. A precipitate formed at once. The mixture was centrifuged for 20 minutes at 2000 R.P.M. The supernatant liquid was decanted and the precipitate suspended in sufficient 16.7 per cent NaCl solution (20 gm. NaCl per 100 ml. H_2O) to make one liter volume. The pH was adjusted to 6.0 whereupon all the precipitate peptized to give a clear sol. The pH was again adjusted to 4.5 and the suspension centrifuged for 20 minutes. The precipitate was peptized by 16.7 per cent NaCl solution at pH 6.0 and made up to 500 ml. volume with the solvent. Sediment forming at this point was centrifuged out since the preliminary experiments had shown it to be relatively inactive. The pH was then adjusted to 4.5 and the suspension centrifuged for 20 minutes. The precipitate was peptized by the 16.7 per cent NaCl solution, made up to 250 ml. with solvent and the pH adjusted to 6.1. Again there was considerable sediment which was centrifuged out. Activity determinations were made on the final liquid and on the original rennet extract. The solution was stored in a cold room at 2° C. Portions were dialyzed "free" of NaCl as needed.

Determination of rennin activity: Rennin activity was determined by pipetting 10 ml. portions of fresh, raw skim milk into suitable test tubes in a constant temperature water bath held at 40°+ C., allowing three minutes to come to 40° C., adding 0.25 ml. $CaCl_2$ solution (containing 94.5 mgm. $CaCl_2$) to each tube, letting stand three minutes more and then adding 0.5 ml. of the rennin sol of different dilutions until a coagulation time of approximately 10 minutes was obtained. The $CaCl_2$ treated milk had pH 5.75–5.80 by glass electrode pH meter. Actual coagulation times were determined by stop watch. The activity of each sol was calculated on the basis of the dry organic matter required to produce coagulation in 10 minutes. The organic matter in the various milk coagulating sols was de-

[1] Kindly furnished by the Chr. Hansen's Laboratory, Inc., Milwaukee, Wisconsin.

termined on suitable volumes (*e.g.*, 30 ml.) after dialysis in viscose sausage casings against running distilled water, any residual NaCl being deducted after a volumetric Cl determination using a standard $AgNO_3$ solution and K_2CrO_4 indicator.

Determination of peptic activity: Peptic determinations on the purified and original dialyzed samples were carried out as follows: To a 50 ml. centrifuge tube containing five ml. of two per cent egg albumin solution, which had been boiled for ten minutes and cooled, were added 15 ml. of 0.05 N HCl solution, and two ml. of the enzyme preparation. After incubation for three hours at 40° C. the mixture was centrifuged and tyrosine determined by the Folin-Marenzi (9) method on 20 ml. of the supernatant liquid. Blanks were run on the coagulated, acidified, incubated egg albumin sol containing boiled pepsin sol. Color comparisons were made with a Cenco Photelometer, using a set of standards of crystalline tyrosine for comparison. Known concentrations of crystalline pepsin prepared from Armour's 1–10,000 pepsin[2] produced a tyrosine liberation curve from the egg albumin under standard conditions, from which the amount of pepsin per unit weight of dry rennin preparation could be estimated. The method has the advantage of detecting minute amounts of pepsin in an unknown sample, it being possible to determine accurately 10 μg. pepsin, the limit of sensitivity being about 5 μg. The rennin preparations had to be dialyzed because NaCl interferes with the color formation. Anson's (8) procedure for pepsin differs in that he used a phenol reagent for determining tyrosine, and employed a colorimeter for the color comparison.

PROPERTIES OF THE PURIFIED RENNIN

The results of the rennin and peptic activity determinations on the original extract and purified rennin are shown in table 1. It is seen that the rennin activity was increased 4.55 times and the peptic activity decreased to

TABLE 1

Enzyme activity of original rennet extract and purified rennin

Enzyme Preparation	Rennin activity determinations				Peptic activity determinations			
	Wt. dry substance in 0.5 ml.	Total dilution	Clotting time	Calc. Activity*	Wt. dry substance in 2.0 ml.	Wt. tyrosine liberated	Estimated wt. pepsin	Estimated conc. pepsin**
	mg.	$\times 10^6$	*min.*	$\times 10^6$	*mg.*	*mg.*	*mg.*	*%*
Original rennet	6.98	14.3	9: 00	15.9	1.984	1.654	0.073	3.68
Purified rennin	1.47	68.0	9: 25	72.3	12.240	1.005	0.028	0.23

* Million parts of milk clotted per part of rennin in 10 minutes.

** Concentration of pepsin on dry weight basis.

[2] First crystallizations using the method of Northrop (10).

6.25 per cent of that of the original extract. The purified rennin was 99.77 per cent "pure" from the standpoint of pepsin contamination.

Further examination of the properties of the purified rennin showed that it behaved as a globulin, being (a) soluble in dilute salt solutions, (b) precipitating at pH 4.5, (c) insoluble in saturated salt solutions, and (d) precipitating out upon electrodialysis. This is in marked contrast to previous reports in the literature (1, 2, 3). The reddish brown color was not found to be associated with the rennin activity as believed by Richardson and Palmer (10). Most of this colored material was found to be left in the first supernatant liquor obtained at pH 4.5, where the major portion of the original peptic activity was separated. Another impurity found to be present was sodium benzoate, which crystallized out as benzoic acid at pH 2.5.

Cataphoresis experiments were carried out on the purified rennin using the apparatus described by Briggs (12). A small quantity of the dialyzed sol was diluted, 1 to 10 with distilled water, the pH adjusted to the desired value by adding 0.1 N NaOH solution and the undispersed particles followed cataphoretically with the microscope. Between pH 6.0 and 7.5, the zone studied, there was a progressive increase in zeta potential. At pH 6.5 rennin and calcium caseinate exhibited approximately the same magnitude of negative zeta potential, namely, -14.25 mv. and -13 to -16 mv., respectively. These results seem to make untenable the conclusion (10) that the isoelectric point of rennin is pH 6.9 to 7.0 and that at pH 6.5 a positively charged rennin micelle exists. A more complete report of electrophoretic studies of rennin action on caseinate sols will be given in another paper.

Very little loss in activity was noted after three months' storage of the purified rennin in 16.7 per cent NaCl solution at 2° C.

DISCUSSION

Previous investigators (3) have objected to the use of commercial rennet extracts from which to prepare purified rennin. Our experiments indicate that such objections are not valid if the nature of the major impurities is recognized and suitable methods employed for their removal. In our experience dialysis and centrifuging will remove the major impurities which are not removed with the foreign proteins by suitable salting out procedures at controlled pH. A rennet extract such as we employed already represents a highly concentrated sol of milk coagulating enzymes in comparison with the crude extracts of calves' stomach mucosa used by other investigators, which are certain to have been much more highly contaminated with mucin and other foreign substances.

The relative milk coagulating activity of rennins purified by various investigators has no exact significance. Not only has the substrate been different but in none of the investigations were the precise optimum condi-

tions determined or employed either with respect to the type of milk substrate or the relations between pH and Ca ions, all of which are known to affect the rapidity of coagulation and also its completeness. The problem is no doubt further complicated by the presence of pepsin for which the optimum conditions no doubt differ. These criticisms apply to our own study in which the dry organic matter of the starting material apparently already had an activity about 3.5 times that of the purified rennin of Tauber and Kleiner (3), if one compares activity by the method we employed with activity by their method. Each study, therefore, is more or less a unit in itself with respect to comparative rennin activities and will show only the degree of concentration of rennin obtained in that study.

The method described in this paper of purifying rennin, at least from the standpoint of pepsin contamination, seems to have the following advantages over methods previously described:

(a) Speed of purification is not an essential factor.

(b) No inorganic solvents are employed which markedly denature the rennin.

(c) The pH is controlled within the zone of greatest stability (4.5 to 6.5) so that pepsin does not inactivate the rennin as is the case in highly acid and highly alkaline solutions.

(d) The use of electrolytes is an advantage, rather than detrimental (as when organic precipitants are employed), for they improve dispersability, and act as bactericidal agents.

(e) The yield of coagulating activity is high, both actual and in relation to original dry organic matter. We recovered 30 per cent of the original activity in seven per cent of the original dry organic matter. It is not possible to say how much of the activity lost was due to pepsin removed.

(f) The method does not necessitate drying and thus avoids loss in activity which may accompany the denaturation involved in this procedure.

(g) The purified rennin retains its activity for many weeks at low temperature in approximately one-half saturated NaCl solution.

SUMMARY

A stable purified rennin sol was prepared from a commercial rennet extract by means of an isoelectric precipitation procedure. One part of dry rennin coagulated 72,300,000 parts of fresh raw skim milk at pH 5.75–5.80 (obtained by added $CaCl_2$) in 10 minutes at 40° C., which was 4.55 times that of the dry organic matter of the original extract. The peptic activity, measured by a method which estimates quantitatively 10 μg. crystalline pepsin, was only 6.25 per cent of the original dry organic mixture. The rennin was therefore 99.77 per cent "pure" from the standpoint of peptic activity. The purified rennin exhibited the properties of a globulin. Its isoelectric point in 16.7 per cent NaCl solution was pH 4.5. The rennin sol,

freed from NaCl by dialysis, showed a progressive increase in negative zeta potential from pH 6.0 to 7.5, and at pH 6.5 showed about the same negative zeta potential as calcium caseinate particles. Rennin and calcium caseinate, therefore, do not exhibit opposite electropotentials at the pH of milk.

REFERENCES

(1) FENGER, FREDERIC. A Comparison Between the Chemical and Physiological Characteristics of Pepsin and Rennin. Jour. Amer. Chem. Soc., **45**: 249–255. 1923.

(2) LÜERS, H., AND BADER, J. Über die Reinigung des Chymosins. Biochem. Ztschr. **190**: 122–142. 1927.

(3) TAUBER, HENRY, AND KLEINER, ISRAEL S. Studies on Rennin. I. The Purification of Rennin and Its Separation from Pepsin. Jour. Biol. Chem., **96**: 745–753. 1932.

(4) TAUBER, HENRY, AND KLEINER, ISRAEL S. The Chemical Nature of Rennin. Jour. Biol. Chem., **104**: 259–266. 1934.

(5) HOLTER, HEINZ. Über die Labwirkung. Biochem. Ztschr., **255**: 160–188. 1932.

(6) RAO, C. N. BHIMA, RAO, M. V. LAKSHMINARAYAN, RAMASWAMY, M. S., AND SUBRAHMANYAN, V. Purification and Chemical Nature of Rennin. Current Sci., **10**: 215–219, 1941; Chem. Abs., **35**: 6274–6275. 1941.

(7) SCHÖBERL, ALFONS, AND RAMBACHER, PAUL. Über die Reinigung des Labfermentes durch chromatographische adsorption. Biochem. Ztschr., **305**: 223–226. 1940.

(8) ANSON, M. L. The Estimation of Pepsin, Trypsin, Papain, and Cathepsin with Hemoglobin. Jour. Gen. Physiol., **22**: 79–89. 1938.

(9) FOLIN, OTTO, AND MARENZI, A. D. Tyrosine and Tryptophane Determinations in One-tenth Gram of Protein. Jour. Biol. Chem., **83**: 89–102. 1929.

(10) NORTHROP, JOHN H. Crystalline Enzymes. p. 129. Columbia Univ. Press, New York. 1939.

(11) RICHARDSON, G. A., AND PALMER, L. S. Rennin Action in Relation to Electrokinetic Phenomena. Jour. Phys. Chem., **33**: 557–576. 1929.

(12) BRIGGS, DAVID R. A Pyrex All-glass Microelectrophoresis Cell. Indus. and Engin. Chem., Analyt. Ed., **12**: 703–705. 1940.

THE PHOSPHATASE TEST—EXTENT OF USE IN NORTH AMERICA[1, 2]

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In order to determine how universally the phosphatase test is being used in North America, 454 questionnaires were mailed out to individuals in official city, state, provincial, county, private, and milk plant laboratories. (List furnished through the courtesy of the American Public Health Association Committee on Standard Methods for the Examination of Dairy Products.)

A total of 281 questionnaires or 62.1 per cent of the number mailed were returned. Fifty-five laboratories reported that they were not using the test. Eighteen of these were no longer active or were not doing milk work. Of the other 37 laboratories not using the phosphatase test, 17 were official city laboratories (16 U. S., 1 Canada), 5 were State Laboratories (one of these reported it did not use the test routinely, but did not state the modification which was occasionally used), 9 were private laboratories, and 6 were plant laboratories.

It is interesting to note the rapidity with which the phosphatase test has been taken up by various laboratories charged with the responsibility of safeguarding the quality of milk supplies.

Laboratories reporting as using the phosphatase test or one or more of its modifications are classified in table 1.

In a similar though less comprehensive survey made in 1940 (1) there

TABLE 1

Laboratories reporting the use of the phosphatase test

Kind of laboratory	Number
State laboratories (including Hawaii and Puerto Rico)	32
County laboratories	14
City laboratories (including private laboratories doing contract city work)	122
Private laboratories	8
Milk plant laboratories	35
Canadian provincial laboratories	7
Canadian city laboratories	7
Canadian milk plant laboratories	1
Total	226

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[1] Presented at the Fifth Session of the 70th Annual Meeting of the American Public Health Association, October 16, 1941.

[2] A detailed tabulation of the data furnished by the various laboratories reporting the use of the phosphatase test is available in mimeographed form and may be had by writing to the author.

were 22 city laboratories which reported that they were using the phosphatase test, from which no report was received in the present survey. One laboratory returned the blank questionnaire with a notation of having filled out such a questionnaire once before, and if the information was desired again, it would be forthcoming.

In view of the fact that we have no record of a laboratory reporting as discontinuing the test once they have used it, we may assume that there are at least 22 additional laboratories in the United States which are using the test.

PASTEURIZATION TEMPERATURE

The official pasteurization temperature and time reported by the various laboratories as in effect in their city or state are tabulated in table 2. Reports were obtained from 133 cities and 41 states, including Hawaii and Puerto Rico.

TABLE 2

Official pasteurization time and temperature reported
Method of pasteurization

Low temperature long hold				High temperature short hold			
Temperature	Time	States	Cities	Temperature	Time	States	Cities
°F.	*min.*	*No.*	*No.*	°F.	*sec.*	*No.*	*No.*
140	30	.....	3	160	15	16	42
140	40	.....	1	160	16	.....	1
140 –145	30	1	7	160	30	.....	1
142	30	8	22	160	15–30	.....	1
142 –144	30	.....	1	160–163	15	.....	1
142 –145	30	7	11	160–165	10–15	.....	1
142 –146	30	.....	1	161	15	.....	1
142.5	30	1	2	161	16	.....	4
142.5–145	30	.....	1	161–162	15–16	.....	1
143	30	18	52	162	12	.....	1
143 –144	30	.....	1	162	15	.....	1
143 –145	30	.....	6	162	17	.....	1
143.5	30	1	14	165	15	.....	2
144	30	.....	3	165	16	.....	1
145	30	1	8	165	30	1	.....

TABLE 3

Official pasteurization temperature and time reported for Canadian provinces and cities

Temperature	Time	Provinces	Cities
°F.			
140–145	20–30 min.	1	.....
143	30 min.	1	2
143–146	25–30 min.	1	.....
145	30 min.	2	5
162	20 sec.	.....	1
163	20 sec.	1	.....

Four states did not report an official pasteurizing temperature and time, and only 17 of the 41 states reported an official temperature and time for high temperature, short hold pasteurization.

From Canada reports for official pasteurization temperature and time were received for 5 provinces and 7 cities. These are reported in table 3.

Although the legal pasteurization temperature may be below 143° F. in 49 cities and 17 states, a number of questionnaires contained the information that the prevailing temperature employed in the plants was 143° F. or higher, and the use of the phosphatase test had been instrumental in getting the plants to use these higher temperatures.

PHOSPHATASE TEST USED

The various phosphatase tests used by the 226 laboratories reporting the use of the test are classified in table 4.

TABLE 4

Classification of the phosphatase tests used

Test used	Number of laboratories
Original Kay and Graham	10
Kay, Graham & Neave modification	5
Gilcreas & Davis modification	36
Scharer field test	82
Scharer laboratory test	33
Scharer field and laboratory test	35
Scharer field and Gilcreas & Davis	13
Scharer laboratory and Gilcreas & Davis	2
Scharer field and laboratory and Gilcrease & Davis	3
Scharer field and Kay, Graham & Neave	1
Scharer laboratory and Kay & Graham	2
Scharer field and Kay, Graham & Neave and Kay & Graham	1
Kay & Graham and Leahy	1
Kay, Graham & Neave and Gilcreas & Davis	1
Leahy	1
Total	226

The majority of the laboratories followed the directions as given in the 7th Edition of Standard Methods for Milk Analysis (2). There were 38 laboratories, however, that used some modifications of these directions.

Most of the modifications were in connection with the Scharer field test, and dealt generally with incubation time. Seven laboratories reported increasing incubation time to 20 minutes. Six used 30-minute incubation. Three used one hour incubation. One used 30–40 minutes, and two used 15 minutes.

The modifications of the various tests reported are summarized in table 5.

The use of a centrifuge in the place of filtration reported by two laboratories using the Gilcreas and Davis test should prove to be of considerable value in cutting the cost of making the tests and also in reducing the time

TABLE 5

Summarization of the modifications of various tests reported

No. laboratories	Incubation		Other modifications
	Time	Temperature	
Modifications of the Scharer field test			
1	30–40 min.	.....	Uses 2 drops B.Q.C.
1	10 min.	56° C.	
1	10 min.	56° C.	Stands 15 min. after adding B.Q.C.
6	30 min.	.....	
6	20 min.	.....	
3	1 hour	.....	
1	15 min.	110° F.	
1	15 min.	100° F.	
2	10 min.	110° F.	0.2 cc. B.Q.C. used. No alcohol extraction
1	20 min.	105° F.	Uses isoamyl alcohol
Modifications of the Scharer laboratory test			
1	.....	.....	Uses a photelometer
1	1.5 hrs.	.....	
1	12.0 hrs.	.....	
1	.....	.....	Uses different color standards
Modifications of the Scharer Field and Laboratory test			
1	.....	.....	Uses 0.1 cc. of (0.04 gm. B.Q.C. in 25 cc. alcohol)
1	.....	.....	Increase incubation time
1	.....	.....	Uses crystalline buffer instead of solution, also acidified alcoholic solution of B.Q.C.
Modifications of the Gilcreas & Davis test			
2	.....	.....	Use centrifuge instead of filtration
1	.....	.....	Uses ½ quantities and increase incubation
1	.....	.....	Uses photelometer
Modifications of the Kay and Graham tests			
2	4.0 hrs.	.....	
Modification of the Key, Graham and Neave test			
1	.....	.....	Report use of Na_2CO_3
Modification of Kay and Graham and Kay, Graham & Neave Test			
1	.....	.....	Uses photelometer

involved when a large number of samples are run. It could also be applied to all of the various laboratory tests, such as the Kay and Graham, Kay, Graham and Neave, and the Scharer laboratory tests.

PRODUCTS REPORTED TESTED BY THE VARIOUS LABORATORIES USING THE PHOSPHATASE TEST

The majority of the laboratories use the test for milk or for milk and

cream only. Quite a few apply the test to ice cream and chocolate milk also. Only a few use it for butter and cheese. See table 6.

TABLE 6

Summary of the use of the various tests and the number of samples tested by each method

Product tested	Laboratories total No. testing	Total samples tested by the various tests as follows:						
		Leahy	Gilcreas & Davis	Kay & Graham	Kay, Graham & Neave	Two methods other than Scharer	Two or more, one or more of Scharer	One or more of Scharer only
Milk	201	2,260	25,635	2,409	4,133	3,474	37,699	155,060
Cream	142	41	6,274	1,692	2,283	64	4,074	81,378
Milk and Cream*	.....	.....	1,485	16,851	.....	.....	7,701	73,886
Ice Cream**	35	.....	22	.....	.....	.....	499	6,007
Chocolate Milk	47	.....	32	.....	.....	.....	121	7,732
Butter	6	.....	.....	.....	.....	.....	.....	1,131
Cheese	5	.....	.....	.....	.....	.....	.....	423
Buttermilk	1	.....	.....	2	.....	.....	.....	.....
Skim milk	1	.....	.....	.....	12	.....	.....	.....
Cremo (half & half)	.....	.....	.....	.....	.....	.....	.....	102
Dairy Products†	2	.....	332	.....	.....	.....	.....	19,296
Total Samples		2,301	33,782	20,954	6,428	3,538	50,094	345,015

* Not reported separately
** Including frozen desserts
† Kind not designated

Number of samples run by the various groups of laboratories and the per cent positive reported are given in table 7. (Reports generally were for 1940.)

In addition, the following number of samples was reported tested, but the per cent positive was not stated.

Milk	36,700	
Cream	900	
Milk and Cream	20,995	(not reported separately)
Ice Cream	875	
Chocolate Milk	250	
Dairy Products	27,239	(kind not reported)
Total	86,959	

Thus, a total of 462,115 samples of dairy products was reported tested by the various laboratories. Most of the reports were for the year 1940.

In conclusion, this survey has shown that the phosphatase test has been adopted with a surprising rapidity by many laboratories in North America. Nearly a half million samples were reported tested during the past year.

The Scharer Field Test was the one most generally used. In answer to question 6, "Have you found the Scharer Field Test reliable?", 123 replied "Yes," 10 "No," 3 said it was "Fair," 1 "Doubtful," and 1 "Limited."

TABLE 7

Summary of samples tested by the various laboratories and the per cent of positive tests found

Product tested	Kind of laboratory												Total	
	State[1]		County		City		Private		Milk plant		Canadian			
	Samples tested[2]	% pos.	Samples tested	% pos.	Samples tested[3]	% pos.	Samples tested	% pos.	Samples tested[4]	% pos.	Samples tested[5]	% pos.	Samples tested	% pos.
Milk	26,719	4.29	5,603	13.67	82,984	2.97	2,060	2.71	74,181	0.62	6,960	6.51	198,507	2.69
Cream	4,950	3.17	979	3.88	18,364	4.97	1,435	1.60	61,172	0.57	1,111	4.32	88,011	1.73
Milk & Cream[6]	3,000	0.53	1,878	3.24	41,761	1.37	511	5.67	.....	.....	5,462	3.16	52,612	1.61
Milk, cream, Choc. milk[7]	.....	.....	.....	.....	2,512	20.72	.....	.....	12,930	0.49	4,300	7.69	19,742	4.64
Ice Cream	650	7.84	2	0.00	1,664	1.38	260	0.38	3,024	0.10	53	5.66	5,653	0.53
Choc. milk	954	1.99	2	100.00	1,997	2.05	30	0.00	3,191	1.78	342	0.00	6,516	1.82
Butter	623	32.26	.....	.....	.....	.....	250	1.20	258	35.27	.....	.....	1,131	25.99
Cheese	15	0.00	.....	.....	.....	.....	.....	.....	408	0.49	.....	.....	423	0.47
Cremo (half & half)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	102	1.96	102	1.96
Skim milk	.....	.....	.....	.....	.....	.....	.....	.....	12	0.00	.....	.....	12	0.00
Buttermilk	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	0.00	2	0.00
Dairy Products[8]	.....	.....	.....	.....	2,445	9.83	.....	.....	.....	.....	.....	.....	2,445	9.83
Total	36,911	4.27	8,464	10.24	151,727	2.96	4,546	2.68	155,176	0.61	18,332	5.54	375,156	2.48

[1] Includes Hawaii and Puerto Rico
[2] An additional 11,750 tests were reported but the per cent positive was not stated
[3] An additional 27,239 tests were reported but the per cent positive was not stated
[4] An additional 45,995 tests were reported but the per cent positive was not stated
[5] An additional 1,975 samples were tested but the per cent positive was not stated
[7] Per cent positive not reported separately
[8] Kind not designated

In reply to the other part of question 6, "Does it compare in reliability with other tests when samples are incubated under controlled conditions for 20 or more minutes?", 47 stated "Yes," 3 "No," 1 said "30 minute incubation was," 2 said "Nearly," and 1 "Usually."

One state laboratory stated that they used the Scharer Field and Laboratory tests for detecting underpasteurization, and used the findings as a basis for prosecution.

The answers to question 10, "Have you noticed any decrease in the number of samples improperly pasteurized as compared to the first time tests were made?" indicates that the phosphatase test has been of decided value in insuring proper pasteurization. One hundred thirty-seven answered "Yes" while 32 answered "No." The majority of those stating the amount of decrease reported that it is more than 50 per cent.

REFERENCES

(1) BURGWALD, L. H. A Critical Review of the Phosphatase Test. Internatl. Assoc. Milk Dealers, Assoc. Bul. No. 14, Feb. 24, 1941.

(2) Standard Methods for the Examination of Dairy Products, 7th Ed. Published by the American Public Health Assoc., 1939.

METABOLISM STALLS

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In balance trial and digestion trial work at the Virginia Agricultural Experiment Station prior to 1938 the experimental animals were kept in a room with a level concrete floor where attendants caught the excreta on shovels and in buckets. To carry out a trial without loss of excreta on the floor was extremely difficult. Since our work is entirely with cows, we found it impossible to collect satisfactorily with rubber ducts. Furthermore a stove, which was located on one side of the room, overheated the cows nearest the stove, causing them to pant, and allowed the attendant of the cows on the side of the room away from the stove to become chilled. How much this affected the results of digestion trials could only be surmised.

To avoid these conditions and to reduce the cost of continuous calcium and phosphorus balance trials the metabolism stalls described in this article were built. A basement room 22 feet square was used for this purpose. This room has three outside exposures; the east side adjoins the main stable. As is shown in the floor plan, the metabolism stalls are arranged so that cows can be led into this room from the west side. The second illustration shows the sections through the first stall.

Four stalls are elevated three feet above the floor level by means of retaining walls and dirt, excavated from the rear of the stalls, used for filling. Over this foundation a slab of reinforced concrete was poured with sunken areas in each stall for laying cork brick floors. In the rear of each stall, a storm grating was fitted with a copper funnel underneath to direct the excreta into thirty-gallon garbage cans. Because of corrosion by sulphates, these copper funnels had to be replaced by galvanized iron. Doors which can be raised to allow the attendant to remove the collection cans were hinged to the outside walls in a position level with the rear of the stall.

These stalls were separated by concrete curbs over which wooden stall partitions were located. Wooden frames covered with sheet metal were made to fit the rear of the stall and to direct the excreta downward to the storm grating. The rear half of the wooden stall partitions was also covered with sheet metal to prevent the excreta from one cow from reaching the adjoining stall. When these stalls were used for digestion trials where the feces and urine must be collected separately, the splash boards were removed from the rear of the stalls to allow the attendant to have access to the cow. A litter carrier track installed at the rear of these stalls connected with the track system of the barn. Wooden mangers were provided of such

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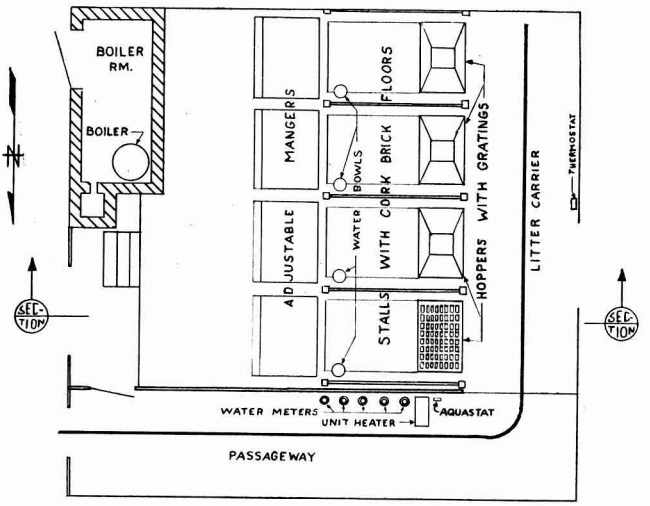
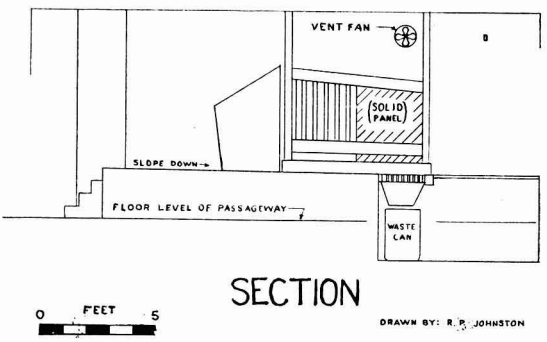
a width that they would telescope into the front of the stall between the partitions thereby adjusting the length of the stall to the individual cow. Short pieces of pipe were sunk into the concrete at intervals of three inches into which door bolts fastened to the manger could be inserted, preventing



PLAN



SECTION

animals from shoving the mangers forward and backward. The details of manger construction are given in a previous paper (1).

Ventilation is provided by a "Jamesway" ventilator in the south wall of the room. This ventilator fan is regulated by thermostatic control, which starts the fan when the temperature reaches 56° F. and stops it when the temperature falls to 52° F.

The room is heated by a hot water supply boiler. A "Trane" unit heater, installed in the hot water line, is so located as to direct the heat past

the end of the partition which separates the metabolism stalls from the work room from which the metabolism stalls are entered at the rear. A wall thermostat controls the unit heater. The water in the system is circulated by a "Thrush" circulator located in the boiler room which is directly ahead of the metabolism stalls. The water circulator in turn is operated by a reverse acting aquastat in the returning hot water line between the unit heater and the circulator. An overhead expansion tank allows for the necessary change in volume due to heating of the water and a check valve prevents hot water from being forced back into the cold water line which feeds the system.

The combined action of the "Jamesway" ventilator and of the heating system results in a very uniform temperature and in low humidity except when the outside air is excessively humid.

Four "Trident" water meters measure the water which flows to water cups placed in the four stalls. Another meter measures the water which might be used for washing the stalls. This would be necessary if water from the pipe line were used for washing purposes as the water is very "hard." During the two years which the stalls have been in use, distilled water has been used for washing the stalls daily before the excreta is weighed. The use of the meters to the water cups makes it possible to estimate the amount of calcium supplied to each cow through the water.

A severe case of mastitis developed in one cow. Since occasional cases of mastitis develop in the main herd the metabolism stalls may not have been the cause in this case. However the storm gratings are good conductors of heat and help to chill the udder. A chimney effect is produced by the funnel and a draft results. This might be prevented by a canvas attached to the funnel and gathered tightly around the can by an elastic band.

SUMMARY

Four metabolism stalls were constructed with storm gratings placed in the rear of each stall and with funnels underneath which direct excreta into garbage cans. A fan ventilator with a thermostatic control regulates the humidity quite satisfactorily. A hot water boiler and unit heater provided with a water circulator controlled by a reverse acting aquastat provide satisfactory heat.

These stalls have been used for two years for balance trials, successfully eliminating the need for constant attendants.

When used for digestion trials in which the feces and urine are collected separately, these stalls helped to eliminate errors by making it possible to recover quantitatively either feces or urine which the attendant failed to catch.

REFERENCE

(1) PRATT, A. D. A Manger for Experimental Feeding. JOUR. DAIRY SCI., 21: 10, 643–644. 1938.

THE CAROTENOID CONTENT OF MILK FAT FRACTIONS

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The separation of milk fat by fractional crystallization into different fractions was recently reported in connection with the study of the effect of the properties of the fat on lipolytic activity in milk (11). This study indicated a definite variation between the fat constants of different fractions. However, that paper did not contain data concerning the carotenoid content and the melting point of the fractions.

Since it was observed that the color intensity of the fractions varied inversely with the temperature of crystallization, it was thought desirable to learn if there is a relationship between the carotenoid content and the properties of the fractions.

For this study the carotenoid content of different fractions was determined by Hand and Sharp's method (8), and is reported in mg. per liter of melted fat. The melting points were determined using the A.O.A.C. (1) method.

The data presented in figure 1 indicate that there is an inverse relationship between the carotenoid content and the melting points of different fractions. They also show that the carotenoid values curve is definitely parallel to that showing the iodine number.

The iodine numbers show a definite tendency for unsaturated acids to concentrate in the liquid fractions.

The Reichert-Meissl numbers show that the volatile soluble acids are also concentrated in the fractions having lower melting points. However, this tendency to concentrate in the liquid phase practically disappeared below 15° C., while the tendency for unsaturated acids and carotenoid to concentrate in the liquid phase continued down to the lowest temperatures studied. These results suggest, therefore, that the solubility of carotenoid in the fraction is to a large extent dependent upon the concentration of unsaturated fats.

Several investigators have studied the seasonal variations in the carotene and vitamin A content of the milk fat (2, 3, 7, 10, 13). These studies indicated that with the change from dry feed to pasture, both the carotene and the vitamin A content of milk fat increased rapidly. However, it is generally recognized that the feed of the cows exerts a definite influence on the chemical properties of milk fat. Hunziker, Mills, and Spitzer (9) were the early workers to show that the change from dry feed to pasture caused an abrupt increase in the iodine numbers and a decrease in Reichert-Meissl numbers of milk fat.

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Although these experiments are not directly comparable, nevertheless it appears that the seasonal variations in carotene and vitamin A content seem to parallel those in iodine numbers of milk fat.

In view of the fact that only a small fraction of ingested carotene appears in the milk fat (3, 12), the above observations, and the data presented in figure 1, suggest the possibility that the absorption of carotene by an animal body and consequently the carotene and the vitamin A content in

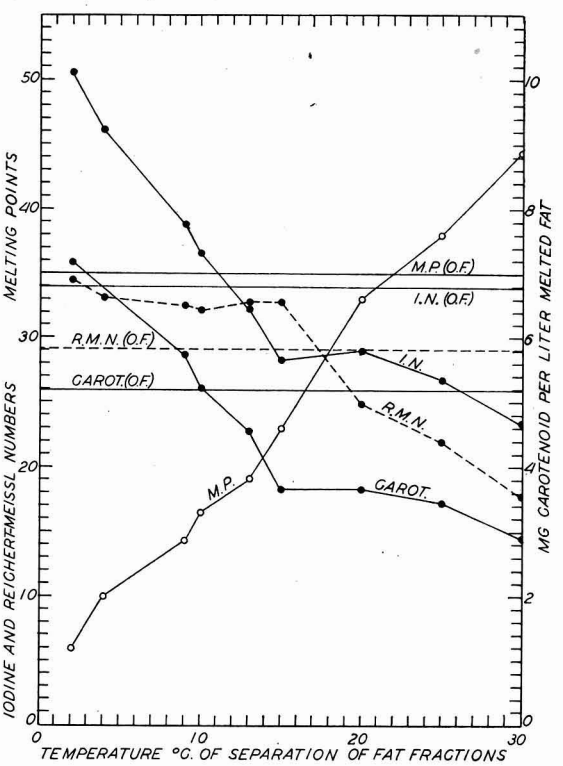

FIG. 1. Fat constants and carotenoid content of different milk fat fractions. (M.P.—melting points; I.N.—iodine numbers; R.M.N.—Reichert-Meissl numbers; O.F.—original fat.)

the milk fat could be affected not only by the carotene content of the feed but by the character and amount (5, 6, 14) of the fat in the diet as well.

Some interesting results were obtained with respect to the keeping qualities of milk fat fractions. These fractions, after two years' storage in an incubator at 4° to 5° C., were tested and scored. It was found that generally the intensity of the oxidized flavor varied inversely with the iodine number and the carotenoid content of the fractions. The fraction having the lowest melting point and which also showed the highest iodine number and carotenoid content appeared to be the best one with respect to flavor.

These observations seemed to indicate that the carotene might be the substance responsible for the reduction in the susceptibility of the fat fraction to oxidized flavor. However, in view of the recent work of Brown *et al.* (4) there is a possibility that some other substance associated with carotene is concentrated in the liquid fraction and is responsible for this effect.

The extreme low temperature fractions might well serve as the starting point in an attempt to identify the highly unsaturated acids and the antioxidant of butter fat.

SUMMARY

A study was made of the relationship between the carotenoid content and the physico-chemical properties of different milk fat fractions.

The data indicate an inverse relationship between the carotenoid content and the melting points of the fractions. They also indicate a definite relationship between the carotenoid content and the iodine numbers of the fractions.

The data suggest that the efficiency of absorption of carotene by an animal from its feed might be influenced by the degree of unsaturation of the fat present in the feed.

The flavor score of different fractions at the end of two years' storage at 4°–5° C. revealed that the intensity of the oxidized flavor varied inversely with the carotenoid content of the fractions. It appears that the substances responsible for the reduction in the susceptibility of the fat to oxidized flavor are concentrated in the liquid fraction.

The extreme low temperature fractions might well serve as the starting point in an attempt to identify the highly unsaturated acids and the antioxidant of butter fat.

REFERENCES

(1) Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 1935.

(2) Bauman, C. A., and Steenbock, H. The Carotene and Vitamin A Content of Butter. Jour. Biol. Chem., **101**: 547–560. 1933.

(3) Bauman, C. A., Steenbock, H., Beeson, W. M., and Rupel, I. W. The Influence of Breed and Diet of Cows on the Carotene and Vitamin A Content of Butter. Jour. Biol. Chem., **105**: 167–176. 1934.

(4) Brown, W. Carson, Vanlandingham, A. H., and Weakley, C. E., Jr. Oxidized Flavor in Milk. Jour. Dairy Sci., **24**: 925–935. 1941.

(5) Drummond, J. C. The Fat Soluble Vitamins. Ann. Rev. of Biochem., **7**: 335. 1938.

(6) Dyer, F. J., Key, K. M., and Coward, K. H. The Influence of the Solvent on the Vitamin A Activity of (a) Carotene and (b) Cod-liver Oil. Biochem. Jour., **28**: 873–881. 1934.

(7) Gillam, Albert Edward. A Modified Spectrophotometric Method for the Assay of Carotene and Vitamin A in Butter. Biochem. Jour., **28**: 79–83. 1934.

(8) Hand, David B., and Sharp, Paul F. Rapid Photoelectric Determination of Carotenoid in Milk Fat. Jour. Dairy Sci., **22**: 729–735. 1939.

(9) HUNZIKER, O. F., MILLS, H. C., AND SPITZER, GEORGE. Moisture Control of the Butter-Maker. Effect of Season of Year on the Chemical Composition of Butter Fat. Purdue Agr. Expt. Sta. Bul., 159, 16: 285–360.

(10) KNUDSEN, S., AND NELSON, S. Some Measurements of the Seasonal Variations in the Color of Butter Fat. Kgl. Vater.-og. Landbohoiskole Aarskrift. 122–131. 1936. Milchwirtschaftlicher Literaturbericht, **109**: 241. 1936.

(11) KRUKOVSKY, VLADIMIR N., AND SHARP, PAUL F. Effect of the Properties of the Fat and of the Fat Globule Surface on Lipolytic Activity in Milk. JOUR. DAIRY SCI., **23**: 1109–1118. 1940.

(12) REED, O. E. Carotene Requirements of Dairy Cattle for Normal Reproduction. Report of the Chief of the Bureau of Dairy Industry, U. S. Dept. Agr., August, 1940.

(13) SHARP, PAUL F., AND HAND, DAVID B. The Carotenoid Content of Milk Fat. JOUR. DAIRY SCI., **22**: 737–741. 1939.

(14) SMITH, M. C., AND SPECTOR, HARRY. Some Effects on Animal Nutrition of the Ingestion of Mineral Oil. Arizona Agr. Expt. Sta., Tech. Bul. 84. 1940.

THE EFFECT OF HIGH-TEMPERATURE SHORT-TIME FOREWARMING OF MILK UPON THE HEAT STABILITY OF ITS EVAPORATED PRODUCT

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The stabilizing effect of high temperatures of forewarming on milk which is to be concentrated and sterilized has been pointed out by Grindrod (2). During the development of his impact process of sterilization he observed that, when high velocity steam was injected into a stream of milk, stabilization to the heat of sterilization occurred. Made by his process evaporated milk had a heat stability four times that obtained by the usual treatment. Grindrod attributed the stabilizing action of the jets of steam largely to the apparent redispersing action upon coagulated albumin, calcium salts, and to a lesser extent coagulated casein. He states, "It appears to be very desirable, if not absolutely essential, that the steam should heat the material instantly by direct contact as distinguished from processes in which part of the milk is heated by direct contact with a hot body and the rest becomes heated by convection or distribution due to its being mixed with the previously heated material" (3). Grindrod noticed that these evaporated milks of high stability often showed a body too thin for a commercially acceptable milk.

Recently, methods of rapidly heating fluids to high temperatures in tubular heaters without allowing the fluid to come in direct contact with steam have been perfected (5). Since equipment of this type was made available in these laboratories it was believed that a detailed study of the effect of high heat treatment or "high temperature forewarming" of milk upon the heat stability of evaporated milk made from it would be of value to the industry. The results reported in this paper are concerned with the effect of high temperature forewarming with a 25-second holding period upon the heat stability of evaporated milks of 18 per cent solids-not-fat content.

EXPERIMENTAL

The milk used in this investigation was produced by the Bureau of Dairy Industry herd at the National Agricultural Research Center, Beltsville, Maryland. The cows were healthy except for a few cases of chronic mastitis. No milk was received from cows suffering from acute mastitis.

Skim milk was used in the early work. After it was noted that skim milk did not always produce the same type of heat stability curve as whole milk, most of the experiments were conducted with whole milk.

The fresh whole milk was standardized by the Babcock test and a hydrom-

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eter reading (4) to a fat: solids-not-fat ratio of 1: 2.29. It was then forewarmed under the desired experimental conditions, concentrated under 28 to 29 inches of vacuum to less than one half its initial weight, quickly heated to 80° C.,[1] homogenized at once at 2500 pounds per square inch pressure, cooled and standardized with water to 26 per cent total solids. The skim milks were forewarmed, concentrated, cooled and standardized with water to 18 per cent solids content. Approximately 70 pounds of whole milk or skim milk was used for each sample.

The control samples were prepared in the same way except that the milk was forewarmed to 95° C. and maintained at this temperature 10 minutes. This forewarming treatment corresponds to the usual commercial procedure. A few samples of milk were also forewarmed at 65° C. for 10 minutes. All samples referred to in this paper as having been forewarmed below 100° C. were so treated with thorough agitation in a steam jacketed kettle.

Samples reported as forewarmed above 100° C. were high-temperature forewarmed by forcing the milk through 0.18-inch I. D. stainless steel tubing with a reciprocating pump. The capacity of this pump was 1.5 gallons per minute but the rate of flow of the milk through the tubing was governed by the relationship between the resistance to this flow and the pressure required for the milk to by-pass through an homogenizing valve. The actual rate of flow of milk through the tubing was approximately 1 gallon per minute.

The heating coil was installed in an insulated metal chamber into which high pressure steam could be admitted through a reducing valve. The holding period depended on the seconds required for the liquid to pass through a known length of tubing wound around a jacketed metal post. The cooling coil was immersed in rapidly flowing tap water.

Temperature readings were obtained by means of thermocouples screwed into tees in the stainless steel tubing, a cold junction, a millivoltmeter and suitable connections. Four thermocouples were employed. One was inserted between the pump and the heater, another between the heater and the holding coil, the third at the end of the holding coil and the fourth at the end of the cooling coil.

After the thermocouples had been standardized it was only necessary, when a certain temperature was desired, to divide that number by a conversion factor to determine the corresponding reading on the scale of the millivoltmeter.

For the most part the milk was heated to the desired temperature in 4 seconds, held 25 seconds and cooled to a temperature of less than 38° C. in 4 seconds.

Heat stability determinations were made by heating the samples of concentrated milk in small cans (208 × 208) in a pilot sterilizer until coagulation was observed. The reel of the sterilizer was equipped with 2 chutes

[1] °F. = (°C. × 1.8) + 32.

which held 10 cans each and which were built so that one or more cans could be removed at any time without disturbing the remaining samples. The stability data were recorded in minutes of heating required to produce the first signs of coagulation. Data were accurate to approximately ± 4 per cent of the coagulation time. The sterilization temperature for whole milk was 115° C. One hundred twenty degrees centrigrade was used for skim milk in order to avoid long heating periods.

When stabilizing salts were used, they were added as standard solutions to 130 ml. of milk measured into each can. A total of 1 ml. of salt solution or water was added per can to keep the milk solids concentration constant. Milks receiving 1 ml. of $\frac{1}{3}$ M $Na_2HPO_4 12H_2O$ or 1 ml. of $\frac{1}{2}$ M $CaCl_2$ per 130 ml. of milk received an equivalent of about 14 ounces and $6\frac{1}{2}$ ounces of the dry salt respectively per 1000 pounds of milk.

RESULTS

The data presented in table 1 show the magnitude of the stabilizing effect produced by high forewarming temperatures. The heat stability of the test samples was generally about 2 and occasionally as much as 6 times greater than that of the control samples.

Data representing the effect of some variations in forewarming fresh whole milk upon the heat stability of its evaporated product have been plotted in figure 1. Observations on the body and physical condition of

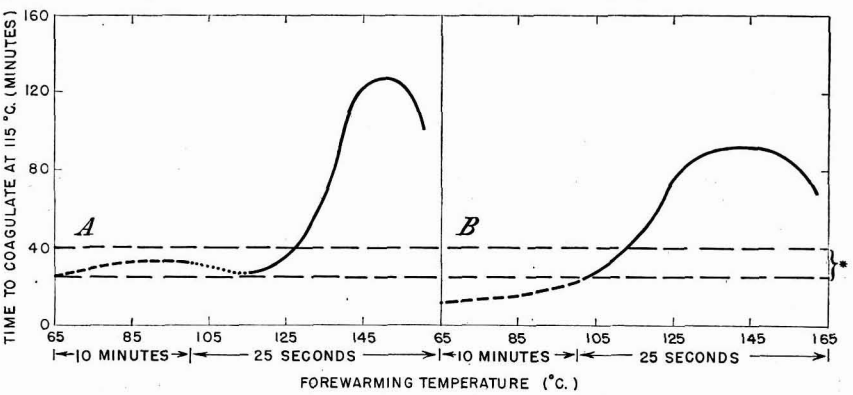

FIG. 1. The effect of variations in forewarming upon the heat stability of two types of evaporated whole milk. Broken lines indicate low temperature, long time forewarming. Solid lines indicate high temperature, short time methods. (* Stability range required for production of milk of good body.)

many samples after sterilization indicated that milks falling within the stability range of 25 to 40 minutes possessed a commercially acceptable body. Milks below this range showed a slight grain or were of excessive viscosity. Milks with a stability greater than 40 minutes were thin at the end of an 18 minute sterilization period.

The destabilizing effect of the fat phase and the effect of dispersal of the fat by homogenization of the concentrated milk were studied. Some data have been plotted in figure 2. Skim milk was always more stable than whole milk. Generally, the whole milk and skim milk curves were of the same type

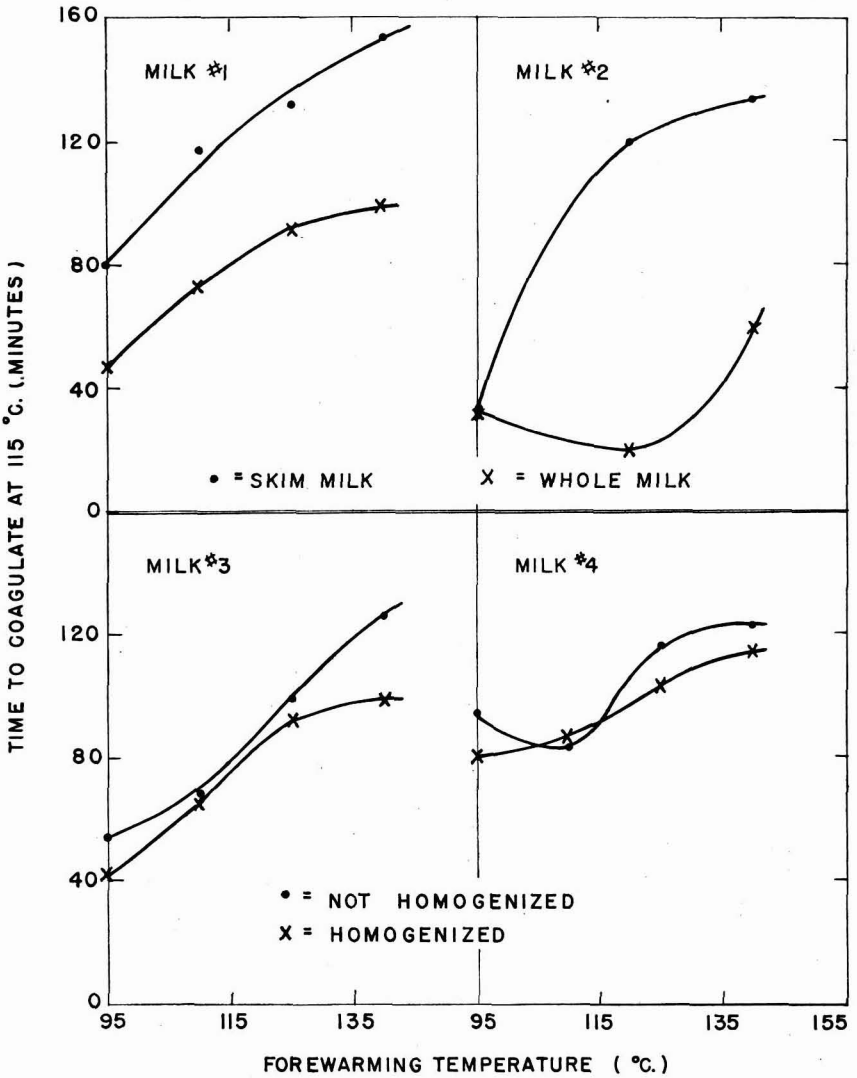

FIG. 2. Some effects of milk fat and its homogenization upon the heat stability of four evaporated milks made from milk forewarmed to different temperatures. Portions of milks #1 and #2 were separated before forewarming to obtain the skim milk. Milks #3 and #4 were each divided into 2 parts after forewarming and concentrating but before homogenization.

but milk #2, figure 2, shows a whole milk destabilized by forewarming to 120° C., while its skim milk was greatly stabilized by the same forewarming treatment.

It is known that homogenization decreases the heat stability of evaporated milk (1). Data plotted in figure 2, milks #3 and #4, indicate that, while homogenization generally destabilizes the milk, this effect may under some conditions be negligible. The greater stability of homogenized milk #4 over the unhomogenized sample at 110° C. forewarming is so small as to be within the limits of experimental error.

Although each batch of concentrated whole milk made during the course of this work was heated to 80° C. before homogenization, this pre-homogeni-

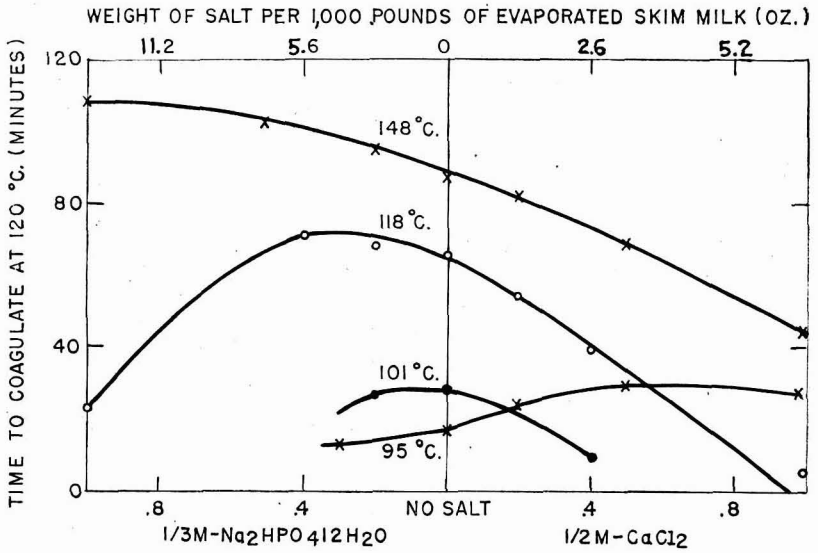

FIG. 3. The effect of stabilizing salts and forewarming treatments upon the heat stability of evaporated skim milks.

zation heat treatment actually had but slight stabilizing action. A large batch of whole milk forewarmed to 130° C. was condensed and divided into four parts, which were heated to different temperatures before homogenization at 2500 pounds per square inch pressure. Samples homogenized at 37° C., 50° C., 65° C. and 80° C. coagulated when sterilized at 115° C. in 35 minutes, 35 minutes, 37 minutes and 38 minutes respectively.

The stabilizing effect of added salts and of high forewarming temperatures is shown in the graphs of figures 3 and 4. Figure 3 indicates that high forewarming shifts the curve of a normally forewarmed skim milk, which is stabilized by calcium to curves which show some stabilization by phosphate.

It has been noted (6, 7, 9) that a calcium-stabilized milk could be changed to one stabilized by phosphate through the addition or the development of acid in the milk. This condition may be again noted by comparing the fresh and aged samples of skim milk #2, figure 4.

Milks of excessive acidity coagulated when subjected to high temperature forewarming, but normal milks of good quality withstood temperatures of 150° C. to 160° C. without coagulating.

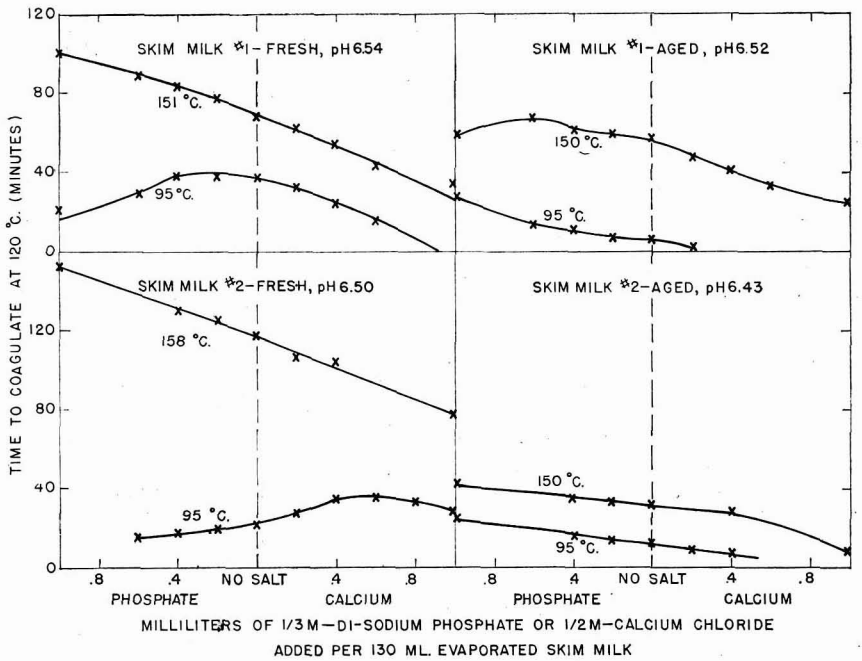

FIG. 4. The effect of stabilizing salts upon the heat stability of two evaporated skim milks. A portion of each milk was processed when fresh and another portion after a ripening period. The pH values were determined before processing.

The color, flavor and pH of milks forewarmed to 140° C. for 25 seconds and to 95° C. for 10 minutes did not show important differences but there were undesirable changes in color and flavor and an increase in acid intensity when temperatures above 140° C. were used.

DISCUSSION

Table 1 includes representative data obtained from milks produced at intervals during one year. No milks were found which did not show greater stability than the control when they were forewarmed to a temperature of 140° C. or higher for 25 seconds. The degree of stabilization brought about by high-temperature forewarming was not the same in various samples of

TABLE 1

The effect of forewarming treatment upon the heat stability of evaporated milk. Control samples were held at 95° C. for 10 minutes. All other samples were held 25 seconds at the indicated temperatures

Skim milk. T.S. = 18%			Whole milk. T.S. = 26%		
Date	Forewarming temperature	Time to coagulate at 120° C.	Date	Forewarming temperature	Time to coagulate at 115° C.
	°C.	min.		°C.	min.
12/23/40	95	8*	3/17/41	95	33
	150	26*		120	30
12/31/40	95	10		140	105
	150	76		157	121
1/ 6/41	95	28	4/ 8/41	95	33
	152	67		120	21
1/13/41	95	27		140	60
	145	88	4/14/41	95	43
1/16/41	95	15		120	35
	101	24		140	65
	118	60	4/29/41	95	36
	133	67		110	55
	141	72		125	92
	148	75		140	98
1/31/41	95	22	5/ 7/41	95	81
	158	117		110	86
2/14/41	95	12		125	104
	150	32		140	114
2/18/41	95	37	5/26/41	95	20
	112	58		130	85
	151	68		140	92
4/29/41	95	80†		150	91
	110	117†	6/ 2/41	95	81
	125	132†		140	130
	140	154†	6/23/41	95	37
				140	84
			8/11/41	95	35
				150	70
			9/25/41	95	44
				120	98
				140	94
			10/ 1/41	95	30
				130	54
			10/27/41	95	28
				120	16
				140	58
			11/ 3/41	95	36
				140	45
			12/11/41	95	74
				140	93

* Time to coagulate at 125° C.
† Time to coagulate at 115° C.

milk. The average values for the stability of the whole milks forewarmed to 95° C. and to 140° C. were 45 and 86 minutes respectively.

The range of forewarming temperature which can be used to produce a milk of proper body depends upon the nature of the forewarming-stability curve of the milk. Figure 1 shows two types of forewarming curves. Milk A fell within the proper stability range when it was forewarmed at any temperature between 65° C. and 127° C., while milk B showed the required stability over the range, 102° C. to 113° C. From available data it is reasonable to assume that the forewarming-stability curve for every milk is different. As a milk is aged and acid is developed the entire curve is lowered. Factors such as changes in salt equilibrium shift the curve to the right or left. If curve A is shifted downward its steepest part can be made to pass through the 25 to 40 minute stability area over a range of only 2° C. in forewarming temperature. An operator, confronted with such a milk, would find it difficult to estimate what forewarming temperature should be used to obtain the required stability. The manufacturer's problem is further complicated by his desire to secure the same type of body in every batch. This would probably make it necessary for him to adjust the permissible stability in a somewhat narrower range than the 25 to 40 minute period given in figure 1.

It is probable that greater difficulty will be encountered in obtaining proper body in a high-temperature short-hold forewarmed milk than in a normally forewarmed milk because the optimum high forewarming temperature must be more closely estimated. This can be done by setting each day's high forewarming temperature after consideration of the previous day's run. When no clue is available on the stability of a milk, the safest procedure would be to forewarm to 130° C.–145° C. for 25 seconds.

Experimental work of the type reported in this paper is more easily conducted with skim milk than with whole milk. After consideration of the data presented in figure 2, however, it seemed desirable to use whole milk until such time as the relationship between the fat phase, heat stability and various manufacturing procedures was better understood. The experiments in which skim milk was used were conducted early in the investigation. Recent trials indicate that the general relationships shown in figures 3 and 4 apply also to whole milk.

The stabilizing effect which may be obtained by selection of the optimum high forewarming temperature was found to be greater than the stabilizing influence secured by addition to the milk of the most favorable quantity of calcium or phosphate salt.

The following tabulation of data from figures 3 and 4 should be of interest. It shows the average heat stability of the control and high-temperature forewarmed samples together with the average heat stability of these

milks when the amount of stabilizing salts giving optimum stability was used.

Forewarming treatment	Time to coagulate when no stabilizing salts were used	Time to coagulate when the optimum quantity of stabilizing salts was used
	minutes	*minutes*
Control—95° C.—10 min.	19	32
Heated to approximately 150° C.—25 sec.	74	93

The milk used in this work was equivalent to market milk and was of a different grade than that received in some condenseries. The effect of a slight development of acidity in the Beltsville cows' milk upon the nature of the flash forewarming results was investigated. The skim milk of table 1, dated 1/31/41 (also figure 4, No. 2) had a reaction of pH 6.50. After part of it was held at 1° C. two weeks and then at room temperature several hours, its reaction was pH 6.43. When this milk was processed on 2/14/41, its heat stability was again greatly increased by high-temperature short-hold forewarming. The sample of whole milk in table 1, dated 5/26/41, was aged 2 hours at 32° C. with added lactic starter and subsequently held at 1° C. overnight before subjecting it to the usual manufacturing procedure. The ripening treatment changed its reaction from pH 6.58 to pH 6.56. Stabilizing salts would have been necessary to produce a commercial evaporated milk of satisfactory body from the control sample but the high-temperature forewarmed samples showed more than ample heat stability. The effect of high-temperature forewarming upon the heat stability of a skim milk in which a slight amount of acid has developed is also shown in figure 4, No. 1.

The results on mildly aged milks indicate that forewarming to the proper high temperature is an effective means of stabilizing concentrated milk toward sterilization. Unless the milk has developed an excessive amount of acid which it may be necessary to neutralize, stabilization by high-temperature forewarming would appear to be better practice than stabilization by salts. In some cases it is possible that both stabilizing methods might be necessary.

Stabilization by heat during forewarming possesses this disadvantage over salt stabilization of the concentrated milk—the operator must guess from past experience what the optimum forewarming treatment of the raw milk should be. No certain and accurate tests are available for determining this. Stabilization by salts is carried out by adding small increments to numerous samples of the concentrated milk just prior to sterilization. Pilot sterilizer runs are made and the exact amount of salt needed is accurately determined.

Available studies (8) on the effect upon heat stability of mixing different grades of milk indicate that if 2 milks of the phosphate-stabilized type are mixed, the milk of higher stability will increase the stability of the poorer milk somewhat in proportion to the quantity of more stable milk used. It is probable that a milk condensery could adjust the stability of its milk by sterilizing the proper blend of normally forewarmed and high-temperature forewarmed, concentrated milk. The heat stability and body of many evaporated milks could be controlled in this way without the use of stabilizing salts. High-temperature forewarming 25 per cent to 50 per cent of the milk received would, in most instances, be sufficient to make a stable concentrated mixture suitable for sterilization.

During the course of a year's experimental work, there was consistent improvement in the heat stability of high-temperature forewarmed milks over milks forewarmed below 100° C. It is not to be expected that the data could be exactly duplicated when milks are used which are produced under different seasonal or geographical conditions. It is believed, however, that the basic relationships observed in this work will be found to exist wherever high-temperature forewarming methods are applied.

A study of the development and maintenance during storage of a smooth body, viscous enough to retard fat separation in evaporated milk, was not made during the course of this work. The effect of different forewarming treatments of whole milk upon the viscosity of its evaporated product during storage is being investigated.

The remarkable heat stability of high-temperature forewarmed milks of 26 per cent total solids content indicates the possibility of processing milks of higher solids concentration. Accumulated data show that the time a milk is held at the high-forewarming temperature exerts a great influence upon its subsequent heat stability. A general study of the relationships between conditions of forewarming, solids content and heat stability is in progress and will be reported later.

SUMMARY

1. The heat stability of evaporated whole milk of 26 per cent total solids content was increased as much as 6 times that of control samples by high-temperature short-hold forewarming the fresh milk. The control samples were forewarmed to 95° C. (203° F.) and held 10 minutes; the test samples were forewarmed over a range of temperatures from 101° C. (213.8° F.) to 165° C. (329.0° F.) with a heating time of 4 seconds, a holding time of 25 seconds and a cooling time of 4 seconds.

2. The relationship between the high forewarming temperature and the heat stability of evaporated milk differs with each milk. A study of this relationship indicates that the high-forewarming temperature required to produce an evaporated milk of a certain desired viscosity may be within

limits of 2° C. (3.6° F.) for one milk or within limits as wide as 60° C. (108° F.) for another milk. Milks forewarmed to produce excessively high stability will be too thin, while those with too low stability will be rough after sterilization.

3. Use of the optimum high-forewarming temperature brought about, in the milks tested, a greater increase in heat stability in the evaporated milk than could be attained by the addition of the optimum quantity of stabilizing salt to a normally forewarmed milk.

4. High forewarming should be a useful commercial procedure for increasing the heat stability of milks which are difficult to sterilize without the addition of stabilizing salts. When a coming-up time of 4 seconds and a holding time of 25 seconds are used, the optimum high-forewarming temperature for most milk will probably fall between 120° C. (248° F.) and 140° C. (284° F.).

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REFERENCES

(1) DEYSHER, E. F., WEBB, B. H., AND HOLM, GEORGE E. The Relations of Temperature and Time of Forewarming of Milk to the Heat Stability of Its Evaporated Product. JOUR. DAIRY SCI., **12**: 1, 80. 1929.

(2) GRINDROD, GEORGE. Sterilization of Milk by Impact of Steam. Creamery and Milk Plant Monthly, **16**: 9, 38. 1927.

(3) GRINDROD, GEORGE. U. S. Patent #1,854,189. 1932.

(4) LAMPERT, LINCOLN M. Tables and Nomograph for Sharp and Hart's Equation for the Calculation of Total Solids in Milk. JOUR. DAIRY SCI., **24**: 5, 417. 1941.

(5) MALLORY, W. E. Ann Arbor, Michigan. U. S. Patent #2,270,540. 1942.

(6) MILLER, P. G., AND SOMMER, H. H. The Coagulation Temperature of Milk as Affected by pH, Salts, Evaporation and Previous Heat Treatment. JOUR. DAIRY SCI., **23**: 5, 405. 1940.

(7) SOMMER, H. H., AND HART, E. B. Heat Coagulation of Evaporated Milk. Wis. Agr. Expt. Sta., Res. Bul. 67. 1926.

(8) WEBB, BYRON H. Heat Coagulation of Evaporated Milk as Affected by Mixing Different Grades of Raw Milk. JOUR. DAIRY SCI., **11**: 6, 471. 1928.

(9) WEBB, BYRON H., AND HOLM, GEORGE E. The Heat Coagulation of Milk. II. The Influence of Various Added Salts Upon the Heat Stabilities of Milks of Different Concentrations. JOUR. DAIRY SCI., **15**: 5, 345. 1932.

THE BACTERIOLOGY OF BRICK CHEESE. II. COMPARISON OF WASHED-CURD AND CONVENTIONAL METHODS OF MANUFACTURE[1]

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INTRODUCTION

The bacteriology of Brick cheese during manufacture by the conventional procedure has been discussed in a previous report (2). In these experiments cheese made by this method always developed excessive acidity when the moisture content remained above 39 per cent, even when different starters and cooking temperatures were used. While some experienced cheese makers have been able to make a "sweet" cheese containing this much moisture, many cheese makers have had difficulty in doing so. The fermentation of the lactose in the cheese by lactic acid bacteria during manufacture and storage usually ceases only with the exhaustion of the milk sugar. In our experiments, when the moisture content of the cheese remained above 39 per cent, fermentation of all the lactose lowered the pH to about 4.8, and an acid cheese with a short, crumbly body resulted. In an effort to prevent the development of excessive acidity, a washing procedure was introduced to remove part of the lactose from the curd before draining. Comparisons of the changes in pH and bacterial numbers in the cheese during manufacture by both the conventional and washed-curd procedures will be considered here.

METHODS

Methods of sampling curd and cheese and the procedure of bacteriological analysis have been described previously (2). Incubation temperatures of 22° C. and 47° C. were used for the cultural counts. Garey (1) established that *Streptococcus lactis* and *Streptococcus thermophilus* could be separated quantitatively in this way, since at 22° C. the growth of *Str. thermophilus* was inhibited but that of *Str. lactis* was not; while at 47° C. the reverse occurred.

The milk used was from the mixed milk supply of the University of Wisconsin Creamery. The conventional procedure for making Brick cheese was that described by Wilson and Price (6) and by Langhus (3). The washed-curd method differed from the conventional procedure only in the treatment of the curd between cutting and dipping. The following washing procedures were tried:

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"One-time wash"—40 to 45 pounds of whey were removed per 100 pounds of milk and replaced with an equal volume of water at the temperature of setting.

"Mild wash"—25 to 28 pounds of water per 100 pounds of milk were added to the curd plus whey in the vat. Then 50 to 55 pounds of whey were removed and replaced by an equal volume of water warmed to 90° F.

"Double wash"—40 to 45 pounds of whey were removed per 100 pounds of milk and replaced by 80 to 85 pounds of water per 100 pounds of milk.

The double wash procedure was used for the cheese described here because it was known to control the high-acid defect; but cheese made by this method frequently developed defects due to insufficient acidity. The mild wash method was introduced to leave more sugar in the curd, so that the acidity would increase slightly.

For the determination of the rate of disappearance of lactose from the cheese, slivers about one-eighth inch thick were sliced longitudinally from the center of a plug from the side of the loaf, placed on three by five inch index cards, and then heated at 104° C. for three to four hours. The degree of brownness in the heated slivers due to caramelization of the lactose indicated the amount of sugar present at any given sampling period. In recording the appearance of the cheese slivers, the relative degrees of browning were indicated by different numbers of plus signs, as shown in the footnote to table 1.

RESULTS

Development of the Washed-Curd Method

The washed-curd method was developed to regulate the sugar content of the curd and thus control the final acidity of the cheese. A pH of 5.0 to 5.15 was usually the safest range to insure a cheese that was not too acid yet would not show defects due to too low acidity.

Analyses of cheese made by the conventional method showed that the lactose content of the curd at dipping was usually about 6.5 per cent of the dry matter. Fermentation of this amount of sugar lowered the pH to about 4.8, which was much too acid to permit normal ripening. The "one time wash" and the "double wash" procedures removed approximately half the lactose and permitted maintenance of the moisture content of the cheese at a much higher level than the legal limit without excessive acidity, but the curd developed an unnatural "corky" feeling before dipping. The "mild wash" procedure removed slightly over one-third of the lactose with no harm to the curd and the quality of the cheese. This procedure allowed an excess of 2 to 4 per cent moisture over the legal limit without danger of a sour flavor and a weak or crumbly body.

Bacterial Numbers, Acidity, Moisture Content and Lactose Content of Cheese Made by the Conventional and Washed-Curd Methods

Cheese made with Streptococcus lactis. Representative data on two lots of cheese made from the same batch of raw milk by the conventional and double washed-curd processes with *Streptococcus lactis* starter are shown in table 1. In the cheese made by the conventional method there was a steady

TABLE 1

Comparison of the effects of the conventional and washed-curd methods of manufacture on the bacteriological and chemical changes in cheese made from raw milk with one per cent Streptococcus lactis starter and cooked to 106° F.

Age of cheese		Acidity pH	Numbers of bacteria per gram (000 omitted). Cultural counts at 2 different incubation temperatures		Moisture content (per cent)	Residual lactose*
Days	Hrs.		22° C.	47° C.		
Conventional method						
0	2	5.77	110,000	1.5	.....	.....
0	4	5.25	.....	.....	.....	.....
0	6	5.12	.....	.....	.....	.....
0	8	4.96	.....	.....	.....	.....
0	10	4.88	6,050,000	2.5	.....	+
1	.....	4.80	10,500,000	8	46.10	+
7	.....	4.87	3,700,000	45	41.43	–
14	.....	4.90	3,000,000	10	.. ..	–
21	.....	4.76	400,000	2.5	40.40	–
28	.....	4.81	1,500,000	3	41.60	–
35	.....	4.78	3,300,000	.....	40.80	–
42	.....	4.78	700,000	1.5	40.60	–
Wash-curd method						
0	2	5.88	1,050,000	12	.....	.....
0	4	5.27	.....	.....	.....	.....
0	6	5.15	.....	.....	.....	.....
0	8	5.12	.....	.....	.....	.....
0	10	5.12	3,750,000	85	.....	+
1	.....	5.10	4,000,000	90	45.64	–
7	.....	5.22	3,550,000	50	41.22	–
14	.....	5.24	250,000	40	40.90	–
21	.....	5.16	150,000	20	41.10	–
28	.....	5.27	350,000	15	40.70	–
35	.....	5.22	240,000	.....	40.10	–
42	.....	5.27	600,000	17	40.00	–

* Designations of relative degrees of browning:

		Approximate percentage of lactose in the dry matter
Dark brown	++++	Above 2
Medium brown	+++	1–2
Brown	++	0.3–1
Light brown to mere trace	+	Less than 0.3
White	–	None

and rapid drop in pH to 4.80 at one day, and little change thereafter because of complete disappearance of the lactose after the first day. The bacterial

counts showed a rapid increase in numbers of the starter organisms parallel to the decrease in pH up to one day, little change for two weeks, and a gradual diminution thereafter. The numbers of organisms capable of growing at 47° C. were rather low throughout the ripening period. These consisted of *Str. thermophilus, Str. bovis, Str. fecalis* and *Str. liquefaciens,* present originally in the milk. This lot of cheese was criticized for an acid, bitter flavor, short body and excessively close texture.

With the exception of those in pH the course of changes in the washed-curd cheese was not significantly different from that described for the conventional method. As might be expected, the lactose disappeared more quickly from the washed-curd cheese whose pH dropped more slowly than it did from the cheese made by the conventional method. The minimum pH of 5.1 was reached at one day, after which it could go no lower because of the lack of sugar. This cheese lacked the undesirable high acidity of that made by the conventional procedure, but was criticized for lack of flavor.

TABLE 2

Comparison of the effects of the conventional and washed-curd methods of manufacture on the bacteriological and chemical changes in cheese made from raw milk with one per cent Streptococcus thermophilus starter and cooked to 106° F.

Age of cheese		Acidity pH	Numbers of bacteria per gram (000 omitted). Cultural counts at 2 different incubation temperatures		Moisture content (per cent)	Residual lactose*
Days	Hrs.		22° C.	47° C.		
Conventional method						
0	2	5.45	9,500	140,000	.....	.....
0	4	5.32	.....	.....	.....	.....
0	6	5.34	.....	.....	.....	.....
0	8	5.21	.....	.....	.....	.....
0	10	5.20	9,500	280,000	.....	++++
1	.....	5.22	13,000	135,000	44.64	++++
7	.....	5.11	475,000	10,000	40.80	++++
14	.....	5.03	700,000	2,000	39.40	+++
21	.....	5.01	800,000	3,000	38.70	++
28	.....	5.04	1,200,000	24,000	39.20	++
35	.....	4.99	500,000	3,000	39.20	++
42	.....	4.98	680,000	900	39.00	++
Wash-curd method						
0	2	5.75	7,000	14	.....	.....
0	4	5.62	.....	.....	.....	.....
0	6	5.56	.....	.....	.....	.....
0	8	5.53	.....	.....	.....	.....
0	10	5.52	11,000	90,000		++
1	.....	5.50	185,000	59,500	45.67	++
7	.....	5.30	1,500,000	30,000	42.00	+
14	.....	5.42	900,000	49,000	43.10	−
21	.....	5.45	1,200,000	42,000	40.10	−
28	.....	5.47	1,200,000	50,000	39.50	−
35	.....	5.43	1,400,000	90,000	40.60	−
42	.....	5.42	800,000	40,000	39.80	−

* See footnote to table 1.

Both lots of cheese were lower in moisture than is desirable. However, it is apparent that the pH in the cheese made by the conventional procedure would have been much lower had the moisture content been near the legal limit.

Cheese made with Streptococcus thermophilus. Representative data on two lots of cheese made from the same raw milk by the conventional and washed-curd processes with *Streptococcus thermophilus* starter are shown in table 2. In the cheese made by the conventional method there was a very rapid increase in the starter organisms during the first few hours after manufacture, then a sharp decrease. Low temperature organisms (those growing at 22° C.) increased slowly but persisted in large numbers throughout ripening. The pH eventually went as low as 4.98, and considerable lactose remained. This cheese was criticized for unclean and bitter flavors, short body and an open texture.

In the cheese made by the washed-curd method the changes in numbers of bacteria capable of growing at both 22° C. and 47° C. followed the same general trend during the period of increase as was found in the conventional cheese. However, the numbers of both types persisted at higher figures for a longer time in the washed-curd cheese. The lactose content of the washed-curd cheese naturally was lower and the sugar disappeared entirely after one week; hence the pH remained at 5.3–5.4 throughout ripening. This condition allowed the development of anaerobic spore-forming bacteria in great abundance, and blowing of the cheese resulted. The final product was criticized for a very undesirable bitter flavor, tough, rubbery body, and an extremely open texture.

Cheese made with a combination of Streptococcus lactis and Streptococcus thermophilus. The changes in the cheese made with *Str. lactis* and *Str. thermophilus,* as shown in table 3, followed the course usually taken in cheese made with a combination of the two starter organisms (2). In both the cheese made by the conventional method and that made by the washed-curd method the high temperature organisms were mainly the thermoduric starter bacteria. These decreased to insignificant numbers after one week, while the low temperature forms persisted longer and in much greater numbers. The pH of the cheese made by the conventional method dropped steadily until the lactose had almost disappeared at seven days, and very slowly thereafter. A very slight amount of lactose persisted near the rind of this lot of cheese throughout ripening, apparently due to inhibition of the lactic acid bacteria by the higher salt concentration in that area. The pH of the washed-curd cheese dropped steadily for one week, after which little further change occurred. The cheese made by the conventional method was criticized for excessive acidity and a short body; the washed-curd cheese had a satisfactory body but little flavor and a few pinholes.

A comparison of the three lots of cheese described above shows that the

TABLE 3

Comparison of the effects of the conventional and washed-curd methods of manufacture on the bacteriological and chemical changes in cheese made from raw milk with a combination of 0.5 per cent Streptococcus lactis and 0.5 per cent Streptococcus thermophilus and cooked to 106° F.

Age of cheese		Acidity pH	Numbers of bacteria per gram (000 omitted). Cultural counts at 2 different incubation temperatures		Moisture content (per cent)	Residual lactose*
Days	Hrs.		22° C.	47° C.		
Conventional method						
0	2	5.58	280,000	15,000	.....	.....
0	4	5.29	.....	.....	.....	.....
0	6	5.25	.....	.....	.....	.....
0	8	5.26	.....	.....	.....	.....
0	10	5.10	850,000	700,000	.....	++
1	.....	5.07	1,300,000	700,000	44.32	++
7	.....	4.92	1,550,000	5,000	41.02	+
14	.....	4.80	800,000	1,850	39.50	+
21	.....	4.76	1,100,000	1,500	39.20	+
28	.....	4.78	1,100,000	1,400	39.00	+
35	.....	4.72	800,000	1,700	38.80	+
42	.....	4.73	750,000	700	39.20	+
Wash-curd method						
0	2	5.58	220,000	31,500	.....	.....
0	4	5.54	.....	.....	.....	.....
0	6	5.43	.....	.....	.....	.....
0	8	5.33	.....	.....	.....	.....
0	10	5.27	1,450,000	350,000	.....	++++
1	.....	5.17	2,050,000	65,000	44.12	+
7	.....	5.14	2,150,000	20,000	40.75	−
14	.....	5.12	900,000	900	39.40	−
21	.....	5.11	1,900,000	1,000	39.20	−
28	.....	5.12	900,000	500	38.80	−
35	.....	.....	..	500	.....	−
42	.....	5.12	300,000	160	.....	−

* See footnote to table 1.

washed-curd method is successful in controlling the acidity, but may enhance other defects. The best results were obtained in both methods with a mixture of the starter organisms.

DISCUSSION

The process of washing Brick cheese curd with water before dipping originated in an effort to manufacture a product with a uniformly desirable flavor, body and texture containing as much moisture as legally allowable but lacking the usual acid defects of cheese with high moisture content. Preliminary studies of the starter organisms (1) had shown that this could not be accomplished by alterations of the manufacturing methods then being used. Fermentation of the residual lactose in high-moisture cheese made by the conventional procedure lowered the pH out of the range compatible with normal ripening, and a very acid cheese with a short, crumbly body and

close texture resulted. When *Str. lactis* was used as part or all of the starter the lactose disappeared completely within the first few days of ripening, and the acidity was high enough to prevent the development of undesirable bacteria. When *Str. thermophilus* was used alone as the starter it ceased growing a few hours after dipping, leaving considerable lactose in the cheese and the pH at a relatively high figure (5.30–5.45). The acidity did not increase until low temperature organisms (*Str. lactis* and others capable of growing at the curing temperature) developed and fermented the remaining lactose. These changes often required several weeks, thus giving ample opportunity to other organisms present in the milk to grow and produce undesirable changes in flavor and texture.

It was realized that a method must be developed to lower the pH rapidly to a point at which undesirable fermentations could not occur, but not too low to prevent normal ripening changes. This was done by removing part of the lactose by the curd washing process. Preliminary trials showed that washing more than once would not be practicable in the ordinary cheese plant because of the extra water and labor required; hence the wash water was applied only once. In the first experiments most of the whey (40–45 pounds whey per 100 pounds of milk) was removed first, and then replaced with an equal or greater quantity of water warmed to 90° F. Cooking was then carried on as usual. This procedure left about one-half the lactose, which was sufficient to yield the desired acidity, but had the disadvantage of causing the curd particles to mat when the whey was first drawn off. The curd prepared in this way always had an unnatural "corky" feeling when pressed together in the hand rather than the usual firmness characteristic of curd particles in the conventional method of manufacture.

The best product was prepared by the "mild wash" method. In this procedure about 25 pounds of water were added per 100 pounds of milk; then twice this amount of whey was removed and replaced with an equal volume of water. Thus sufficient liquid remained in the vat during washing to suspend the curd particles and prevent their matting. There was no undesirable effect on the curd particles. About 35 per cent of the lactose was removed by washing and the remainder disappeared as quickly as it did in the conventional method to give the desired pH (usually less than one day). Washing had no harmful effect on the activities of the starter bacteria. After six weeks of ripening the flavor of this cheese was clean and mild, the body was smooth and soft, and the texture was medium close. The smooth, soft body was attributable to the relatively high moisture content and to a greater degree of proteolysis than occurred in high-acid cheese.

It is recognized that most of these experiments were performed under laboratory conditions with milk of high quality; hence the methods cannot be recommended without reservation for all field conditions. It was generally observed that when a defect due to undesirable bacteria appeared in

cheese made by the conventional method, this defect was even more pronounced in cheese made from the same batch of milk by the washed-curd method. Differences in acidity of the cheese probably explain this observation. Many defects such as late gas formation by anaerobic spore-forming bacteria usually can be prevented by lowering the pH to 5.0. It is natural, then, if this or similar defects occur in conveniently made cheese with its relatively higher acidity that they would occur to an even more marked degree in cheese made by the washed-curd method with its lower acid content.

Usually, then, the washed-curd method satisfactorily controls the acid defect in Brick cheese, and with milk of good quality yields a desirable product. But with milk of poor quality the method cannot be recommended without modification. Any defect other than excessive acidity in cheese made by the conventional method is merely enhanced when the same milk is used with the washed-curd procedure. The success attained with the washed-curd method to date is sufficiently encouraging to warrant its trial under practical factory conditions, and it is believed that cheese makers will find it advantageous in producing a uniformly better product with a higher percentage yield.

SUMMARY

1. In an effort to remedy the acid defect common in high moisture Brick cheese a process was introduced for washing the curd with water before dipping. After this treatment enough fermentable lactose remained in the curd to lower the pH to about 5.00–5.15 in cheese with a moisture content of 40–42 per cent.

2. Of the several washing procedures tried the "mild wash" (25 pounds of water added per 100 pounds of milk, 50 pounds of whey removed and replaced with 50 pounds of water) gave the best product. The cheese made by this method had a mild and clean flavor, soft and smooth body and a medium close texture.

3. The washing process had no noticeable effect upon the rate of development of the starter bacteria as detectable by the cultural counts, but there was a slightly slower rate of acid formation in the washed-curd cheese. Acid formation ceased soon in the washed-curd cheese because of the exhaustion of the lactose. A combination of *Str. lactis* and *Str. thermophilus* was a better starter than either alone.

4. The occurrence of undesirable fermentations was more pronounced in the washed-curd than in the conventional cheese, due probably to the relatively lower acidity in the former. However, with milk of good quality undesirable fermentations did not appear when the mixed starter was used, and the washed-curd cheese was superior to the other in flavor and body as well as in moisture content.

REFERENCES

(1) GAREY, J. C. Bacteriology of Brick Cheese During Its Manufacture. Ph.D. thesis, University of Wisconsin. 1940.

(2) GAREY, J. C., FOSTER, E. M., AND FRAZIER, W. C. The Bacteriology of Brick Cheese. I. Growth and Activity of Starter Bacteria. JOUR. DAIRY SCI., 24: 1015–1025. 1941.

(3) LANGHUS, W. L. Study of *Streptococcus lactis, Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the Manufacture of Brick Cheese. M. S. thesis, University of Wisconsin. 1938.

(4) Methods of Analysis. A.O.A.C. 4th edition. 1935.

(5) SHAFFER, P. A., AND HARTMAN, A. F. The Iodometric Determination and its Use in Sugar Analysis. II. Methods for the Determination of Reducing Sugars in Blood, Urine, Milk and other Solutions. Jour. Biol. Chem., 45: 365–390. 1920.

(6) STILES, H. R., PETERSON, W. H., AND FRED, E. B. A Rapid Method for the Determination of Sugar in Bacterial Cultures. Jour. Bact., 12: 427–439. 1926.

(7) WILSON, H. L., AND PRICE, W. V. The Manufacture of Brick Cheese. U. S. Dept. Agr. Cir. 359. 1935.

THE BACTERIOLOGY OF BRICK CHEESE. III. THE BACTERIA INVOLVED IN RIPENING[1]

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INTRODUCTION

The function of microorganisms in the manufacture of cheese of all types is well recognized. It has been pointed out (7) that certain lactic acid forming bacteria are used in the manufacture of Brick cheese because of the desirable effect of their acid production upon the nature of the curd and upon subsequent changes during ripening. Also, it is generally accepted that microorganisms are responsible for many of the physical and chemical changes that occur during the ripening of all types of cured cheese. This study was made to ascertain the nature of the bacterial flora in the interior of Brick cheese during ripening. The surface flora, which is believed by Langhus (14) to function chiefly in flavor production, is not considered here.

The literature on the ripening of hard cheese has been reviewed recently by Orla-Jensen (16). Studies have been made on the bacterial flora of cheese similar to Brick, but no reports have been found on the role of bacteria in curing Brick cheese. Studies on Tilsiter cheese (9, 10, 11, 19) showed the lactic acid bacteria predominant throughout the ripening period. During the earlier part the lactic acid streptococci were most numerous, but during the later part the numbers of rod forms multiplied considerably while the numbers of coccus forms diminished. Micrococci usually were present throughout ripening.

Dalla Torre (5) reported that changes in the bacterial flora of Bel Paese cheese were similar to those in Tilsiter. The lactic acid bacteria, particularly *Streptococcus lactis,* predominated throughout ripening, but rod forms increased as the cheese aged. A few yeasts and appreciable numbers of gelatin liquefying cocci also were present.

A close resemblance between the course of chemical and bacteriological changes in Wilster Marschkäse and in related types of cheese (Tilsiter, Bel Paese, and Brick) was shown by the investigations of Boysen (3). Lactic acid streptococci, usually *Str. lactis,* predominated in this cheese during all stages of ripening but rod forms appeared after two to three weeks and increased slowly thereafter. Micrococci were never found after one day by the ordinary plating methods.

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METHODS

The methods of manufacture of Brick cheese have been described previously (6, 7). Analyses were made of cheese manufactured by the conventional and double washed-curd methods from raw and pasteurized milk with *Str. lactis* and *Str. thermophilus* alone and in combination as the starter cultures.

The organisms predominant in the cheese were isolated from the tube cultures prepared for colony counts (as described in 7) and from each temperature of incubation (22°, 37° and 47° C.). To remove the agar one arm of a sterile, small-bore, V-shaped glass tube was inserted to the bottom of the culture tube alongside the agar; then gentle blowing into the other arm forced the agar into a sterile Petri dish, where selected colonies could be picked into litmus milk. Incubation of the isolated cultures was at the incubation temperature of the tube from which the culture had been made. At least one colony of each kind of bacterium present was picked, and the number of colonies selected per kind of organism was in proportion to the total number of its colonies in the tube.

Litmus milk cultures showing growth were examined microscopically; then triplicate subcultures were made in fresh litmus milk, and these were incubated at 22°, 37° and 47° C., respectively. From the microscopic appearance, the action in litmus milk and the temperature of growth it was possible to place all but a few of the cultures in one of eight groups. From these groups 66 representatives were selected for identification, but only 52 are represented in tables 1 and 2. As nearly as possible the cultures of each group represented different lots of cheese. The following observations were made on part or all the cultures:

Minimum and maximum temperatures for growth were determined by incubating cultures growing in buffered glucose carrot-liver extract broth at 10°, 20°, 30°, 37°, 40°, 47°, 50° and 55° C., respectively, and observing microscopically at frequent intervals for the amount of growth. Since the optimum temperature of the lactobacilli is a specific differential characteristic, representatives of each rod culture growing in the above medium were incubated at the three available temperatures apparently nearest their optima. Turbidity measurements were made at frequent intervals with an Evelyn photoelectric colorimeter and the temperature which favored the most rapid rate of growth was considered the optimum.

Methylene blue and salt tolerance were determined by the methods of Yawger and Sherman (20).

Ammonia formation from peptone was tested by the method suggested by Ayers, Rupp and Mudge (2).

Active proteolysis was determined by examination of milk cultures for caseolysis.

Hemolysis. Plates of blood agar were inoculated by the spot method and examined for the type of hemolysis.

Final pH in glucose broth. The medium suggested by Ayers, Johnson and Davis (1) was used and the acidity measured with a Beckman pH meter.

Final pH and titratable acidity in milk. Each organism was inoculated into sterile skim milk and incubated at its optimum temperature. After 11 days the final pH was determined with a Beckman pH meter and the titratable acidity measured by titration with N/10 NaOH.

Lactate utilization. Each culture was inoculated into tubes of a broth medium containing 20 grams peptone, 5 grams yeast extract and 12 grams sodium lactate per liter and incubated at its optimum temperature. Unless a culture showed definite turbidity within one week it was not considered capable of utilizing lactate.

RESULTS

The eight groups of cultures isolated as described above included five species of streptococcus and three of lactobacillus. Sherman's review on streptococci (17) and Orla-Jensen's monograph on lactic acid bacteria (15) provided most of the tests necessary for identification of the cultures, but the scheme given in Bergey's "Manual of Determinative Bacteriology," 5th Edition, was followed in the final classification and naming of each organism. Emphasis was placed on such observations as growth in lactate broth, action in litmus milk, growth in high concentrations of salt, production of acid in milk and growth at different temperatures, since all of these reflect something of the behavior of the organisms in the cheese. Sufficient additional tests were included to permit final identification in all cases except one or two, when two species were separable only by means of a single fermentation test.

Lactic streptococci. *Str. lactis* and *Str. cremoris* are listed by Sherman (17) as the lactic streptococci. These two species were not separable by the means used in this study; but since their differences are largely quantitative their separation was not considered of great importance. The six cultures identified as *Str. lactis* were selected from a group of 545 of this type, and were isolated from five lots of cheese. Cultures A-107, B-162, and E-111 were taken from cheese made with *Str. lactis* starter; the other three cultures were taken from cheese made with *Str. thermophilus* alone. Because the characteristics of *Str. lactis* are well known it was thought advisable to identify only a few of these cultures and place more emphasis on those which have been studied less extensively. All of the 545 cultures considered to be *Str. lactis* exhibited the usual diplococcus form in milk, showed the characteristic reduction of litmus milk before curdling, and grew only at temperatures considered typical for this organism.

Table 1 shows that the characteristics of the cultures identified as *Str. lactis* coincide rather closely with those described by Sherman. During the

TABLE 1

Characteristics of the coccus forms

No. of culture	Growth at			Growth in presence of		NH_3 formed from peptone	Strong reduction	Lactate utilization	Final acidity in			Name of organism
	10° C.	40° C.	45° C.	6.5 per cent NaCl	0.1 per cent methylene blue				Glucose broth	Milk		
									pH	*pH*	*T.A.**	
214	+	−	−	−	+	+	+	−	4.00	4.22	0.85	*S. lactis*
249	+	−	−	−	+	+	+	−	4.25	.....	.....	" "
A107	+	+	−	−	+	+	+	−	4.33	4.12	0.96	" "
B162	+	+	−	−	+	+	+	−	4.39	4.31	0.78	" "
C173	+	+	−	−	+	+	+	−	.....	4.23	0.85	" "
E111	+	+	−	−	+	+	+	−	4.48	4.12	0.85	" "
B80	−	+	+	−	−	−	−	−	4.31	3.90	1.09	*S. thermophilus*
C110	−	+	+	−	−	−	−	−	4.64	3.98	1.03	" "
E38	−	+	+	−	−	−	−	−	5.01	4.00	1.00	" "
E84	−	+	+	−	−	−	−	−	4.28	3.96	1.09	" "
E88	−	+	+	−	−	−	−	−	4.80	3.94	1.08	" "
E146	−	+	+	−	−	−	−	−	4.60	3.94	1.10	" "
A120	−	+	+	−	−	+	−	−	4.77	4.75	0.59	*S. bovis*
B105	+?	+	+	−	−	−	−	−	4.26	4.73	0.56	" "
C86	−	+	+	−	−	−	−	−	4.28	4.86	0.54	" "
D58	−	+	+	−	−	+	−	−	4.30	4.98	0.51	" "
D86	+	+	+	−	−	−	−	−	4.29	4.72	0.59	" "
180	+	+	+	+	+	+	+	+	3.89	4.11	0.96	*S. fecalis*†
240	+	+	+	+	+	+	+	+	4.03	4.48	0.71	" "
A70	+	+	+	+	+	+	+	+	4.27	4.64	0.66	" "
B147	+	+	+	+	+	+	+	+	4.15	4.92	0.53	" "
C50	+	+	+	+	+	+	+	+	4.23	4.64	0.66	" "
C151	+	+	+	+	+	+	+	+	4.28	4.67	0.64	" "
C184	+	+	+	+	+	+	+	+	4.22	4.64	0.66	" "
D185	+	+	+	+	+	+	+	+	4.28	4.59	0.70	" "
E151	+	+	+	+	+	+	+	+	4.20	4.57	0.67	" "
A177	+	+	+	+	+	+	+	+	4.22	4.73	0.92	*S. liquefaciens*‡
C88	+	+	+	+	+	+	+	+	4.10	4.80	0.87	" "
D84	+	+	+	+	+	+	+	+	4.20	4.68	0.83	" "
E152	+	+	+	+	+	+	+	+	4.15	5.04	0.75	" "

* Titratable acidity expressed as per cent lactic acid.
† No cultures proteolytic.
‡ All cultures strongly proteolytic.

early part of ripening this species appeared at times in numbers as high as two or three billions per gram of cheese, and even at the end of ripening often occurred in numbers of several hundred millions. The presence of so many *Str. lactis* organisms during ripening might lead to the belief that they function in the actual curing processes. Although very slight proteolytic action has been demonstrated for this species, most investigators consider acid formation to be its chief function in cheese manufacture and ripening.

Viridans streptococci. *Str. thermophilus* and *Str. bovis* were the viridans streptococci found in Brick cheese and identified as shown in table 1. The six cultures of *Str. thermophilus* represent a group of 119 isolations

from six lots of cheese; the five cultures of *Str. bovis* were selected from a group of 87 found in four lots of cheese. All cultures considered to be the same species agreed very well on the different tests, with the exception of a few minor variations between strains of *Str. bovis*. The main differential features between these two species were the changes in litmus milk and the final acidity produced in milk. In litmus milk cultures *Str. thermophilus* rapidly produced acid and coagulation, then slowly reduced the litmus either partially or completely. *Str. bovis*, on the other hand, grew much more slowly, seldom curdled milk at 37° C. but produced coagulation in four days at 47° C., and had very slight reducing power. *Str. thermophilus* produced much more acid in milk than did *Str. bovis*.

Str. thermophilus seldom was found unless it was added in the starter, and usually was present in numbers no greater than a few thousands per gram of cheese after two weeks, even when there had been two or three billions per gram present at 12 hours. *Str. bovis*, on the other hand, developed frequently from the original flora of the milk, especially in cheese made from pasteurized milk. It usually was found after the first week of ripening in numbers of one to fifty millions, and these numbers remained fairly constant throughout the curing period. The prevalence of this organism in cheese made from pasteurized milk is an indication of its high heat resistance, and helps to explain the high numbers of thermoduric organisms sometimes present in this type of product.

Enterococci. The members of the enterococcus group found in Brick cheese, chiefly in raw milk cheese, were *Str. fecalis* and *Str. liquefaciens*. Table 1 shows results of differential tests on 9 cultures representing a group of 98 isolations of *Str. fecalis* from 7 lots of cheese and on four cultures taken from a group of 19 isolations of *Str. liquefaciens* from 4 lots. The chief difference between the species was proteolysis, *Str. liquefaciens* showing active caseolysis in milk and liquefaction of gelatin, while *Str. fecalis* showed neither. Both organisms utilized lactate readily, a characteristic observed consistently only with these and one of the lactobacillus species.

The function of enterococci in the normal ripening of Brick cheese is not readily explainable. The marked proteolytic action of *Str. liquefaciens* indicates that it may take part in the ripening activities, but its irregular appearance and the unpleasant nature of its products make its action appear undesirable. When this organism grew occasionally to numbers of several millions per gram, a bitter flavor developed in the cheese. Although *Str. fecalis* sometimes was present in Brick cheese in numbers of several hundred thousands to a hundred million per gram, its function in the cheese was not determined. Its proteolytic ability is negligible and usually it was not isolated until all the lactose had disappeared. Hence it probably is not important either in protein breakdown or in acid formation. The ability of *Str. fecalis* to utilize lactate readily probably explains its appearance in consider-

able numbers in the cheese; and its occurrence in the numbers mentioned above certainly must have an effect on the other flora present and on flavor production.

Lactobacilli. Rod-shaped, lactic acid forming bacteria usually were detectable after one or two weeks in Brick cheese made from raw milk, but were not found in significant numbers in cheese from pasteurized milk. The species found were *Lactobacillus casei, Lactobacillus brevis* and *Lactobacillus lactis,* the first two appearing most consistently and the third in only a few instances. Table 2 shows some of the characteristics of the organisms that

TABLE 2

Characteristics of the rod forms

No. of culture	Temperatures of growth in degrees C.			CO_2 formed in milk	Growth in presence of 0.1 per cent methylene blue	Milk curdled in 11 days	Strong reduction	NH_3 formed from peptone	Lactate utilization	Final acidity in			Name of organism
	Minimum	Optimum range	Maximum							Glucose broth	Milk		
										pH	*pH*	*T.A.**	
186	10	28–35	45	–	–	+	+	+	–	4.00	3.94	1.13	*L. casei*
252	10	28–32	45	–	–	+	+	–	–	3.96	3.80	1.28	" "
A131	10	28–37	45	–	–	+	+	+	–	4.09	3.75	1.36	" "
A167	10	28–35	45	–	–	+	+	–	–	4.25	3.76	1.29	" "
B89	10	28–35	45	–	–	+	+	–	–	4.40	3.80	1.24	" "
B153	10	28–35	45	–	–	+	+	–	+	4.21	3.81	1.29	" "
C56	10	28–35	45	–	–	+	+	–	–	4.14	3.95	1.12	" "
C140	10	28–35	45	–	–	+	+	–	–	4.09	3.93	1.14	" "
D123	10	28–35	45	–	–	+	+	–	+	4.10	3.90	1.21	" "
D147	10	28–35	45	–	–	+	+	–	–	3.80	3.90	1.22	" "
D209	10	28–35	45	–	–	+	+	+	–	4.10	3.84	1.20	" "
216	10	28–32	40	+	–	–	–	+	+	4.90	5.14	0.47	*L. brevis*
237	10	28–32	40	+	–	–	–	–	+	4.19	4.81	0.60	" "
253	10	28–32	40	+	–	–	–	–	+	4.03	4.68	0.67	" "
B109	10	28–32	45	+	–	–	–	+	+	4.49	5.15	0.42	" "
C71	10	28–32	45	+	–	–	–	+	+	4.10	4.71	0.61	" "
D162	10	28–32	45	+	–	–	–	–	+	4.10	4.76	0.57	" "
E166	10	28–32	40	+	–	–	–	+	+	4.55	5.25	0.40	" "
B125	20	37–40	50	–	–	+	+?	–	–	3.89	3.63	1.54	*L. lactis*
B162	20	37–40	50	–	–	+	+	–	–	4.10	3.60	1.58	" "
B200	20	37–40	50	–	–	+	+	+	–	4.00	3.61	1.56	" "
C108	20	37–40	50	–	–	+	+	–	–	3.90	3.71	1.51	" "

* Titratable acidity expressed as per cent lactic acid.

belong to these species. The 11 cultures classified as *L. casei* represent a group of 90 isolations of this type. They had an optimum temperature of about 30° C. and curdled milk in two to four days with reduction of litmus. All cultures produced 1.1 to 1.5 per cent lactic acid in milk. The seven cultures of *L. brevis* selected from 44 isolations also grew best at 30° C., but they formed gas and only small amounts of acid in milk, which was not curdled even at the optimum temperature. Carbon dioxide was produced

in fairly large quantities and lactate was utilized by all cultures of this species. *L. lactis* was found in four lots of cheese, but never in very large numbers. Of the fourteen isolations of this organism, the four identified had high optimum (37° to 40° C.) and maximum (50° C.) temperatures, and produced a large amount of acid (1.5 to 1.6 per cent) in milk. At 37° C. these cultures caused rapid coagulation of milk and reduction of litmus in one to three days. The main differential features between the three lactobacillus species were the optimum and maximum temperatures of growth, carbon dioxide formation in milk, coagulation of litmus milk with reduction of the litmus, and the total acidity produced in milk. Considerable variation was noticed between the different strains of certain species in such tests as lactate utilization and the production of ammonia from peptone. None of them was able to grow in sterile skim milk containing 0.1 per cent methylene blue.

There are many references in the literature to the role of lactobacilli in cheese ripening (3, 8, 9, 10, 12, 13, 14, 16). Certain of these organisms have been shown to be able to produce significant proteolytic changes in cheese when they are present in large numbers. Hence, the detection of *L. casei* in numbers as high as 100,000,000 per gram indicates that this organism is important in the proper curing of Brick cheese. It is doubtful whether the other species of lactobacilli found in this study have much importance in the ripening, because their numbers were much smaller than were those of *L. casei* and their appearance more inconsistent. In fact, the gas production of *L. brevis* probably is undesirable in the cheese.

Table 3 summarizes the results of all the tests. It is believed that these tests, when accompanied by the usual observations of the microscopic appearance of the organisms and their action in litmus milk, yield sufficient information to serve as a scheme for the differentiation and identification of the bacteria that occur in Brick cheese.

Anaerobic spore forming bacteria. Throughout this investigation, at all seasons of the year, it was observed that agar culture tubes incubated at 22° and 37° C. frequently showed gas formation, at times sufficient to break the agar into small pieces. This condition prevailed only when the cheese had a relatively low acidity (above pH 5.20) and was shown to be due to anaerobic spore formers. These organisms grew readily in cheese made with *Str. thermophilus* alone because of its lower acidity. Because of the effect on the acidity, the use of pasteurized milk and the washed-curd manufacturing method also favored anaerobic gas forming bacteria. Cheese made under these conditions frequently showed cracks and splitting due to excessive gas formation, and a very undesirable fermented flavor after two to four weeks. These organisms have been reported previously in milk (7) and in cheese (3, 4, 7, 11). Since they are very resistant to heat it would appear that the best means of controlling them is to prevent, as far as possible, their entrance into the milk and to insure proper salting and acid production in the cheese.

TABLE 3

Differential cultural characteristics of the organisms that comprise the bacterial flora of Brick cheese

Name of organism	Temperature ranges of growth—degrees C.			Milk curdled in 11 days	Growth in presence of 0.1 per cent methylene blue	Active proteolysis	Strong reduction	Lactate utilization	CO_2 formed in milk	NH_3 formed from peptone	Final acidity in		
	Minimum	Optimum	Maximum								Glucose broth	Milk	
											pH	*pH*	*T.A.**
S. lactis	8–10	30–35	37–40	+	+	−	+	−	−	+	4.5–4.0	4.3–4.1	0.75–0.95
S. thermophilus	15–20	37–40	47–52	+	−	−	−	−	−	−	4.8–4.2	4.0–3.9	1.00–1.10
S. bovis	10–15	37–45	50–52	+	−	−	−	−	−	±	4.5–4.2	5.0–4.7	0.50–0.60
S. fecalis	8–10	30–40	45–50	+	+	−	+	+	−	+	4.3–3.8	5.0–4.5	0.50–0.80
S. liquefaciens	8–10	30–40	45–50	+	+	+	+	+	−	+	4.2–4.1	5.0–4.5	0.75–0.95
L. casei	8–10	28–35	40–45	+	−	−	+	±	−	±	4.4–3.9	4.0–3.6	1.10–1.50
L. brevis	10–15	28–35	40–45	−	−	−	−	±	+	±	4.9–4.1	5.3–4.6	0.40–0.70
L. lactis	18–20	37–40	50–52	+	−	−	+	±	−	±	4.1–3.9	3.7–3.6	1.50–1.60

* Titratable acidity expressed as per cent lactic acid.

Defects due to bacteria of the coli-aerogenes group will be discussed in a following paper.

DISCUSSION

As in Tilsiter cheese (8), the normal ripening of Brick cheese apparently depends upon the development of a desirable bacterial flora with its members occurring in the proper sequence and proportion. The lactic acid starter bacteria which are added to the milk function chiefly in acid formation. Not only is the acid necessary to obtain the proper drainage of whey from the curd but also to prevent the subsequent occurrence of undesirable fermentations. These organisms persist in the cheese for varying periods during ripening, but probably do not play an important role in the curing.

Most of the ripening changes that are due to bacterial action depend upon the development of the desirable organisms from the natural flora of the milk. Probably the most important of these organisms is *Lactobacillus casei* to which has been ascribed much of the proteolysis and flavor production in other types of cheese. This organism always developed in substantial numbers in cheese made from raw milk, and if the acidity was favorable its growth was accompanied by the formation of a desirable body and flavor. However, when the milk was pasteurized, extensive development of *L. casei* was infrequent, and in its absence a product with a poor body and little or no flavor resulted. Thus, it may be said that the presence of *L. casei* is necessary for the normal ripening of Brick cheese. Other rod forms have been observed, but they appeared inconsistently and in relatively small numbers, hence they probably function little in the normal curing processes.

Of the other organisms present in the milk, *Str. bovis, Str. fecalis, Str. lactis, Str. thermophilus* and *Str. liquefaciens* were found at times in significant numbers. None of these except *Str. lactis* appeared consistently. *Str. fecalis* and *Str. liquefaciens* can utilize lactate as a food and can grow after the available sugar has disappeared. *Str. fecalis* is not proteolytic, hence probably functions only in flavor development. *Str. liquefaciens,* however, is strongly proteolytic and its action is accompanied by the formation of undesirable bitter flavors as shown in the few instances in which this organism developed in considerable numbers. It is believed that this species corresponds to Gorini's *acidoproteolytic* cocci and the gelatin liquefying cocci reported in the obligate ripening flora of Tilsiter (9) and Bel Paese (18) cheese. However, the effects of its growth in Brick cheese are such that doubt is cast upon its desirability. Anaerobic spore forming organisms invariably were present in the milk and developed when the acidity was low. They produced quantities of gas sufficient to split the cheese and caused an undesirable fermented flavor.

Completion of a study of this type makes it evident that certain phases of the work should be expanded and clarified. Closer examination of the physiological activities of the groups of organisms that appear during ripening should give a clearer understanding of their specific role in curing.

This would also involve the addition of these organisms to the cheese and following the course of their activity at intervals. It is possible that the addition of certain of the ripening organisms in small amounts with the starter might hasten the curing and make it possible to obtain a better product in a shorter time.

It should be emphasized here that most of the milk used in this investigation was of good quality. Perhaps if milk of poorer quality were used a different picture of the bacterial flora would be obtained. It is believed that the use of milk with a more varied and numerically greater initial flora than that employed in this study would demonstrate more clearly the role of lactate fermenting bacteria in the ripening processes, as well as the function of some of the other organisms.

SUMMARY

Examination of 1016 cultures isolated at different stages of ripening from 18 lots of Brick cheese yielded the following results:

1. *Str. lactis* was the predominant organism throughout the ripening of cheese in which it was used as part or all of the starter. Its numbers rose steadily to one to three billions per gram during the first one to four days, and declined again after three to four weeks. It also increased slowly in cheese to which it had not been added and reached numbers of several hundred millions per gram after one to two weeks.

2. When *Str. thermophilus* was used as the starter its numbers increased rapidly to a maximum of one to two billions per gram at twelve hours, then after one to two weeks they decreased sharply and the organisms seldom were found later in the ripening period.

3. Lactobacilli were found in the raw milk cheese after the first one or two weeks, but they seldom appeared in cheese made from pasteurized milk. *L. casei* occurred most frequently and in numbers up to ten to one hundred millions per gram; *L. brevis* was found less consistently than *L. casei* and usually in much smaller numbers; *L. lactis* appeared in a few of the samples after three to four weeks in insignificant numbers.

4. *Str. liquefaciens* was found in six of the lots of cheese after two to three weeks in numbers sometimes as high as 10,000,000 per gram. A few instances of bitter flavor probably were due to excessive numbers of this organism.

5. *Str. bovis* and *Str. fecalis* usually appeared in the cheese after one to two weeks, and ranged in numbers from a few thousands to as high as one hundred millions per gram. These organisms were most prevalent in cheese made from pasteurized milk.

6. Late gas formation by anaerobic spore forming bacteria occurred in all cheese in which the pH did not drop below 5.3 during the first three days of ripening. This condition was most pronounced in cheese made with *Str. thermophilus* starter by the washed-curd method from pasteurized milk.

The main function of the starter bacteria is a rapid and steady production of lactic acid during manufacture. The most important organism in bringing about the changes in body within the cheese during ripening is *L. casei,* and this organism also contributes to the flavor.

REFERENCES

(1) AYERS, S. H., JOHNSON, W. T., JR., AND DAVIS, B. J. The Thermal Death Point and Limiting Hydrogen Ion Concentration of Pathogenic Streptococci. Jour. Infect. Dis., **23**: 290–300. 1918.

(2) AYERS, S. H., RUPP, P., AND MUDGE, C. S. The Production of Ammonia and Carbon Dioxide by Streptococci. Jour. Infect. Dis., **29**: 235–260. 1921.

(3) BOYSEN, O. Über die Mikroflora des Wilster Marschkäses und ihre Entwicklung während des Reifens. Milchw. Forsch., **18**: 150–168. 1936.

(4) CSISZÁR, J. Das Verhalten der anaeroben Blähungserreger des Schmelzkäses der Hitze, Säure und den Konservierungsmitteln gegenüber. Milchw. Forsch., **15**: 201–227. 1933.

(5) DALLA TORRE, G. On the Microbial Flora of Bel Paese. 8th World's Dairy Congress Proc. London, pp. 462–463. 1928.

(6) FOSTER, E. M., GAREY, J. C., AND FRAZIER, W. C. The Bacteriology of Brick Cheese. II. Comparison of Washed-curd and Conventional Methods of Manufacture. JOUR. DAIRY SCI. **25**, 4: 313–322. 1942.

(7) GAREY, J. C., FOSTER, E. M., AND FRAZIER, W. C. The Bacteriology of Brick Cheese. I. Growth and Activity of Starter Bacteria. JOUR. DAIRY SCI., **24**: 1015–1025. 1941.

(8) GRATZ, O. Beitrag zur Frage der Hartkäsebereitung aus dauererhitzter Milch mit Bakterienreinkulturen. Süddeut. Molkerei Ztg. **52**: 1269–1272. 1931.

(9) GRENZ, A. Zur Mykologie des Tilsiter Käses. III. Mitteilung. Die Mykologie fehlerhafter Tilsiter Käse. Milchw. Forsch., **14**: 140–163. 1932.

(10) GRIMMER, W., AND ARONSON, E. Zur Mykologie des Tilsiter Käses. II. Mitteilung. Milchw. Forsch., **4**: 538–546. 1927.

(11) GRIMMER, W., AND PRINZ. Forsch. auf dem Jrb. des Milchw. und des Molkw., **1**: 377. 1921. (Cited by Grimmer and Aronson, 1927.)

(12) HANSON, F. E., SPICER, D. W., AND PRICE, WALTER V. Observations on the Splitting of Brick Cheese. JOUR. DAIRY SCI., 21: 433–444. 1938.

(13) LANE, C. B., AND HAMMER, B. W. Bacteriology of Cheese. II. Effect of *Lactobacillus casei* on the Nitrogenous Decomposition and Flavor Development in Cheddar Cheese Made from Pasteurized Milk. Iowa Agr. Expt. Sta. Res. Bul., 190. 1935.

(14) LANGHUS, W. L. The Surface Ripening of Brick Cheese. Ph. D. thesis. Univ. of Wis. 1942.

(15) ORLA-JENSEN, S. The Lactic Acid Bacteria. Copenhagen. 1919.

(16) ORLA-JENSEN, S. Die Reifungstheorie der Hartkäse. Milchw. Ztg. Wien (July, No. 2), 33–37. 1939.

(17) SHERMAN, J. M. The Streptococci. Bact. Rev., **1**: 3–97. 1937.

(18) SHERWOOD, I. R. The Bacterial Flora of New Zealand Cheddar Cheese. Jour. Dairy Res., **10**: 426–448. 1939.

(19) STÖLTING, J. Über die Streptokokken des normal reifenden Tilsiter Käses. Milchw. Lit. Nr. 106–117. p. 42. (Abstr.) 1936.

(20) YAWGER, E. S., JR., AND SHERMAN, J. M. *Streptococcus cremoris.* JOUR. DAIRY SCI., **20**: 205–212. 1937.

THE BABCOCK TEST; A REVIEW OF THE LITERATURE[1]

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The Babcock method for estimating the fat content in milk was one of the outstanding contributions to Dairy Science. When this method was 21 years old, Russell (181) stated that it was the only test used in the United States and Canada and was introduced into Argentina and South America and accepted in Australia and New Zealand before it was adopted in this country.

The Babcock test has served the dairy industry for more than 50 years without significant changes in technic and procedure. However, the American Dairy Science Association in annual meeting in 1938 recommended a study of this method with the objective of evaluating its accuracy. This recommendation was made because the dairy industry had questioned the progressiveness of the method in comparison with other important technological advances. A committee of the American Dairy Science Association is studying the procedure of the Babcock test and a detailed report will be forthcoming.

VOLUMETRIC METHODS PRECEDING THE BABCOCK TEST

Europe

The first record of a volumetric method of estimating fat in milk is that of Marchand (140, 141). His method was based on the assumptions that small amounts of sodium hydroxide had no serious effect on the fat, and that butterfat was soluble in ether and insoluble in water. He devised two test bottles called lactobutyrometers. One was of 10–11 mm. diameter throughout and divided into three parts. The lower one-third was calibrated for the milk, the middle for the ether, and the upper portion for the alcohol, the remaining volume of the tube being used for mixing. The upper part of the tube was calibrated in tenths and the very uppermost in hundredths of a cubic centimeter. The other instrument was somewhat different, having a lower bulb of 25 mm. diameter, 110 mm. in length and with a capacity of 53–54 cc. The detached graduated tube was calibrated, of 8 mm. diameter and had a capacity of 6 cc. The calibrated portion of the tube was graduated in tenths and arbitrarily referred to as degrees. The technique of Marchand's method consisted of adding 10 cc. of milk, followed by a drop or two of sodium hydroxide. Next the 10 cc. of ether were added, the tube stoppered and the contents well mixed. This was followed by the addition of 10 cc. of alcohol of specific gravity 86–90, and the contents carefully

[1] Published with the approval of the Director of the Vermont Agricultural Experiment Station.

mixed. The tube was placed vertically in a water bath at 40°[2] until it cooled to 30°. When the ether-fat layer had separated fully, the number of divisions on the graduated neck were read as degrees, and converted by a formula to weight of fat in grams. In a later paper (142) he gave more detail of how he arrived at the formula used. He stated that the amount of fat held below the ether-fat layer was 0.126 gm. per 10 cc. of milk, and the amount of ether and fat that separated was a constant. The formula he submitted was:

$$X = (N \times 2.33) + 12.6$$

X = value sought.
N = No. of degrees on the instrument.
2.3 = weight in grams of fat for each degree on the tube.
12.6 = grams of fat that remained in the aqueous phase per liter.

It is evident that Marchand's method is empirical. He cited the statement of Quevenne that milk contained an average of 34 grams of fat per liter.

Marchand's work stimulated more research on volumetric methods during the ensuing years. Bouchardat and Quevenne (34) reported comparisons of Marchand's with what was termed a chemical method but the results were divergent.

Schmidt (186) used five different lactobutyrometers, two were constructed according to Marchand's original instrument, one was made from a burette, and two were modified to provide more room for mixing the milk and reagents. He modified Marchand's reagents by using 3–5 drops of 5 per cent acetic acid instead of sodium hydroxide, used 90–92 per cent alcohol and read the tests at 20° instead of 40°. Forty estimations were made and compared with the gravimetric method of Tollens. The gravimetric method gave higher results. From these results he suggested another formula for the Marchand method so that the results would agree more closely with the gravimetric method. Applying this new formula to the 40 tests previously made, the two methods agreed within 0.1 per cent in 26 cases. He further stated that the Marchand lactobutyrometer did not give reliable results for adulterated milk.

Schmidt and Tollens (187) compared Marchand's with a gravimetric method on 30 samples of milk and they reported differences of 0.2 per cent and the greatest number did not agree within 0.1 per cent. They observed that separation of the fat was slower with alcohol of 86–90 per cent as compared to 91–93 per cent alcohol, and that the concentration of alcohol influenced greatly the estimations in milk with a high fat content. Using 92 per cent alcohol and reading the tests in a water bath at 20°, the results were compared with the gravimetric method and another formula was submitted. The application of this formula to the 30 samples of milk previously esti-

[2] All temperatures in this paper are in degrees Centigrade.

mated, resulted in three-fourths of the comparisons agreeing within 0.05 per cent. Krämer and Schulze (127) compared the Marchand lactobutyrometer with a gravimetric method and obtained results averaging 0.32 per cent lower with the former method.

Tollens and Grote (215) compared Marchand's with a gravimetric procedure and concluded that the volumetric method showed reliable results for all practical purposes; however, their limited data indicated the volumetric yielded lower results than the gravimetric method. They emphasized the importance of shaking the contents vigorously until the ether and milk formed a uniform mixture for the moment. The tests were kept in a water bath at 40° for 5–10 minutes and the ether-fat layer read and the per cent fat obtained from a prepared table. The reagents and milk were placed in the lactobutyrometer with pipettes. No marked differences were revealed when the tests were read at 20° and at 40°.

Schmoeger (188) tested 110 milks with the gravimetric and the Marchand method using the butyrometer modified by Tollens and found that the latter gave results 0.1 per cent lower. Using 92 per cent alcohol, the volumetric test yielded the highest results, but both 90 and 92 per cent alcohol contributed results lower than the gravimetric method. He stated that the modified method submitted by Schmidt and Tollens (187) gave more accurate results when 3 drops of 15 per cent potassium hydroxide were used and separation of the ether took place at 20°, otherwise, their method gave results 0.2 per cent too low.

Dietzsch (53) compared Marchand's with Soxhlet's aerometric method and reported agreement within 0.5–0.1 per cent on numerous samples. He recommended leaving the butyrometers in the water bath at 38° for 10–15 minutes, and emphasized the importance of mixing reagents and milk, otherwise, reliable results were not obtained. Marchand's method was used in the Anglo-Swiss condensed milk laboratory. Peter (169) used essentially the same procedure and obtained close agreement on the majority of 84 comparisons with a gravimetric procedure. Sjostrom (201) modified Marchand's method for milk containing 1.78 to 4.31 per cent fat.

Liebermann (133) contributed a method whereby 50 cc. of milk were placed in a large cylinder and 5 cc. of sodium hydroxide and 50 cc. of ether were added. The contents were shaken from one to two minutes to a homogenous yellow color. After allowing the contents to settle for 10–15 minutes, a 20-cc. aliquot was evaporated on a water bath at 40–50°, then dried for 15 minutes at 100–105°. The air-free fat was measured in a carefully calibrated flask and converted to per cent by weight, assuming a constant value for the specific gravity of butterfat. Close agreement was obtained with Hoppe-Seyler's gravimetric procedure on five samples of milk.

Cronander (44) constructed a test bottle of 200–250 cc. with two glass tubes, one calibrated and the other to carry hot water to force fat into the

graduated neck, 10 cc. of potassium hydroxide were added to 100 cc. of milk and thoroughly shaken with 30 cc. of ether. The flasks were allowed to stand quietly for 30 minutes, then placed in a water bath at 60–65° for 1–1½ hrs. and at 80° for 30 minutes. The fat was forced into the measuring tube and the estimated volume converted to per cent from a table. Close agreement was obtained with Soxhlet's method.

Schmid (185) contributed a method that involved the use of a 50-cc. tube, graduated in tenths. Ten cc. of milk or 5 cc. of cream were measured into this tube and 10 cc. of concentrated hydrochloric acid added and the mixture heated until a dark brown color appeared. The contents were cooled and 30 cc. of ether added and mixed. When the separated ethereal layer was clear, 10 cc. was pipetted into a tared dish and evaporated over a water bath at 100°. The results checked within 0.1 per cent of the gravimetric procedure and required about 15 minutes. The name of the Helvetia Milk Condensing Company, Highland, Illinois, appeared at the end of this paper. Stokes (211) modified this method slightly and reported excellent results and Hill (96) reported satisfactory results with a slight modification of Schmid's (185) method. Léze (132) submitted a similar method whereby milk was heated to near boiling with two volumes of hydrochloric acid, then saturated with ammonium hydroxide and the contents became clear as the fat rose to the surface. He suggested the use of the centrifuge to speed the separation of the fat.

The volumetric methods cited up to this point have been empirical, rather time-consuming and cumbersome. De Laval (54) in 1885 patented a method (55, 56) for estimating the fat content of milk. He was the first to describe the use of the centrifuge in a volumetric fat determination. He used a disc that revolved in place of the bowl in his centrifugal milk separator. The original method called for the use of an equal amount of milk and a mixture of 20 parts concentrated acetic and one part of sulphuric acid. Ten cc. of milk at 15° were placed in a boiling tube containing 10 cc. of the acid mixture, heated to 80° for 15–18 minutes with occasional agitation. Next the boiling tubes were placed in water at 60° until the contents reached this temperature. The tubes were well mixed and some of the contents poured into small cups; next the small graduated test bottles were filled by pushing them into these cups and the excess flowed through the top into a suitable receptacle. The centrifuge was heated with hot water or steam to keep the temperature above 50° and whirled at 6,000 r.p.m. for 3–5 minutes. The test bottles were taken from the centrifuge and read while the small hole at the top was closed with the finger. These bottles were empirically calibrated in 0.1 per cent divisions to check with the gravimetric procedure. Tests could be read to 0.02 per cent accuracy. De Laval named this the lactocrite test. Eugling and Klenze (66) stated that the lactocrite procedure was cheaper and checked closely with Soxhlet's method. Sebelien (192) re-

ported close agreement between the lactocrite and a gravimetric procedure; similar results were reported by Soxhlet (205), while Frahm (82) stated that the lactocrite required more expensive equipment. Nilson (160) supplied a table of corrections so that the method was more nearly accurate on low testing milk. Blyth (33) and others (128, 189) reported close agreement between the lactocrite and the gravimetric procedure, while Soxhlet (205) reported similar results with his aerometric method. Faber (67) reported close agreement between the lactocrite and a gravimetric method and also lauded the simplicity of the former. Gerber's method (85, 86) apparently had not received extended experimental attention beyond a description of the butyrometer and the reagents used and did not assume its present form until 1895 (22). Barthel (22) has described the lactocrite method in detail. Oven (163) has contributed a review of the literature of some of the methods used in Europe.

United States

While European research workers were seeking to develop rapid methods of estimating fat in milk, chemists in America were engaged in similar activities.

Short's method (197) consisted of saponifying the fat in 20 cc. of milk with 10 cc. of an alkali that contained 250 grams of sodium hydroxide and 300 grams of potassium hydroxide dissolved in 1809 grams of water. The saponification process continued for two hours. The insoluble fatty acids were obtained by boiling one hour with 10 cc. of an acid mixture consisting of equal parts of sulphuric and acetic acids. Tubes were filled within one inch of the neck and allowed to stand in hot water without boiling. The fat column was estimated in millimeters and from the weight of the milk and the specific gravity of the insoluble fatty acids, the per cent of fat was calculated. Short (197) submitted analysis on a number of samples of milk and the results were approximately 0.04 higher than the gravimetric method. Results obtained by his assistants did not agree so closely. Manns (139) compared Short's method with a gravimetric procedure and the greatest variation was 0.23 per cent. Farrington (70) on a few samples found that Short's method yielded results that were too high and similar results were reported by Frear and Holter (83). Myers and Magruder (153) reported close agreement between Short's, the lactocrite and the lactobutyrometric methods.

Parsons (165) criticized Short's method for not being accurate for skimmilk and buttermilk containing less than 0.50 per cent fat. He developed a method whereby 10 cc. of cream were measured into a 250-cc. flask with the addition of 10 cc. of 50 per cent sodium hydroxide, plus 5 cc. of 95 per cent alcohol containing one ounce of castile soap per gallon, and finally 50 cc. of gasoline. The flask was shaken vigorously for a few seconds and five or six times at equal intervals within the next half hour. If the fat-gasoline

layer did not separate, repeated additions of the alcoholic soap solution were made until separation occurred. Twenty-five cc. of the fat-gasoline material were placed in a flask to evaporate, and the residue dried at 245–255° for 30 minutes. The fat was collected in a calibrated tube cooled to the first evidence of solidification, held in the hand for a moment and estimated to the uppermost meniscus; this reading was converted to per cent fat from a table. Close agreement with the gravimetric and with Soxhlet's method was obtained on milk, cream, skimmilk and buttermilk. It was estimated that 40 analyses could be made daily. Professor E. H. Farrington collaborated in this work. Collier (40) experienced difficulty with this method as originally described, so he used 15 cc. of the alkali and 10 cc. of the alcoholic soap solution. Unskilled workers obtained results that averaged 0.03 per cent from the gravimetric method.

Failyer and Willard's (69) method is similar to that of Schmid (185). They used hydrochloric acid and milk in a tube similar to that devised by Short (197), except the graduated neck was of smaller diameter. The test bottle was 10 inches long with the graduated portion being 3½ inches long and 4–5 mm. in diameter. Ten cc. of milk were added to the bottle and heated to near boiling over a flame with 8 cc. of acid carefully added. The contents were heated cautiously for about five minutes with careful agitation and cooled. Next 15–20 cc. of gasoline were added and the bottle gently agitated to dissolve the fat. The bottles were placed in an inclined position in a boiling water bath and air blown into the tube to evaporate the gasoline. Water was added to the base of the neck and the bottle whirled (time not stated) a few times and allowed to stand in a boiling water bath for a few minutes. Boiling water was added to bring the fat into the graduated neck. Readings were made in the water bath. Single analyses require about 25 minutes. It was suggested that the test bottles could be graduated in fractions of a cubic centimeter with 0.87 as the specific gravity of the fat. The results checked closely with a gravimetric procedure used by Babcock.

Cochran (42) contributed a method whereby milk was heated at about 100°, for 10–15 minutes in a special flask with 5 cc. of a mixture of equal parts of acetic and sulphuric acid and until the contents assume a coffee color. The flasks were cooled and 4 cc. of ether added; boiling was continued for 10–15 minutes. The fat was poured into a flask with a side tube and boiling water added to bring the fat into the graduated neck. Estimations were made at 65.5° and converted to per cent with the aid of a specially prepared table. Caldwell (36) reported a preference for this method over those of Parsons (165), Short (197) and Failyer and Willard (69), while Magruder (137) obtained variable results with this method and Farrington (70) and Frear and Holter (83) reported close agreement on a few samples. This method was standardized to agree with the Adams gravimetric method and was used in about 50 creameries in southwestern Pennsylvania. It was claimed that 60 determinations could be made in 2–3 hours.

Patrick (166) released details of the Iowa test in June, 1889, through the Farm Journal Homestead. This test utilized an acid mixture to dissolve the solids-not-fat, consisting of 9 volumes of 90 per cent acetic acid, 5 volumes of concentrated sulphuric and 2 volumes of concentrated hydrochloric acid and the solution saturated with sodium sulphate. The test bottles had a bulb with a capacity of 21 cc. and two-thirds the distance from the bottom a small orifice was located, 1 to 1½ mm. wide, and closed with a rubber band. The graduated neck was 6 to 6½ cm. long, being carefully calibrated to 1 cc. capacity. The table above the neck was 14½ cm. long. A 10.8-cc. pipette was used and later changed to hold 10.4 cc. to allow the method to check more closely with the gravimetric procedure. After adding the acid, the bottles were placed in a hot sand bath and allowed to boil slowly for 4–6 minutes. The fat rose and the bottles were again allowed to boil gently for 5–8 minutes to clarify the fat. The tests could be read directly from the sand bath, but more accurate results were obtained by immersing the bottles in water at 140° for 10–15 minutes and for more exact results immersion for 30 minutes was recommended. The fat column was estimated from the extreme lower to the extreme upper part. Farrington (70, 71) reported close agreement with the gravimetric method on a few samples using this method. Patterson (168) found that the methods of Beimling (5) and Patrick (166) agreed closely with the gravimetric method.

Leffmann and Beam (130, 131) submitted specifications for a test that they claimed was devised in 1889. Test bottles of 30 cc. capacity with calibrated necks containing divisions equivalent to 0.1 per cent fat were used. The test was conducted by measuring 15 cc. of milk into the bottle followed by 3 cc. of a mixture of equal parts of amyl alcohol and "strong" hydrochloric acid, mixed well, filled nearly to the neck with concentrated sulphuric acid and further mixed with a rotary motion. The bottles were filled to about the zero point with diluted sulphuric acid, and whirled in a centrifuge for 1–2 minutes. The estimations were made with dividers, allowance being made for the meniscus. Results agreed within 0.1 per cent of the Adams method.

A test was described by the Vermont Agricultural Experiment Station (5) that involved the use of the same kinds of reagents as the methods of Leffmann and Beam (130, 131) and of Gerber (85); but the glassware in the Gerber method was different from the other two. The centrifuge was patented by H. F. Beimling, an Austrian chemist living in Philadelphia, Pennsylvania, and was sold by the Creamery Package Manufacturing Company (43). About 200 of these centrifuges were sold from 1890–1902. Special glassware was devised by the Vermont Station (5). It was stated that 75 per cent of the results agreed within 0.1 per cent of the Adams gravimetric method. Magruder (137) expressed a high regard for what he called the Beimling method (5). Russell (181) stated that Babcock devel-

oped a method similar to Soxhlet's aerometric procedure whereby the milk was made strongly alkaline and the fat was extracted with ether. The ether was evaporated and the fat estimated in bottles similar to those used in the regular Babcock test and without transferring the contents. This method was discarded because the results did not check in all instances with the gravimetric method.

Thus, the literature reveals a background of much research on volumetric methods of estimating fat before Babcock made his contribution to dairy science.

STANDARDS OF ACCURACY FOR BABCOCK GLASSWARE

When Babcock (10, 11) contributed his test, there were two volumetric dimensions in use, namely, the Mohr cubic centimeter of one gram of water at 15°, and the true centimeter of water at 4°. The use of both created considerable confusion among manufacturers of glassware and state officials. Holland (103) recognized the difficulties involving the use of two volumetric units and pioneered in obtaining the adoption of one fundamental standard for calibrating Babcock glassware. He wrote to three prominent manufacturers and found that the Wagner Glass Works used one cubic centimeter as equal to 13.59 grams of mercury at 15.5°, which is the Mohr unit and the Kimball Glass Company used the true cubic centimeter and calibrated with mercury, specific gravity 13.5463 at 20°. The Emil Greiner Company (64) reported to Holland (103) that they made the first test bottles for Babcock who told them to graduate the neck of 2 cc. capacity into 50 parts with each 5 parts to represent 1 per cent butterfat and they used the Mohr unit. Babcock (12) stated that the neck of the 10 per cent milk test bottle represented a volume of 2 cc. that is equivalent to 2 grams of water or 27.18 grams of mercury, specific gravity 13.59. The temperature was not stated but it was assumed that 15.5° was intended.

Holland (103) realized the necessity of having one absolute unit of volume so that test bottles and pipettes could be calibrated on a scientific basis. Consequently he had a recommendation made to the Bureau of Standards at Washington, D. C., which was adopted, that the true cubic centimeter of water at 4° and weighing 0.998877 be the unit of graduation for all Babcock glassware. In addition he made recommendations for methods of calibrating and for standards of accuracy of glassware, approved by Babcock, which appeared in the Association of Official Agricultural Chemists (8) (A.O.A.C.) in 1909 and have remained unchanged, with minor exceptions, to the present time. In this connection it should be stated that Professor Hunziker (116) rendered monumental service to the dairy industry from 1910 to 1926 in the capacity of Chairman of the Committee on Official Methods of Testing Milk and Cream for Butterfat in the Official Dairy Instructors Association (O.D.I.A.), now the American Dairy Science Association (A.D.S.A.). This committee called a meeting in Washington, D. C., in 1911 with members of the O.D.I.A., the U. S. Bureau of Dairying, the

U. S. Bureau of Standards and manufacturers of glassware. Standard specifications for Babcock glassware were formulated and adopted and pronounced official and published by the A.O.A.C. (8) and by Standard Methods of Milk Analysis (210) (S.M.M.A.). Hunziker's committee also drafted a standard procedure for the Babcock test that has been universally adopted with or without minor modifications and was published by the A.D.S.A. (3) in 1917. These refinements in glassware and procedures marked a distinct milestone of progress since 1902 when the A.O.A.C. (8) stated that due to the wide publication of Babcock's method it was not deemed advisable to publish the detailed procedure. Andrews (4) advocated state control and supervision of Babcock testing equipment and procedures.

In view of the fact that this discussion will involve the manipulation of the various technics of the test, it was thought advisable to state the present procedure for the Babcock test so that persons in countries where it is not common may more easily follow the context. The procedure for milk in the A.O.A.C. (8) in 1940 is outlined as follows:

1. Transfer 18 grams of milk with a 17.6-cc. pipette to the test bottle, blowing out the last drops after free outflow has ceased.

2. Add 17.5-cc. sulphuric acid at 15–20° (sp. gr. 1.82–1.83 at 20°) preferably not all at one time, pouring it down the side of the bottle neck to wash all traces of milk into the bottle.

3. Shake bottles until the curd has disappeared, place them in the centrifuge and counterbalance each bottle.

4. After the proper speed has been attained, whirl for five minutes. Add soft water at 60° or above to fill the bulb of the bottle. Whirl two minutes. Add hot water until the liquid fat approaches the top graduation on the neck. Whirl one minute longer at 55–60°.

5. Immerse the bottles in a water bath maintained at 55–60° to the level of the top of the fat column until the column is in equilibrium and the lower fat surface has assumed final form.

6. Remove the bottle, wipe it, and measure the fat column with calipers from its lower surface to the highest point of the meniscus. The fat column at this time should be translucent, of golden yellow or amber color, and free from suspended particles.

The procedure for cream is essentially the same in principle, except that it is weighed instead of measured by volume.

The discussion to follow is a summary of the technics that have been used in the sequence of conducting the Babcock test.

TECHNICS

Preparation and Care of the Sample

The A.O.A.C. (8) in 1908, 1916, 1920 and the A.D.S.A. (3) in 1917 emphasized the importance of careful mixing until a homogeneous sample

was obtained. Since 1925 the A.O.A.C. (8) specified in addition that the sample should be tempered to 20°, and if lumps of cream prevailed, heat to 38° and cool to 20° before pipetting; similar recommendations were made by S.M.M.A. (210) in 1928, while the A.D.S.A. (3) in 1917 specified that abnormal samples should be heated to 38–49°, mixed thoroughly and pipetted at once.

Babcock (10, 11) emphasized the importance of securing a representative sample by pouring from one container to another at least three or four times, and milk with a cream layer should be given this treatment until no lumps appeared on the surface, but he cautioned against excessive agitation. Farrington (72) and others (35, 52, 63, 80, 84, 91, 135, 143, 159, 206, 234) recommended pouring milk from one vessel to another at least three or four times. Crowe and Davis (45) recommended slow pouring at 16–21° to avoid excessive incorporation of air, while Ball (21) supplemented the pouring process by shaking the samples at 18–24° and if necessary, heating to disperse the fat and cooling to this temperature before sampling. Smith (202) gently rotated the sample and poured it through a fine wire sieve. Dahlberg (46) specified heating and sampling preserved composite milks at 38–43° because they could not be cooled at 21° without some destabilization of the fat; later Dahlberg and Powers (49) suggested sampling at 32.2–37.7°. Hunziker (115) and others (157, 212) stated that partially churned milks and those that had a dried cream layer should be heated slowly at 29–43° and mixed carefully to avoid further injury to the emulsion. Kerr (124) stated that milk should be pipetted immediately after shaking to obtain an accurate sample. Caldwell and Herreid (37) sampled milks at 4.4, 21.1, 37.8 and 60° and found that those sampled at 60° tested 0.08 per cent lower than at 4.4°, while at 37.8° the test was 0.04 per cent lower than at 4.4°. Liverance (135) and others (35, 52, 91, 113, 122, 164, 212) suggested sampling temperatures ranging from 10–27°.

Composite sampling was conceived of as the dairy industry grew in commercial prominence with demands for increased efficiency through the use of labor saving devices. Patrick (167) first proposed the use of composite samples in this country. Babcock (13) suggested that a satisfactory composite could be obtained by using a test bottle twice the usual size for each patron and measuring into this bottle 5 cc. of milk daily for seven days. Shutt (199) suggested a similar procedure by taking 2.93 cc. of milk daily for six days.

Heinreich (93) reported that milk could be measured into test bottles and left for weeks without affecting the accuracy of the results. Furthermore he reported that finished tests could be kept for a considerable time and reread by placing in warm water and centrifuging. Hills (99) used this procedure on tests that were kept for three years and exposed to temperatures from –18 to 38°. Each year the tests were heated and read. Two

years did not seriously affect the results, but after three years the tests were of no value.

Kent (123) compared the aliquot and dipper methods for preserved composite samples with the dipper giving the lowest results, while Potts (173) obtained only slight differences. Tracy and Tuckey (218) concluded that aliquot sampling was not necessary but that inaccurate sampling may lead to erroneous results. Marquardt and Durham (144) stated that stirring milk in the weigh can was not necessary, while Bailey (18, 19, 20) reported that agitation was essential. Webster (224) reported significant differences in the fat content of milk from different parts of the weigh can. Powers (174) concluded from an extensive survey that there was a tendency for non-agitated samples to test higher, but that in general, sampling was affected by other conditions in individual plants.

The care of preserved composite samples received early consideration by Babcock (15) who recommended keeping them in a cool place. The A.D.S.A. (3) in 1917 and in 1922 stated that composite samples should be kept cool and be the product of not more than one week of milk deliveries. Sanmann and Overmann (182, 183) submitted data to show that composite samples should be stored at 10° or lower.

With the advent of composite sampling, a number of preservatives received experimental attention. Patrick (167) studied a number of preservatives including borates, salicylates and bichloride of mercury. He concluded that bichloride of mercury in concentrations of 10–15 grains to 200–400 cc. of milk was the most satisfactory. Jackson (119) reported no difference in results by varying the concentration from 0.5 to 1.5 grams bichloride of mercury per 150–300 cc. of milk. Campbell *et al.* (38) and others (65, 115, 146, 147, 152, 182, 183, 220, 225) reported the use of this preservative. Grélot (88) stated that small amounts of ammonium chloride expedited the solution of bichloride of mercury.

Farrington (72) used a preservative consisting of a mixture of 2 ounces of bichloride of mercury, 2 ounces of fine "salt," 8 ounces of finely ground borax and 1.5 drams of aniline red. He used 15–20 grains of this mixture for each sample jar.

Alén (1) preserved milk by adding 0.5 grams of potassium dichromate to 250–500 cc. of milk and stated his intention of having this method patented. Babcock (15) and others (25, 87, 88, 102, 134, 153, 158, 178, 195, 199, 230, 234, 235, 236) used this preservative in varying concentrations.

Rideal (176) stated that one part of formalin to 10,000 parts of milk kept it fresh for 7 days. Thompson (214) preferred formalin to boric acid, borax, salicylic or benzoic acid. McConnell (154) reported formalin equal to potassium dichromate, but preferred the latter because it colored the milk. Lindet (134) stated that formalin interfered with fat determinations by Gerber's method, while Bevan (31) and Hills (97) added increasing amounts

of this preservative to milk and obtained a parallel increase in total solids. Hills (97) experimented with a number of preservatives and concluded that the most useful were mercuric chloride, a mixture of 10 parts mercuric chloride with 50 parts of borax, potassium chromate and the bichromate, sodium sulphite and bisulphite, copper ammonium sulphate, sodium salicylate and formaldehyde.

Other preservatives used were copper ammonium sulphate by Windisch (230), who has also contributed an excellent review of the literature on preserving milk for chemical analysis; Farrington (73), who used a teaspoonful of 98 per cent sodium hydroxide to each sample; Ronneberg (178), who used potassium permanganate and Neumann (158), who used ammonium hydroxide and ammonium carbonate.

Cream

The sampling of cream for testing received some experimental attention because of its viscous nature. The A.O.A.C. (8), from 1908 to 1916 inclusive, gave no detailed directions for sampling, but referred to the various editions on testing published by Van Slyke (222) and by Farrington and Woll (77).

Van Slyke (222) stated in 1913 that thick cream of low temperature should be warmed to not above 30–35°, poured carefully and quickly sampled, while if the cream was completely separated it should be heated to 40–43°, continuously shaken and cooled to 15.5° and quickly weighed. Farrington's and Woll's (79, 80) recommendations applied chiefly to the sampling of cream from individual patrons.

In 1917 and in 1922 the A.D.S.A. (3) stated that if the cream was in good condition, the samples could be mixed by shaking, pouring or stirring, but if very thick they should be warmed to 30° and then mixed. If lumps of butter were present, or if a large number of samples were to be tested the cream should be heated to 38–50° in a water bath, being careful to avoid "oiling off." It was also recommended that the samples be kept cool and stored in non-adsorptive air-tight containers. The A.O.A.C. (8) in 1925 and S.M.M.A. (210) in 1934 published this procedure that has appeared in subsequent editions.

Babcock (10) stated that cream required thorough mixing and if sour and exposed to the air until the surface had dried, proper sampling was difficult. Hunziker (115) stated that normal fresh cream could be sampled accurately by rotating or by careful pouring, but thick cream should be heated to 29–32°, poured gently a few times and weighed immediately, while cream in poor physical condition should be heated to 43° or above, and continuously shaken while cooling to 16° or lower before weighing. Hills (100) suggested that lumpy cream be passed through a small sieve and mixed by pouring from one cup to another. Webster (223) and others (2, 20, 91, 113, 122, 143) emphasized care in the preparation of the cream sample.

Size of Charge

Milk. In 1902 the A.O.A.C. (8) referred to Wiley (228) who stated that a 17.6-cc. pipette delivered a volume that varied but slightly from the desired weight of 18 grams. In 1908 the A.O.A.C. (8) specified a 17.6-cc. pipette, while in 1916 this organization stated the dimensions of the pipette as specified by the United States Bureau of Standards (221) and by the A.D.S.A. (3) in 1917. This pipette was calibrated to deliver 17.6 cc. of water at 20° in 5 to 8 seconds and since 1925 the A.O.A.C. (8) has specified the pipette to contain 17.6 cc. of water at 20° when the bottom of the meniscus coincided with the line on the draw tube. Since 1923 S.M.M.A. (210) has also published these specifications. Furthermore, the A.D.S.A. (3) in 1921 recommended that the discharge stem of the pipette be made to slip into the test bottle, and thus eliminate the awkward method of holding the tip of the pipette in a slanting position against the neck of the test bottle. It was also suggested that the last drop of milk, after free outflow ceased, should be blown into the test bottle. S.M.M.A. (210) in 1923 and the A.O.A.C. (8) in 1925 incorporated this recommendation.

Babcock (10, 11, 12) stated that when the pipette was filled so that the upper surface of milk coincided with the mark on the draw tube it should contain 17.6 cc., but would deliver slightly less than 17.5 cc. and 17.44 cc. of milk would be equivalent to 18 grams, assuming an average specific gravity of 1.032.

Bailey (17), Dahlberg (50) and Hoyt (111) used pipettes calibrated to deliver 17.6 cc. of water at 20° C. Bailey (17) reported a delivery of 17.924 grams of milk at 21°. He determined the influence of temperature and at 21° the average delivery was 17.937 and at 46° was 17.814 grams of milk. The pipettes were allowed to drain 2 to 3 seconds and the tips were touched against the inside of the necks of the bottles. Dahlberg (50) weighed the deliveries of six samples at 21.1° and at 48.8° and obtained weights of 17.9680 and 17.8425 grams, respectively; the estimated fat content on each were 4.81 and 4.82 per cent. Bartlett (26) and others (12, 35, 52, 80, 84, 91, 92, 113, 122, 135, 143, 159, 206, 212, 231) specified the use of 17.6 cc. of milk, but the intent of calibration of the pipette was not always certain; with few exceptions they recommended blowing the last drops from the pipette. Hoyt (111) obtained an average delivery of 18.015 grams of milk from 36 determinations; the temperature of the milk was not stated, but the pipette was calibrated to deliver 17.6 cc. of water at 20° in 5 to 8 seconds. Bearce (29) has submitted data to show the weight-volume relationship of milk and cream at practical temperature ranges.

Cream. The A.O.A.C. (8) in 1908 stated that cream should be weighed and in 1916 and 1920 this organization specified the use of 9 and 18 grams, depending on the fat content. In 1917 the A.D.S.A. (3) mentioned either

9 or 18 grams, using standard cream test scales set level with a sensitivity reciprocal of 30 mgs. and the weights should preferably be stamped for accuracy by some official organization. In addition to these requirements, the A.O.A.C. (8) since 1925 has specified that the scales be protected from air currents and that the weights consist of material capable of resisting corrosion and be of the low squat type with rounded edges.

Babcock (10, 11) suggested dividing the contents of the 17.6 cc. pipette, including rinsings between three milk test bottles, but recognized the errors involved that could be eliminated by weighing the cream. Eckles (60) made similar suggestions. Winton (232) devised a pipette for cream that delivered 6 grams; 12 cc. of water were added and the remainder of the procedure was the same as for milk. Jones (121) presented data to show the fallacy of measuring cream by volume for the Babcock test. Spillman (207) measured cream by volume and devised a table of corrections for variable contents of fat. Webster (223), Farrington and Woll (80) and others (21, 35, 45, 84, 91, 113, 115, 122, 135, 143, 159, 212, 216) used 9- or 18-gram charges and there was some division of opinion as to the merits of the 9- and 18-gram bottle.

Type of Bottle

Milk. The bottle designed by Babcock (10, 11) was the same shape as that used by Short (197), except it was smaller and of heavier glass. Babcock's bottle was graduated from 0–10 in 0.2 per cent divisions. This same type of bottle was illustrated by Wiley (228) in 1897 as official, and presumably this same bottle was listed as official by the A.O.A.C. (8) in 1908. In 1909 the A.O.A.C. (8) stated that the capacity of each per cent should be 0.2 cc. as calibrated with clean dry mercury at 20°, and the limit of error should be the smallest graduation and not to exceed 0.5 per cent. The O.D.I.A. (161) in 1911 adopted specifications that were restated in detail by the A.D.S.A. (3) in 1917 and 1922 and are summarized as follows: The bottle should have a total height of 150–165 mm. with a bulb capacity of at least 45 cc. and if cylindrical, the body must have a diameter of 34–36 mm., and if conical, the base must have a diameter of 31–33 and a maximum of 35–37 mm. The graduated portion of the neck should be not less than 63.5 mm. long with calibrations in whole, half and tenths from 0.0 to 8.0 per cent. The 0.1 per cent graduations must not be less than 3 mm. long, the 0.5 per cent not less than 4 mm. long and must project 1 mm. to the left, while the whole per cent markings must extend at least half way around the neck to the right and project at least 2 mm. to the left of the tenth per cent graduations. The cylindrical part of the neck must extend 5 mm. below the lowest and above the highest graduated mark and the top must be flared at least 10 mm. wide. The volume of each whole per cent should be 0.2 cc. and the maximum error must not exceed the smallest unit of graduation. These detailed specifications appeared in the A.O.A.C. (8) and S.M.M.A. (210) in 1925 and 1928, respectively.

Mitchell and Walker (148) described a test bottle and a centrifuge whereby water could be added to the bottle while the machine was in motion. This bottle had in addition to the Babcock, a second funnel-shaped neck for receiving water from a spindle. The water was forced outward by the centrifuge and caught by the cone-shaped necks of the bottles. The lower end of this water neck was reduced in size to prevent fat from rising into it. It was claimed that churning of the milk was avoided because the acid flowed down the outer wall of the bottle, thus avoiding immediate mixing. Bartlett (26) devised a milk test bottle with a graduated body that eliminated the need for an acid measure. Whitman (227) designed a bottle for testing as little as 2.5 cc. of milk. The body of this bottle was calibrated for the milk charge and to fit trunnion carriers in a small centrifuge. A ground glass graduated tube was placed into the shoulder of the bottle to collect fat. The results were claimed to be identical to those obtained with the Babcock test bottle. Manchester (138) presented data to show that the length of the graduated neck on the Babcock test bottle was a factor in obtaining accurate results. He reported the errors introduced when the length of graduations in a 0–10 per cent bottle were varied as follows: 60–65 mm. 0.1 per cent, 45–50 mm. about 0.2 per cent, and 22 mm. about 0.55 per cent. Bottles with graduation lengths of 80 mm. were reported as the most accurate.

Cream. The invention of the Babcock test naturally led to its application to cream and other milk products. Winton (234) developed a test bottle that appears to have been the pattern used for the present cream bottle. The committee in the O.D.I.A. (161), previously referred to, in 1911 gave detailed specifications for the 50 per cent 9-gram cream bottle in which the total length, length of neck, capacity and dimensions of the body and general methods of marking were essentially the same as for the whole milk bottle; the essential difference being in the size and capacity of the graduated neck. The same specifications applied to the 9 inch bottle, except the total height was 210–225 mm. These bottle specifications were restated by the A.D.S.A. (3) in 1917 and in 1922 together with dimensions for the 18-gram 50 per cent bottle, all of which appeared in the A.O.A.C. (8) in 1925 and in S.M.M.A. (210) in 1934. These detailed specifications were inspired by the recommendations of Hunziker *et al.* (117) who illustrated five different types of 6-inch and the same number of 9-inch bottles. They condemned both 50 per cent 18-gram 6-inch bottles, one with a bulb in the graduated neck and the other with a straight neck, because the large diameter of the necks made accurate reading very difficult. The 30 per cent 18-gram 6-inch bottle was not recommended because of its limited range.

Bartlett (23, 24) described a test bottle with a neck sufficiently long to estimate cream up to 25 per cent fat. He devised a second bottle for cream containing up to 35 per cent fat, with a removable neck that required a larger centrifuge. Later (26) he contributed a bottle for cream with a smaller

body that was calibrated for the proper amount of acid. Bartlett (26) also submitted a bottle to estimate the fat in butter using an 18-gram sample. Farrington (75) contributed an 18-gram 6-inch bottle with a body of 45 cc. capacity and with a neck calibrated from 0–30 in 0.5 per cent divisions. Bebee (30) used an 18-gram 50 per cent 9-inch bottle, with the zero mark at the top of the neck. Holter (107) devised a test bottle with a large graduated neck for butter and which could be used for cream of high fat content.

Amount, Strength and Temperature of the Sulphuric Acid

Milk. The amount of acid specified by all official organizations was 17.5 cc. of specific gravity 1.82–1.83. Since 1925 the A.O.A.C. (8) and since 1928 S.M.M.A. (210) stated the specific gravity of the acid at 20° and the temperature of adding acid to 15–20°, preferably not to be added at one time. The A.D.S.A. (3) in 1917 and 1922 specified the temperature of the acid at 10–21°. The acid was designated, with few exceptions, to be measured with a graduate or a Swedish acid bottle that would deliver 17.5 cc. The A.O.A.C. (8) in 1916 and 1920 specified that the error of volume for acid measures should not exceed 0.2 cc.

Babcock (10) recommended 17–18 cc. of acid with a specific gravity of 1.82–1.83. He (13) later recommended 17.5 cc. of 1.82–1.83 specific gravity, and with few exceptions, these specifications for acid have been followed by others (21, 22, 35, 45, 63, 74, 84, 113, 116, 143, 159, 212, 234).

Bartlett (26) recommended 20 cc. of acid of 1.82–1.825 specific gravity and advised adding hot water to the base of the bottle neck immediately after mixing acid and milk; this was a slight modification of the Babcock test.

Bailey (17) used varying amounts of acid of 1.80, 1.81, 1.82, and 1.83 specific gravity with acid and milk at 21°. The acid of low and smaller amounts of high specific gravity resulted in practically colorless fat columns that averaged 0.05 per cent lower than when 17.5 cc. of the stronger acid was used.

Petersen (170) found that when sulphuric acid was contaminated with butterfat, lard and the oils of cottonseed, olive, corn, cod, sperm and of mineral, that the fat estimations of milk were increased. Later (171) he prepared a stable emulsion of sulphuric acid, specific gravity 1.83, fat saturated benzine and a water soap solution that also increased the fat readings. This emulsion could not be separated by whirling in an 18-inch centrifuge at 1,000 r.p.m.[3]

Babcock (10, 11, 13) did not mention a specific temperature for milk and acid. Bailey (17) made estimations with acid and milk at 10°, 21° and 32°, using acid of specific gravity 1.83, and found equally good results if the amount of acid was varied inversely with the temperature. He recommended the lower temperature to avoid charring the fat. White (226) ob-

[3] Stated 10,000 r.p.m., but this is an error.

tained the most accurate results when sufficient acid at 15.5° was used to give an almost colorless fat column. Farrington (74) stated that acid of 1.82 specific gravity should be used with milk at 16–21° and if the acid is stronger, cool to a lower temperature. Bartlett (26) and others (35, 45, 74, 122, 159, 202, 206, 212) stated that milk and acid should be tempered to 16–21°, while Farrington and Woll (80) and others (91, 113, 116, 143) suggested temperatures ranging from 13–27°.

Cream. The O.D.I.A. (161) described three methods of adding sulphuric acid that was published by the A.D.S.A. (3). In the first method sufficient sulphuric acid was added until the mixture resembled the color of coffee, containing cream. The amount needed depended on the temperature of acid and cream and the fat content of the cream, but varied from 8–12 cc. for a 9-gram and 14–17 cc. for an 18-gram sample. The second method was only applicable to the 9-gram bottle and consisted of weighing the cream and adding 9 cc. of water, plus 17.5 cc. of sulphuric acid. The third method consisted of adding 8–12 cc. of acid for a 9-gram and 14–17 cc. for an 18-gram charge, or acid was added until the mixture of acid and cream after mixing had a chocolate brown color. After thorough mixing not less than 5 cc. of hot soft water was added, whirled 5 minutes, soft hot water added to near top of neck and whirled 1 minute. Since 1925 A.O.A.C. (8) and since 1934 S.M.M.A. (210) have declared methods two and three as official. The specifications for the sulphuric acid are the same as those for milk. The temperature of the acid was not stated.

Hunziker *et al.* (117) and others (91, 113, 143) stated that the amount of acid varied with the fat content and sufficient acid of 1.82–1.83 specific gravity should be used to give a color similar to coffee after the cream had been added. Marquardt (143) stated that a 9-gram sample required 4–8 cc., and an 18-gram sample, 8–12 cc. of acid, and that after adding acid and mixing, water at 82° should be added to the base of the neck to prevent charring; Crowe and Davis (45) concurred in this method of retarding the action of the acid. Fuller (84) and others (135, 159) suggested diluting cream with 9 cc. of cold water and adding 9 cc. of acid, while Hunziker (116) and others (45, 122, 216) advised 9 cc. of acid for a 9-gram and 17.5 cc. for an 18-gram sample. Hunziker *et al.* (117) tested 96 samples of cream with acid and cream from 5–43°, and found no differences when the amount of acid was regulated by a color similar to that of coffee after the cream had been added. They concluded the best results could be obtained with acid and cream at 21°; similar recommendations were made by others (143, 159).

Centrifuging Procedure

Milk. The A.O.A.C. (8) in 1908, 1916 and 1920 stated that the centrifuge should be operated at a speed in accordance with the diameter of the wheel. In 1925 the A.O.A.C. (8) and in 1928 S.M.M.A. (210) indicated the

revolutions per minute for wheels of different diameters and the diameter was defined as the distance between the inside bottoms of opposing horizontal cups through the center of rotation. In 1922 the A.D.S.A. (3) specified an attached speed indicator, and the A.O.A.C. (8) in 1925 and S.M.M.A. (210) in 1928 adopted this recommendation.

Three whirling periods of 4, 2 and 1 minute, respectively, were stated by the A.O.A.C. (8) from 1908–1920 inclusive and by S.M.M.A. (210) in 1923, with the addition of "boiling" water between whirlings. In 1917 the A.D.S.A. (3) specified whirling periods of 5, 2 and 1 minute, respectively, with the addition of "soft" water at 60.5° or above, between whirlings, and the A.O.A.C. (8) in 1925 and S.M.M.A. (210) in 1928 adopted similar regulations except that "hot" water was stipulated for the second addition. For the first time the A.O.A.C. (8) in 1925 and S.M.M.A. (210) in 1928 stated that the centrifuge be heated electrically, or otherwise, to at least 55° during the whirling process.

The centrifuge described by Babcock (10) contained rigid trunnion cups that were inclined at an angle similar to the angle of inclination in De Laval's (54, 55, 56) machine. The wheel that held the trunnion cups was surrounded by a stationary copper jacket with a cover. This jacket held hot water that could be further heated from beneath. Babcock (10) stated that the test bottles should not be heated to the boiling point of water in the centrifuge during the whirling process, but enough water should be poured in the jacket so that it would be heated to boiling when the whirling process is finished.

Babcock (10) stated that the first whirling should be 6 minutes, the second 1 to 2 minutes and the third for a short time with the addition of hot water between whirlings. He (13) later whirled the bottles for two periods of 5 and 1 minute, respectively, and Emery (63) followed this procedure. Weld (225) designated periods of 7, 3 and 2 minutes as most satisfactory. Farrington (74) and others (17, 21, 35, 90, 116, 143, 159) used centrifuging periods of 5, 2 and 1 minute, respectively, while Tolstrup and Mortensen (216) suggested whirling periods of 5, 2 and 2 minutes, and Fuller (84) recommended whirling periods of 5, 3 and 1 minute.

The temperature of the water added between whirlings has varied. Babcock (10, 14) implied the use of water near the boiling point, while others (17, 26, 74, 80, 91, 92, 175) specified the addition of hot water. Emery (63) recommended the use of soft water at 77° and preferably nearer 100° while Kerr (124) suggested a temperature of at least 82°. Liverance (135) and others (113, 116, 143, 212) suggested temperatures of 54° or above, while Jones and Wright (122) used water at 26.6°. The desirability of using water practically free from minerals to avoid the formation of insoluble salts was emphasized by most investigators.

Babcock (10) believed that heat from the chemical reaction was adequate

if the bottles were immediately whirled, but if they were allowed to cool to 37.6° or lower, he suggests heating to 93.3° before centrifuging and others (21, 35, 80, 135) followed this procedure. Bailey (17) reported that at room temperature milk will have the same reading from a heated or an unheated centrifuge, if whirled immediately after adding the acid and if water at 82.2° is added to the test bottles. Only small differences were obtained with either machine in a room at 10°. Fahl *et al.* (68) and Lucas (136) obtained higher results in heated than in unheated centrifuges; they suggested adding boiling water to the test bottles between whirlings if the room is cold; similar results were reported from the same station (7). Nelson (155) reported that estimations made in a room at 7.22° were 0.019 per cent lower than those made in a room at 28.9°, but he also stated with a temperature in the centrifuge of 37.8°, that 32 samples averaged 0.049 per cent higher than the Mojonnier estimations of the same milk and with the centrifuge at 54°, the samples averaged 0.07 per cent higher. Woll (237) and Farrington and Woll (77) found that a steam turbine centrifuge yielded results 0.1 to 0.3 per cent higher than the gravimetric method; these results were attributed to an abnormally high temperature in the centrifuge. Otis (162) reported that the unheated yielded results 0.2 per cent below the heated centrifuge. Woll (237) stated that manufacturers of centrifuges designed their machines to operate at high temperatures on the inside because they were of the opinion that such machines yielded the best results. Eckles (61) cautioned against the use of excessively hot centrifuges that heat the contents to near the boiling point, because impurities are more apt to be forced into the fat column. Burke (35) also suggested placing the test bottles from unheated centrifuges in water at 82° between whirlings for a few minutes.

Babcock (10) advocated whirling the test bottles at 600–800 r.p.m. and if the diameter of the wheel was less than 20 inches, the speed should be greater or the whirling time extended. Later (12) he proposed 700–1200 r.p.m., depending on the size of the wheel and that an 18-inch wheel should be run at 700–800 r.p.m. On this basis Farrington and Woll (80) calculated that a wheel operating at 800 r.p.m. would exert a force of 30.65 pounds, according to the formula, $F = \frac{WV^2}{32.2r}$, where F is centrifugal force in pounds, W is weight of test bottles and contents in pounds; V = velocity in feet per second; and r is radius of the wheel in feet. Using this formula they calculated the necessary speed for wheels of different diameters, that later appeared in a number of publications (21, 111, 113, 116, 159). Heinreich (93) reduced the speed of the centrifuge from 1500 to 800 r.p.m. with a subsequent reduction of 0.22 per cent fat and Bailey (17) reported similar results while the importance of adequate centrifugal force was further emphasized (27, 63, 143, 202).

Cream. The mechanical specifications outlined by the various official organizations for milk also apply to cream, with the exception of the whirling time. In method three for the addition of acid (see preceding section) only two centrifuging periods of 4 and 1 minute, respectively, were specified as contrasted to three periods of 5, 2 and 1 minute for one and two.

The whirling periods employed by the various investigators were similar to those for milk, with some exceptions. Marquardt (143) and others (21, 113, 122) recommended two whirling periods of 5 and 2 minutes, respectively. Bebee (30) suggested two periods of 5 and 4 minutes, and he found that longer periods were unnecessary.

The temperature of the water added between whirlings has been similar to that for milk, the chief distinction being that some investigators specified "hot" water, while others recommended water at 57° or above.

Bebee (30) placed a thermometer near the outer shell and maintained a temperature of 76.6° inside a steam centrifuge, thus he stated, the bottles were kept at 54.4–60°. Hunziker and co-workers (117) conducted tests in a cool and dry and in a steam-heated turbine centrifuge. There was no difference in the impurities in the fat columns and in the loss of volatile fatty acids between the two centrifuges. Webster (223) studied temperatures of 43.3, 48.8, 54.4 and 60° in the centrifuge. He weighed butterfat into test bottles and skimmilk was added to a total weight of 18 grams, with a calculated butterfat content of 39.8 per cent. The average increase in reading was 0.5 per cent from 43.3–60°. He concluded that the hand-operated centrifuge gave more reliable results than the steam centrifuge because tests from the former were nearer the desired 48.8°. The tests were read directly from the centrifuge. Eckles (61) stated that the fat reading may be increased 1 per cent by raising the temperature from 43–82°, and similar results were reported by Farrington and Woll (78).

Hunziker and co-workers (117) conducted 547 tests in an 18-inch centrifuge to ascertain the effect of centrifugal force. They found that low speeds resulted in significantly lower tests and fat was observed in the liquid below the fat column. These results were corroborated by the recovery of larger amounts of fat from the acid hydrolysate when the speed was reduced. However, Siegmund and Craig (195) obtained lower results as the speed of their centrifuge was increased from 800–1000 r.p.m., and these lower results were attributed to the expulsion of occluded water and acid in the fat column. The diameter of the wheel was not given.

The first power-driven centrifuges according to Babcock (15) were of the steam turbine type, but during recent years the electrically powered and heated types are gradually displacing the steam machines. It is difficult to obtain information when the first electric centrifuges were marketed. The Chicago Department of Health (39) reported instructions for installing electric centrifuges in 1906. Kingsley (125) stated that a motor was at-

tached about 1900 and ten years later the heating element was included. Ziegler (239) stated from old catalogue files of the Cherry-Burrell Corporation, that the electric powered centrifuge was introduced sometime between the years 1900 and 1909, and the heating element appeared in the catalogue as standard equipment in 1923. Bingman (32) stated that the first electric centrifuges were marketed by The Jalco Motor Company in 1913 and the heating elements were included a short time later. Mojonnier (150) stated that his company sold electrically operated centrifuges soon after the year 1916, but this type of equipment was previously available from other companies.

Temperature and Time of Holding Bottles in the Water Bath

Milk. Babcock (10, 11) stated that reading the tests at 43.3–65.5° gave satisfactory results, but the higher temperatures were preferred. He stated that a difference of 22.2° made less than 0.1 per cent difference with milk containing 5 per cent fat. Farrington (74) and Emery (63) recommended tempering the tests to 60°, before reading and Emery (63) believed that one minute was sufficient. Bailey (17) read tests at 54.4°, but calculated this would cause an overreading of 0.032 per cent on milk testing 4.51 per cent, because he found the density of butterfat was 0.9 at 45°. Bailey (17) and others (35, 45, 116, 159) stated that the five minutes were sufficient. The A.D.S.A. (3) in 1917 specified not less than three minutes at 57–60°. Fuller (84) stated the readings could be made direct from a steam centrifuge. Harrison (90) estimated the fat content of 235 samples of milk and 2,068 samples of cream, using an electrically heated centrifuge, and reported no significant differences in the results when the tests were read direct from the centrifuge and from a water bath at 57° after five minutes. Caldwell and Herreid (37) employed water bath temperatures of 37.8°, 60° and 71° and obtained averaged tests of 4.33, 4.37 and 4.4 per cent, respectively, on the same samples of milk. Hays (91) believed it unnecessary to keep the bottles hot in the centrifuge.

Cream. The A.O.A.C. (8) in 1902 made no recommendations while in 1908 this organization cited Farrington and Woll (77) and Van Slyke (222) for procedures. The A.O.A.C. (8) for 1916 and 1920 specified the same procedure as for milk after weighing the cream, but also referred to Farrington and Woll (78) who stated that an error of one per cent (presumably bottle reading) may result due to the expansion of fat in an excessively hot turbine centrifuge with a tight cover, which they stated could be corrected by placing the test bottles in water at 60° for "some minutes." The A.D.S.A. (3) specifications for the time temperatures and exposure of the milk test bottles in the water bath, also apply to cream, with the exception that a few drops of glymol are added and the bottles are read at once with the aid of calipers.

Since 1925 the A.O.A.C. (8) has indicated that if glymol is used, a few drops should be allowed to flow down the inside of the neck just before reading each bottle; the specific gravity of the glymol should not exceed 0.85 at 20° and it may contain a soluble artificial color. The surface separating glymol and fat should be regarded as the upper limit of the fat column that must be free from visible foreign particles. The exposure of the bottle in the water bath is the same as for milk. S.M.M.A. (210) published these specifications in 1934.

Hunziker and co-workers (117) stated that the tests should be held in a water bath at 57.2–60° for five minutes because the bottles are calibrated for 0.9 as the specific gravity of butterfat at 57.2°. They obtained results 0.1 to 0.2 per cent too low, using water-bath temperature of 43.3–48.8°. Beach (28) obtained a reading of 0.42 per cent higher at 82° as compared to 54°. Eckles (61) stated that the exact temperature at which cream tests should be read had not been determined, but that it was near 49°.

Method of Estimating the Fat Column

Milk. The A.O.A.C. (8) in 1908 stated that the fat column should be read at 54–65.5° and in 1916 and 1920 to be read at 57–60°, and S.M.M.A. (210) contained similar specifications in 1923. No mention was made of the method to obtain these temperatures. The A.D.S.A. (3) in 1917 first specified a water bath, with thermometer and equipment to insure proper temperature control at 57.2–60°, and to leave the test bottles in the water bath for not less than three minutes. Since 1925 the A.O.A.C. (8) has stated that the test bottles, after the last centrifuging, should be immersed in the water bath at 55–60° to the level of the top of the fat column and left until the fat reached equilibrium and the lower surface had assumed final form. The bottle is wiped dry and the fat column measured with calipers or dividers from the lower surface to the highest point of the upper dimensions. The fat column must be translucent, golden yellow, or amber and free from visible particles. In 1928 S.M.M.A. (210) incorporated these specifications.

Babcock (10, 11) recommended that the fat column be measured in a perpendicular position with the line between the acid liquid and the column of fat in a horizontal position and the calibrations level with the eye, thus observing the highest and the lowest limits of the fat column. He (13) cautioned, "the reading should be taken at the line where the upper surface of the fat meets the side of the tube and not from the bottom of the dark line caused by the refraction of the curved surface." Babcock (10) stated that the fat column could be easily estimated to half divisions, which was to 0.1 per cent. Dahlberg (46) emphasized the difficulty of reading the meniscus and stated that the human eye cannot determine the extreme uppermost line of the meniscus. He is quoted, "one must assume the extreme upper end of the meniscus of the fat column to be that point at which

the fat seems to meet the wall of the glass rather than the high point to which the fat is drawn along the glass in a film by capillary attraction." Spillman (206) and others (21, 35, 45, 80, 84, 92, 113, 122, 135, 143, 159, 202, 212) recommended reading what they call the extremes of the fat column. White (226) employed better lighting conditions to show the outline of the meniscus more plainly and he thereby reduced the magnitude of the difference between the Mojonnier and the Babcock methods. Hortvet (110) devised an instrument for estimating fat columns, but it was not used extensively in commercial plants. Herreid (94) improved Hortvet's apparatus so that estimations could be made more accurately and under more standard conditions on both milk and cream. Theophilus and Barnett (213) reported the use of a device with a 60-watt blue bulb enclosed in a container with an opening from which the reading was made and a clear definition of the meniscus was obtained. Wilster and Robichaux (229) also reported a device to facilitate reading the fat columns. Mojonnier Brothers Company in Chicago, Illinois, list an apparatus to read the fat column, called the Wagner Junior Columnmeter, but it was not used extensively in commercial laboratories. This piece of equipment has mechanically adjusted calipers, but the points are blunt. Caldwell and Herreid (37) improved this device by replacing the dull points with sharp ones, illuminating the apparatus and adding a magnifying lens. Sanmann and Overmann (184) revealed significant personal differences in reading the Babcock test with the hand calipers. Nelson (156) stated that the probable error by the Babcock method was ∓ 0.02 per cent with possible variations of 0.1 per cent between different technicians, while Dahlberg (47) reported the experimental error on the same sample in different laboratories to be 0.2 per cent.

Cream. Winton and Ogden (235) in their earlier work read the fat column from the extreme top to the bottom. Webster (223) studied the dimensions of the meniscus and reported that readings from the top, middle and the bottom of the meniscus did agree with the gravimetric method. He suggested reading the extremes of the fat column, deducting four-fifths of the meniscus and adding 0.2 per cent to the result. He stated that the fat/water acid interface should be flat when the fat column was read. Liverance (135) advised including one-half of the meniscus. Eckles and Wayman (62) recognized the variability of estimating the large meniscus and recommended the use of amyl alcohol, colored red with fuchsin. Using this technique, the tests had to be read quickly because fat is soluble in the alcohol that in turn is miscible with water (208). The results showed close agreement with the gravimetric method. The chief disadvantage of this reagent is the toxic properties of amyl alcohol. Hunziker (114) advised reading to the bottom of the upper meniscus and adding one-third of the meniscus to the reading. Babcock and Farrington (16) and Smith (202)

recommended the use of fat saturated alcohol to eliminate the meniscus, while Hunziker *et al.* (117) after a thorough investigation recommended glymol, a mineral oil, colored red with alkanet root, that was favorably received by the industry. He also estimated cream tests to the bottom of the meniscus with the aid of a mirror to eliminate parallax and obtained close agreement with the gravimetric method. Doan *et al.* (57) estimated cream tests to the top and to the bottom of the upper meniscus, with glymol, with a fat-saturated alcohol and by including one-third of the meniscus. They found glymol to be the most reliable. Bebee (30) suggested the use of magnifying lens to aid in reading cream tests. Ross and McInerney (179) and others (21, 35, 45, 91, 143, 212, 216) advised the use of glymol.

Modifications of the Babcock Method

Attempts have been made to modify the Babcock test for the sake of expediency. Bartlett (26) submitted a procedure that consisted of using the usual charge of milk and 20 cc. of sulphuric acid of specific gravity 1.82–1.825. After the acid was added and the contents mixed with a rotary motion, the bottles were allowed to set for not less than 5 minutes, followed by a gentle shaking. Hot water was added to the uppermost mark and the bottles centrifuged at 1000 to 1200 r.p.m. for 5 minutes. A heated centrifuge was recommended. The data showed close agreement with the regular Babcock procedure. Hills (98) made 34 comparisons of the Bartlett modification with the Babcock procedure and likewise obtained close agreement but suggested its use by skilled testers.

Holm (106) was concerned with the constituents of milk that contribute to the charred material in the fat columns and stated that the carbohydrates cause a foamy char, the fats a brownish translucent char and the casein a flocculent brownish char. His method of preventing charred fat columns consisted of adding 2 cc. of a mixture of 80 parts glycerin and 20 parts water to the milk in the test bottle. When acid was added the glycerin formed a layer between the acid and fat to prevent charring before the bottle was rotated. No data were presented but he reported good results on several thousand samples.

Siegfeld (194) reported a modified method whereby the milk and sulphuric acid were mixed as usual, followed by 2 cc. of amyl alcohol. Next, sufficient sulphuric acid of specific gravity 1.5 and at 90–100° was added to fill the bottles to the uppermost part of the neck. The centrifuge was whirled for 3 minutes and the tests read. The results agreed closely with the Gerber and with the Adams gravimetric method.

James (120) suggested the addition of 3 cc. of a mixture of amyl alcohol and hydrochloric acid at the time the sulphuric acid was added and centrifuging at ordinary temperatures; thus making the procedure similar to the Beimling method.

Comparisons of the Babcock and the Gravimetric Methods

Milk. Babcock (10) compared his test with the Adams method using paper coils on 29 samples of fresh milk and the results showed that his test averaged 0.0193 per cent higher with 19 of the 29 comparisons agreeing within 0.10 per cent. Snyder (203) made 43 comparisons using asbestos as the absorbent in the Adams method and found that the Babcock method averaged 0.026 per cent lower; later (204) he reported that the Babcock method averaged 0.016 per cent higher. Results reported by Farrington (71), Dahlberg (50), Fisher and Walts (81) and James (120) show that the Babcock test exceeded the gravimetric ether extraction methods by 0.1 per cent or more, while the Iowa Experiment Station (6), Bailey (17), Dahlberg (48), Lucas (136), Phillips (172), Scrott-Fiecht (190), Shiver (196) and Shutt (198) reported that the Babcock test exceeded the gravimetric methods by less than 0.1 per cent. Close agreement was obtained by Mojonnier and Troy (151), Sebelien and Storen (193), Winton (231, 233) and Zehenter (238). Conversely, Patterson (168) reported that the gravimetric averaged 0.132 per cent higher, and Hortvet's (109) results were also 0.05 per cent above the Babcock method. On only 3 samples of milk, Hite (101) obtained higher results by the Adams method.

Bailey (17) obtained a reading of 0.179 per cent more than was contained in the neck of the bottle by including the extremes of the fat column. He calculated a net loss of 0.106 per cent in his experiments, and the difference of 0.073 per cent was above the gravimetric results. Hoyt (111) estimated the extremes of the fat column and by eliminating the upper meniscus with glymol he obtained close agreement with the Mojonnier method, while Phillips (172) using glymol obtained values 0.087 per cent below the Mojonnier and 0.146 per cent below the Babcock method when extremes of the fat column were included. Dahlberg (50) used glymol and obtained results 0.1 per cent too low as compared with a modified Röese-Gottlieb procedure. Jack and Abbott (118) tabulated the differences between results obtained by the Babcock and the various gravimetric procedures as reported by a number of investigators.

Smith (202) weighed purified butterfat into milk test bottles and added water to make a total of 18 grams. All but one of 16 estimations were too high when read to the top of the upper meniscus. He stated that the addition of a few drops of alcohol before reading gave results that compared favorably with the gravimetric method.

Since the earliest comparisons of results obtained by testing preserved and fresh samples of milk by the Babcock and by the ether extraction methods, there has been lack of agreement in the results obtained.

Campbell *et al.* (38), England and D'Ambrogie (65), Fahl *et al.* (68), Holland (104), Kochheiser (126), Meade and Leckie (146), Mecham (147), Monroe (152), Potts (173) and Sproule (209) all reported lower results

on the composite samples, while Babcock (15), Eaton (59), Farrington (73), Ronneberg (178), Neumann (158), Sanmann and Overmann (182), Windisch (230) and Zehenter (238) obtained results that were only slightly lower or agreed with the daily samples. Sanmann and Overmann (183) submitted data to show that composite samples stored at 10°, or below agreed closely with the daily tests. They (183) further showed that samples stored in the receiving room when the temperature varied from 30–38.8° tested lower than those held at 7.2–10°, the differences increased as the storage period was prolonged. Similar results were reported by Campbell *et al.* (38) and others (104, 209). Tracy and Tuckey (218) reported that daily tests on fresh samples agreed closely with their experimental composites, but the routine plant composites tested lower. Holland (104) reported declines of 0.08–0.12 per cent fat in composite samples that he attributed to destabilization of the fat emulsion and which could be avoided by the addition of 0.5 grams of saponin to 250 cc. of milk. Wilster (229) on the other hand, reported no beneficial results from the use of saponin.

With the rise in popularity of homogenized milk, it was necessary to estimate the fat content for commercial purposes. It was observed by Hollingsworth (105) and others (9, 108, 112, 180, 217) that the fat content of homogenized milk was lower than that of the original raw milk by the Babcock method. Trout (219) reported curdy or charred material below the fat column with the regular Babcock procedure, and he found that the specific gravity of this material was higher than the milk fat and was of a tenacious nature. Tracy (217) reported that this condition could be alleviated by adjusting the milk and acid to 21.1° before mixing and by adding the acid in small equal portions to a total of 16 cc. Ruehe (180) stated that if this method is used and the acid is added in five equal portions and the contents shaken thoroughly after each addition, there should be no difference in the results from homogenized and from normal milk. Hood and White (108) obtained clear fat columns with both the acid and the milk at 5–10° and shaking the test bottles thoroughly after the first and after the second whirlings. They reported that milk homogenized at 3500 pounds tested 0.08 per cent lower than the normal milk. Roadhouse and Henderson (177) published essentially the procedure suggested by Hood and White (108) except that the temperature of acid and milk was not stated. The explanation for the lower results from homogenized milk has been ascribed by Hollingsworth (105) to such a fine subdivision of the fat globules that the smaller ones are not separated by the centripetal force. On the contrary, Doan and Swope (58) stated that homogenization had but little influence on the Babcock test and Halloran and Trout (89) reported similar results.

Cream. Babcock (10) compared his method with the Adams gravimetric method on four samples of cream and obtained close agreement, presumably

the whole milk test bottle was used. Hortvet (109) reported that the Babcock averaged 0.4 per cent higher than the Röese-Gottlieb. Ross and McInerney (179) reported results on 58 samples of fresh cream by the Babcock method, using glymol to eliminate the meniscus and a method they called chemical, presumably an extraction method. Both methods checked well, with only eight of the tests varying more than 0.50 per cent. Schrott-Fiechtl (191) reported that the cream test bottle yielded results that compared favorably with the gravimetric method. Close agreement was reported by Fisher and Walts (81) for the Babcock and Mojonnier methods.

Dahlberg (50) estimated the fat content of 34 samples of cream by the Babcock and the Röese-Gottlieb methods and found that the Babcock averaged 0.13 per cent higher, while Mojonnier and Troy (151) reported that the Babcock method averaged 0.33 per cent higher on seven samples of high fat content cream. Doan *et al.* (57) obtained a difference of 0.28 per cent higher with the Babcock method.

Dahlberg *et al.* (48) reported results from three laboratories on 33 samples of fresh cream that showed the average variation of the Babcock from the Röese-Gottlieb was -0.32, $+0.20$, and $+0.34$ per cent. Siegmund and Craig (195) obtained higher results by the Babcock method; they also reported that several additional ether extractions in the gravimetric method did not make any appreciable difference.

Hunziker *et al.* (117) compared daily and preserved composite samples of cream and both agreed when the preserved samples were handled correctly. The composite yielded results that were too high when they were not tightly stoppered, and also when tightly closed but exposed to high temperatures. Lee and Hepburn (129) reported greater variation between composites and individual samples than between two sets of composites, and that there was a tendency for composites to test slightly higher than daily samples in the summer and lower in the winter. Combs *et al.* (41) reported that when composites were prepared in aliquot the producers could expect accurate tests in 60.8 per cent of the deliveries, but the dipper method reduced this accuracy to 45 per cent of the deliveries.

Martin *et al.* (145) in a comprehensive statistical study, from 1599 estimations made on samples obtained from ten gallons of sweet cream and the same cream allowed to sour, found that 97.5 per cent of the readings were approximately within 0.5 per cent of the mean.

DISCUSSION

The early literature revealed much research on volumetric methods of estimating fat in milk before Babcock gave his test to Dairy Science. Babcock's test was a simplification over early methods so that it had immediate practical application. His essential contribution was the use of the one reagent, sulphuric acid, and the application of specified whirling periods with the addition of hot water between whirlings.

The Babcock test originally was standardized for accuracy by comparison with the ether extraction gravimetric method. Some of the comparisons reported in the literature between the Babcock and the gravimetric methods agreed closely, while most of the Babcock results are above and a few below the gravimetric method. The results may be complicated by variable technique in the conduct of the official methods. For example in the laboratory of the Vermont Station it has been shown that weighing four 10-cc. pipettes full of the same milk on the weighing stand in the Mojonnier method, yields lower results for the milk from pipettes numbers 2, 3 and 4. Creaming of the milk with adsorption of the cream to the pipettes during the process of weighing is responsible for these lower results. Pipette number 1 does not remain long enough on the weighing stand for the cream to rise sufficiently so that the results check closely with those obtained by weighing milk directly into the extraction flask. It should be stated that the temperature of the ethers and of the milk may also be a factor of some importance.

The fact that the ether extraction methods also include a portion of the phosphatides in milk, further complicates the comparisons between the Babcock and the official method. Theoretically, the Babcock method should give results that are too high because it was designed to agree with the official procedure. It should also be recalled that the earlier comparisons between the gravimetric and Babcock methods were made when the test bottle was calibrated in 0.2 per cent divisions, thus allowing considerable latitude in interpreting the results.

It is questionable if all the gravimetric methods agree on milk and cream. Theoretically, the gravity separation of the fat-ether solution in the Röese-Gottlieb method should not be as exhaustive as the centrifugal separation in the Mojonnier procedure.[5] It has been observed in the laboratory of the Vermont Station that the Mojonnier vacuum drying oven, operating at 57°, has a small amount of fat in the uppermost surface after a number of determinations have been made; however, the degree of volatilization of fat from each determination must be small so that the error involved may not be great.

The temperature of sampling milk affects the reliability of the results obtained by the Babcock method. Fresh whole milk can be sampled at lower temperatures than preserved milk, because the effect of storage and the action of the preservative changes the physical-chemical nature of the fat emulsion. Therefore, it is necessary to apply heat in order to render the emulsion of fat homogeneous in preserved composites so that a representative sample can be obtained. The composites must be heated to at least 32.2°, and some state regulations specify up to 43°. Composites are also susceptible to handling, consequently, they must be carefully prepared to

[5] Mechanized modification of the Röese-Gottlieb method.

avoid separation of the fat. If they are prepared at 15.6–21.1°, partial churning is apt to occur and the partially churned fat will adhere to the inside of the pipette and the results will be too low.

Another variable factor in comparing the Babcock and the gravimetric methods was the amount of milk used in the Babcock test. Some investigators (17, 50, 111) used pipettes that were standardized to deliver 17.6 cc. of water in 5–8 seconds and this was generally assumed to be the intent of calibration prior to the year 1917. One investigator (172) weighed 18 grams of milk into the test bottles. Since 1925 the A.O.A.C. (8) has specified that the pipette shall contain 17.6 cc. of water at 20°. The method of interpreting the capacity of the pipette has caused some confusion. Babcock (10, 11) considered that the pipette was full when the milk touched the mark on the draw tube, while it is a universal practice for technicians to consider a pipette full when the lowest part of the meniscus is level with the mark.

The centrifuge has undergone considerable change since the inception of the Babcock test. The transition has been from the unheated hand operated to the electrically powered and heated centrifuges. The centrifuge described by De Laval (54, 55, 56) was operated at 50°, while the machine described by Babcock (10) had provision for applying heat. However, a large number of centrifuges were used that could not be heated and finally, the commercial impetus for the use of the heated machines came from the manufacturers who believed that they gave the best results. Many of the early comparisons between the gravimetric and Babcock methods, were made with the unheated centrifuge, and in some instances the whirling mechanism was not inclosed, with trunnion cups and bottles revolving in the open air; thus the contents of the bottles would cool rapidly and subsequent work has indicated that under these conditions the Babcock method yields lower results. Fahl, Lucas and Baten (68, 136) obtained lower results with the unheated as compared to the heated centrifuge, which was verified in the laboratory of the Vermont Station. Thus some of the work reported is complicated by the type of centrifuge used, whether heated or unheated. It is known that some steam-powered centrifuges may operate warmer than others due to the leakage of steam into the chamber. On the other hand, a steam operated centrifuge, open on the top center and not leaking steam, will operate cooler and the results may be lower than those obtained with a heated machine. It is also possible for steam centrifuges to operate too hot. For example, excessive steam leakage into the whirling chamber will heat the test bottles and contents abnormally high and when they are placed in a water bath at 57–60° the contents will recede to a point where the bottom of the fat column is below the lower graduation, thus legal reading is impossible. This condition may be further aggravated by some state regulations that specify the addition of water at 82° or above, between

whirlings, or even require that the centrifuges be operated at higher temperatures. Because of the excessive recession of the fat column, the dimension of the meniscus may be greater and some fat may stick to the glass and thus not be estimated. To again bring the fat column within the graduated scale it is necessary to add water and whirl for about one minute.

The speed of the centrifuge is of importance. If the steam pressure decreases, the speed of the centrifuge is likewise decreased. A similar condition occurs in the electric powered centrifuge when the voltage drops. It was observed in one plant that the speed of the centrifuge decreased when all of the electrically driven equipment was in operation, indicating inadequate power requirements; this would undoubtedly affect the results obtained by the Babcock test. The A.D.S.A (3) in 1922 and the A.O.A.C. (8) in 1925 specified an attached speed indicator, but only one reference (229) reported a centrifuge equipped with a permanently attached tachometer. Some technicians have been observed to judge the desired speed of the centrifuge by the pressure gauge reading, but this is an unreliable procedure.

The water bath was originally intended to standardize the method of estimating the fat columns of a large number of bottles when the unheated centrifuges were used and when laboratories were colder than they are at the present time. The revolving of the centrifuge wheel is a cooling process and the degree of cooling is dependent upon the temperature of the room and whether or not the centrifuge is open or closed in the center. It has been observed that even a modern centrifuge, electrically powered and heated, will decrease in temperature during the first five minute whirling period. It may be possible to eliminate the water bath when readings are made from centrifuges heated to 55–60° in laboratories at 21.1° or above.

The test bottles should be more finely calibrated to reduce the latitude in interpreting the results. A consistent difference of 0.1 per cent in results between two technicians is too much variation for the sake of plant efficiency in accounting for milk fat, and this is within the legal tolerance at the present time. Furthermore, the reading of the Babcock test should be placed on a more scientific basis. There is difference of opinion about the magnitude of the meniscus when estimations are made with the hand calipers in commercial plants. This variable factor can be eliminated by the use of glymol, but the amount of milk used in test will have to be increased so that the results will agree with the official method. The use of the improved Hortvet apparatus (94) has resulted in close agreement between the Babcock and Mojonnier methods (95) with the latter averaging about 0.02 per cent lower. The dimensions of the meniscus can be accurately measured with this device.

The Babcock test is somewhat empirical and some refinements are necessary in order that it may continue to be acceptable to the Dairy Industry and maintain the confidence of all concerned. In this connec-

tion it should be stated that the Gerber test is gaining popularity because of alleged simplicity and speed of obtaining results. The states of New York and New Jersey have legalized this method. It is possible that the Babcock test may be modified so as to shorten the procedure, without sacrificing accuracy.

REFERENCES

(1) ALÉN, J. A. Zentbl. f. Agr. Chem., **21**: 549–550. 1892.
(2) AMBROSE, A. S. Miss. Agr. Ext. Bul. 13. 1919.
(3) AMERICAN DAIRY SCIENCE ASSOCIATION. JOUR. DAIRY SCI., **1**: 38–44. 1917–1918. JOUR. DAIRY SCI., **4**: 165–166. 1921. JOUR. DAIRY SCI., **5**: 175–182. 1922.
(4) ANDREWS, W. N. Y. Produce Rev. and Amer. Creamery, **33**: 2, 90–92. 1911.
(5) ANONYMOUS. Vt. Agr. Expt. Sta. Bul. 21. 1890.
(6) ANONYMOUS. Iowa Agr. Expt. Sta. Ann. Rpt. 30. 1918.
(7) ANONYMOUS. Mich. Agr. Expt. Sta. Bien. Rpt. 25. 1933–1934.
(8) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis.* Washington, D. C. U. S. Dept. Agr. Bur. Chem. Bul. 65, 1902; Bul. 107, 1908; Bul. 122, 1909; Jour. A.O.A.C., **2**: 3, 289–290, 1916; A.O.A.C., 1920, 1925, 1930, 1935, 1940.
(9) BABCOCK, C. J. U. S. Dept. Agr. Tech. Bul. 438. 1934.
(10) BABCOCK, S. M. Wisc. Agr. Expt. Sta. Bul. 24. 1890.
(11) BABCOCK, S. M. Wisc. Agr. Expt. Sta. 7th Ann. Rpt., 98–113. 1890.
(12) BABCOCK, S. M. Wisc. Agr. Expt. Sta. 9th Ann. Rpt., 219–244. 1892.
(13) BABCOCK, S. M. Wisc. Agr. Expt. Sta. Bul. 31. 1892.
(14) BABCOCK, S. M. Wisc. Agr. Expt. Sta. 10th Ann. Rpt., 116–151. 1893.
(15) BABCOCK, S. M. Wisc. Agr. Expt. Sta. Bul. 36. 1893.
(16) BABCOCK, S. M., AND FARRINGTON, E. H. Wisc. Agr. Expt. Sta. Bul. 195. 1910.
(17) BAILEY, D. E. JOUR. DAIRY SCI., **2**: 331–373. 1919.
(18) BAILEY, D. H. Pa. Agr. Expt. Sta. Bull. 310. 1934.
(19) BAILEY, D. H., TWINING, F. M., MITTEN, J. W., AND SWOPE, W. D. 25th Ann. Conv. Internatl. Assoc. Milk Dealers, Lab. Sec. Proc., 157–170. 1932.
(20) BAILEY, D. H., MITTEN, J. W., AND TWINING, F. M. 26th Ann. Conv. Internatl. Assoc. Milk Dealers, Lab. Sec. Proc., 81–95. 1933.
(21) BALL, F. E. Colo. Agr. Expt. Sta. Bul. 295. 1924.
(22) BARTHEL, C. Methods Used in the Examination of Milk and Dairy Products. Translated by Goodwin, W. Macmillan and Co. London, Eng. 1910.
(23) BARTLETT, J. M. Me. Agr. Expt. Sta. Bul. 3. 1891.
(24) BARTLETT, J. M. Me. Agr. Expt. Sta. Bul. 4. 1893.
(25) BARTLETT, J. M. Me. Agr. Expt. Sta. Bul. 15. 1894.
(26) BARTLETT, J. M. Me. Agr. Expt. Sta. Bul. 31. 1896.
(27) BARTLETT, J. M. Me. Agr. Expt. Sta. Rpt., 165–172. 1896.
(28) BEACH, C. L. Conn. (Storrs) Agr. Expt. Sta. Bul. 40. 1906.
(29) BEARCE, H. W. Jour. Agr. (U.S.) Res., **3**: 3, 251–268. 1914.
(30) BEBEE, A. C. Chicago Dairy Produce, **7**: 71, 22. 1901.
(31) BEVAN, E. J. The Analyst, **20**: 152–158. 1895.
(32) BINGMAN, R. G. Private communication. 1941.
(33) BLYTHE, A. W. Ztschr. f. Analyt. Chem., **27**: 91. 1888.
(34) BOUCHARDAT, M., AND QUEVENNE, T. A. Du Lait. Premier Fascicule. Pp. 63–68. Paris, 1857. Cited by Schmidt (186). Original book not examined.
(35) BURKE, A. D. Okla. Agr. Expt. Sta. Cir. 65. 1926.

* Title previous to 1920 was slightly different.

(36) CALDWELL, G. C. N. Y. (Cornell) Agr. Expt. Sta. Bul. 17. 1890.
(37) CALDWELL, W. A., AND HERREID, E. O. JOUR. DAIRY SCI., **21**: 140. 1938.
(38) CAMPBELL, H. C., JAGGARD, G., AND MORRIS, D. Milk Dealer, **21**: 3, 42. 1931.
(39) Chicago Dept. Health Rpt., p. 18. 1906. Cited by Farrington and Woll (80). 1912.
(40) COLLIER, P. N. Y. (Geneva) Agr. Expt. Sta. Bul. 19. 1890.
(41) COMBS, W. B., THURSTON, L. M., GROTH, A. E., AND COULTER, S. T. Minn. Agr. Expt. Sta. Bul. 243. 1927.
(42) COCHRAN, C. B. Jour. Analyt. Chem., **3**: 381–385. 1889.
(43) CREAMERY PACKAGE MFG. CO. The Origin and History of Creamery Package. The Creamery Package Mfg. Co., Chicago, Ill., p. 14. 1937.
(44) CRONANDER, N. Milch Ztg. (Bremen), **15**: 161–163. 1886.
(45) CROWE, L. K., AND DAVIS, H. P. Nebr. Agr. Expt. Sta. Cir. 35. 1927.
(46) DAHLBERG, A. C. 23rd Ann. Conv. Internatl. Assoc. Milk Dealers, Lab. Sec. Proc., 139–146. 1930.
(47) DAHLBERG, A. C. N. Y. State Assoc. Dairy and Milk Insp. Ann. Rpt., **6**: 191–196. 1932.
(48) DAHLBERG, A. C., HOLM, G. E., AND TROY, H. C. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 122. 1926.
(49) DAHLBERG, A. C., AND POWERS, A. J. 25th Ann. Conv. Internatl. Assoc. Milk Dealers, Lab. Sec. Proc., 171–176. 1932.
(50) DAHLBERG, A. O. Jour. Assoc. Off. Agr. Chem., **7**: 159–169. 1923.
(51) DEAN, H. H. Ontario Agr. and Expt. Farm Bull. 76. 1892.
(52) DEAN, H. H. Ontario Agr. Coll. and Expt. Farm Ann. Rpt., 125–127. 1906.
(53) DIETZSCH, O. Repert. d. Anal. Chem., **1**: 33–34. 1881.
(54) DE LAVAL, C. G. P. Kaiserliches Patentamt. Patentschrift. 35810. 1885. Photographic copy obtained from the U. S. patent office.
(55) DE LAVAL, C. G. P. Dinglers Polytechnisches Jour., **261**: 219–220. 1886.
(56) DE LAVAL, C. G. P. Chem. Ztg., **10**: 66, 1003. 1886.
(57) DOAN, F. J., FIELDS, J. N., AND ENGLAND, C. W. JOUR. DAIRY SCI., **6**: 406–411. 1923.
(58) DOAN, F. J., AND SWOPE, W. D. Penn. Agr. Expt. Sta. 40th Ann. Rpt. and Bull. 213. 1927.
(59) EATON, W. H. N. C. Agr. Expt. Sta. Bul. 240. 1918.
(60) ECKLES, C. H. Iowa Agr. Expt. Sta. Bul. 52. 1900.
(61) ECKLES, C. H. Dairy and Creamery, **4**: 2, 2–3. 1902.
(62) ECKLES, C. H., AND WAYMAN, H. S. N. Y. Produce Rev. and Amer. Creamery, **26**: 11, 422. 1908.
(63) EMERY, F. E. N. C. Agr. Expt. Sta. Bul. 113. 1895.
(64) EMIL GREINER CO. Cited by Holland (103). 1908.
(65) ENGLAND, C. W., AND D'AMBROGI, G. D. Md. Agr. Expt. Sta. Bul. 413. 1937.
(66) EUGLING, W., AND KLENZE, V. Milch Ztg. (Bremen), **16**: 509–512. 1887.
(67) FABER, H. The Analyst, **12**: 6–11. 1887.
(68) FAHL, J. R., LUCAS, P. S., AND BATEN, W. D. Mich. Agr. Expt. Sta. Tech. Bul. 158. 1938.
(69) FAILYER, G. H., AND WILLARD, J. T. Kan. Agr. Expt. Sta. 1st Ann. Rpt., 149–164. 1888.
(70) FARRINGTON, E. H. Ill. Agr. Expt. Sta. Bul. 10. 1890.
(71) FARRINGTON, E. H. Ill. Agr. Expt. Sta. Bul. 14. 1891.
(72) FARRINGTON, E. H. Ill. Agr. Expt. Sta. Bul. 16. 1891.
(73) FARRINGTON, E. H. Ill. Agr. Expt. Sta. Bul. 18. 1891.
(74) FARRINGTON, E. H. Ill. Agr. Expt. Sta. Bul. 27. 1893.

(75) FARRINGTON, E. H. Wisc. Agr. Expt. Sta. 19th Ann. Rpt., 147–149. 1902.
(76) FARRINGTON, E. H., AND WOLL, F. W. Wisc. Agr. Expt. Sta. 20th Ann. Rpt., 118–129. 1903.
(77) FARRINGTON, E. H., AND WOLL, F. W. Testing Milk and Milk Products. 6th ed., p. 37. Mendota Book Co., Madison, Wisc. 1897.
(78) FARRINGTON, E. H., AND WOLL, F. W. Testing Milk and Its Products. 18th ed. Mendota Book Co., Madison, Wisc. 1911.
(79) FARRINGTON, E. H., AND WOLL, F. W. Testing Milk and Its Products. 19th ed. Mendota Book Co., Madison, Wisc. 1912.
(80) FARRINGTON, E. H., AND WOLL, F. W. Testing Milk and Its Products. 23rd ed. Mendota Book Co., Madison, Wisc. 1916.
(81) FISHER, R. C., AND WALTS, C. C. Conn. (Storrs) Agr. Expt. Sta. Bul. 131. 1925.
(82) FRAHM, H. Milch Ztg. (Bremen), **16**: 881–882. 1887.
(83) FREAR, WM., AND HOLTER, G. L. Penn. State Coll. Ann. Rpt., 172–187. 1890.
(84) FULLER, J. M. Okla. Agr. Expt. Sta. Bul. 107. 1915.
(85) GERBER, N. Milch Ztg., p. 891. 1892; pp. 363, 656. 1893; pp. 169. 1895. Cited by Barthel (22). Original paper not examined.
(86) GERBER, N. Milch Ztg. (Bremen), **18**: 265–266. 1889.
(87) GIRARD, C. Chem. Ztg., **33**: 586. 1909.
(88) GRÉLOT, M. P. Jour. Pharm. et Chem., 6 ser., **25**: 9, 423–28. 1907.
(89) HALLORAN, C. P., AND TROUT, G. MALCOLM. Proc. 27th Ann. Meet. Amer. Dairy Sci. Assoc., 17. 1932.
(90) HARRISON, T. B. Proc. 27th Ann. Meet. Amer. Dairy Sci. Assoc., 16. 1932.
(91) HAYS, W. P. Mo. Agr. Expt. Sta. Cir. 119. 1924.
(92) HAYWARD, H., AND MCDONNELL, M. E. Penn. Agr. Expt. Sta. Bull. 33. 1895.
(93) HEINRICH, R. Molk. Ztg. (Hildesheim), **7**: 4, 37–38. 1893.
(94) HERREID, E. O. JOUR. DAIRY SCI., **23**: 526–527. 1940.
(95) HERREID, E. O. Unpublished results. Vt. Agr. Expt. Sta. 1941.
(96) HILL, T. E. The Analyst, **16**: 67–73. 1891.
(97) HILLS, J. L. Vt. Agr. Expt. Sta. 11th Ann. Rpt., 350–355. 1898.
(98) HILLS, J. L. Vt. Agr. Expt. Sta. Rpt., 368–370. 1898.
(99) HILLS, J. L. Vt. Agr. Expt. Sta. 16th Ann. Rpt., 287–288. 1903.
(100) HILLS, J. L. Vt. Agr. Expt. Sta. Bul. 100. 1904.
(101) HITE, B. H. W. Va. Agr. Expt. Sta. Rpt., 68–88. 1889.
(102) HITTCHER, DR. Milch Ztg. (Bremen), **22**: 21, 339–341. 1893.
(103) HOLLAND, E. B. Mass. Agr. Expt. Sta. 20th Ann. Rpt. Publ. Doc., **31**: 219–225. 1908.
(104) HOLLAND, R. F. JOUR. DAIRY SCI., **21**: 141. 1938.
(105) HOLLINGSWORTH, J. B. Milk Dealer, **20**: 9, 63. 1931.
(106) HOLM, M. L. Amer. Food. Jour., **2**: 7, 28. 1907.
(107) HOLTER, G. L. Pa. Agr. Expt. Sta. and State Coll. Ann Rpt., 134–137. 1891.
(108) HOOD, E. G., AND WHITE, A. H. Reported by R. J. Quirie, Proc. 28th Ann. Conv. Internatl. Assoc. Milk Dealers, Plant Sec., 67–91. 1935.
(109) HORTVET, J. Jour. Assoc. Off. Agr. Chem., **2**: 238–257. 1917.
(110) HORTVET, J. United States patent 1,611,448. 1924.
(111) HOYT, C. F. Jour. Assoc. Off. Agr. Chem., **6**: 354–362. 1923.
(112) HUDSON, M. H. Food Indus., **3**: 153–156. 1931.
(113) HUNDERTMARK, R. E. Wash. Agr. Expt. Sta. Popular Bul. 75. 1915.
(114) HUNZIKER, O. F. Report Ann. Meeting Official Dairy Instructors Assoc. Milwaukee, Wisc. 1909. Cited by Smith (202).
(115) HUNZIKER, O. F. Ind. Agr. Expt. Sta. Cir. 42. 1914.
(116) HUNZIKER, O. F. Private communication. 1941.

(117) HUNZIKER, O. F., SPITZER, G., MILLS, H. C., AND CRANE, P. H. Ind. Agr. Expt. Sta. Bul. 145. 1910.
(118) JACK, E. L., AND ABBOTT, F. H. Proc. 23rd Ann. Meet. West. Div. Amer. Dairy Sci. Assoc. 1937.
(119) JACKSON, H. C. JOUR. DAIRY SCI., 2: 170–182. 1919.
(120) JAMES, C. C. Ontario Agr. Coll. Expt. Sta. Bul. 61. 1891.
(121) JONES, C. H. Vt. Agr. Expt. Sta. 16th Ann. Rpt., 191–196. 1903.
(122) JONES, H. M., AND WRIGHT, T. H. S. D. Agr. Expt. Sta. Bul. 197. 1922.
(123) KENT, F. L. Ore. Agr. Expt. Sta. Bul. 70. 1902.
(124) KERR, J. M. Jour. Dept. Agr. Vict., 4: 204–206, 211. 1936.
(125) KINGSLEY, P. M. Private communication. 1941.
(126) KOCHHEISER, D. S. 23rd Ann. Conv. Internatl. Assoc. Milk Dealers, Lab. Sec. Proc., 147–166. 1930.
(127) KRÄMER AND SCHULZE. Biedermann's Zentbl. f. Agr. Chem., 9: 232. Cited by Schmidt (186). 1878. Original paper not examined.
(128) KRETZSCHMAR, M. Chem. Ztg. (Göthen), 11: 9, 18–19. 1887.
(129) LEE, C. E., AND HEPBURN, N. W. Ill. Agr. Expt. Sta. Bul. 153. 1912.
(130) LEFFMANN, H., AND BEAM, W. The Analyst, 17: 83–84. 1892.
(131) LEFFMANN, H., AND BEAM, W. Analysis of Milk and Milk Products. P. Blakiston, Son & Co., Philadelphia, Pa., 26–28. 1893.
(132) LÉZE, M. Compt. Rend., 110: 647–649. 1890.
(133) LIEBERMANN, L. Ztschr. f. Analyt. Chem., 22: 383–390. 1883.
(134) LINDET, M. Rev. Gen. Lait, 2: 16, 370–372. 1903.
(135) LIVERANCE, W. B. Mich. Agr. Expt. Sta. Cir. 2. 1909.
(136) LUCAS, P. S. JOUR. DAIRY SCI., 20: 457. 1937.
(137) MAGRUDER, A. C. W. Va. Agr. Expt. Sta. Bul. 4. 1889.
(138) MANCHESTER, T. C. Orig. Commun. 8th Internatl. Cong. Appl. Chem. (Washington and New York), 15: 179–185. 1912.
(139) MANNS, A. G. Ill. Agr. Expt. Sta. Bul. 9. 1890.
(140) MARCHAND, E. Jour. de Pharm. et de Chimie., 26: 344–352. 1854.
(141) MARCHAND, E. Jour. de Chim. Med. Nov. 1855, p. 641. Chem. Zentbl., 1: 319. 1856.
(142) MARCHAND, E. Jour. de Pharm. et de Chimie., 26: 352–359. 1854.
(143) MARQUARDT, J. C. Calif. Agr. Expt. Sta. Cir. 230. 1921.
(144) MARQUARDT, J. C., AND DURHAM, H. L. N. Y. (Geneva) Agr. Expt. Sta. Bul. 605. 1932.
(145) MARTIN, W. H., FAY, A. C., AND RENNER, K. M. Jour. Agr. (U. S.) Res., 41: 147–159. 1930.
(146) MEADE, D., AND LECKIE, J. N. Milk Plant Monthly, 25: 8, 28–30. 1936.
(147) MECHAM, M. C. Unpublished results. Quoted by England and D'Ambrogi (66). 1937.
(148) MITCHELL, J. W., AND WALKER, W. O. Ontario Dept. Agr. Bul. 170. 1909.
(149) MOJONNIER BROTHERS CO. Instruction Manual for Setting up and Operating the Mojonnier Milk Tester. Bul. 101. 1925.
(150) MOJONNIER, F. Private communication. 1940.
(151) MOJONNIER, T., AND TROY, H. C. The Technical Control of Dairy Products. Mojonnier Bros. Co., Chicago, Ill., 49–55. 1922. 2nd ed., 1925.
(152) MONROE, C. F. Ohio Agr. Expt. Sta. Ann. Rpt., 127–128. 1929.
(153) MYERS, J. A., AND MAGRUDER, A. C. W. Va. Agr. Expt. Sta. Bul. 6. 1889.
(154) MCCONNELL, T. F. Ariz. Agr. Expt. Sta. Rpt. 487. 1904.
(155) NELSON, D. H. Calif. Agr. Expt. Sta. Ann. Rpt., 63–64. 1926.
(156) NELSON, D. H. JOUR. DAIRY SCI., 11: 108–109. 1928.

(157) NELSON, D. H. Calif. Agr. Expt. Sta. Cir. 340. 1937.
(158) NEUMANN, J. Milch Ztg., **22**: 453–456. 1893.
(159) NEWLANDER, J. A. Testing Dairy Products. The Olson Publishing Co., Milwaukee, Wisc., 12–33. 1932.
(160) NILSON, L. F. Chem. Ztg., **15**: 37, 649–656. 1891.
(161) OFFICIAL DAIRY INSTRUCTORS ASSOCIATION. 1911. Cited by Farrington, E. H., and Woll, F. W. (81).
(162) OTIS, D. H. Kan. Agr. Expt. Sta. Bul. 125. 1904.
(163) OVEN, A. V. Milchw. Zentbl., **43**: 11, 285–295; 13, 350–356. 1914.
(164) OVERMAN, O. R., AND GARRETT, O. F. Ill. Agr. Expt. Sta. Cir. 390. 1932.
(165) PARSONS, C. L. N. H. Agr. Expt. Sta. 1st Ann. Rpt., 69–83. 1888.
(166) PATRICK, G. E. Iowa Agr. Expt. Sta. Bul. 8. 1890.
(167) PATRICK, G. E. Iowa Agr. Expt. Sta. Bul. 9. 1890.
(168) PATTERSON, L. G. Miss. Agr. Expt. Sta. Bul. 15. 1891.
(169) PETER, H. Jour. f. Landw., **32**: 197–200. 1884.
(170) PETERSEN, W. E. JOUR. DAIRY SCI., **7**: 361. 1924.
(171) PETERSEN, W. E. JOUR. DAIRY SCI., **10**: 261–262. 1927.
(172) PHILLIPS, C. A. JOUR. DAIRY SCI., **6**: 549–555. 1923.
(173) POTTS, A. E. Thesis, Cornell Univ. Library. 1913. Cited by Jackson (119). Original paper not examined.
(174) POWERS, A. J. 26th Ann. Conv. Internatl. Assoc. Milk Dealers, Lab. Sec. Proc., 95–159. 1933.
(175) RAWL, B. H. S. C. Agr. Expt. Sta. Bul. 95. 1905.
(176) RIDEAL, S. The Analyst, **20**: 157–158. 1895.
(177) ROADHOUSE, C. L., AND HENDERSON, J. L. The Market Milk Industry. McGraw-Hill Book Co., Inc., New York. 1941.
(178) RONNEBERG, C. Milch Ztg., **22**: 459. 1893.
(179) ROSS, H. E., AND MCINERNEY, T. J. N. Y. (Cornell) Agr. Expt. Sta. Bul. 337. 1913.
(180) RUEHE, H. A. Milk Plant Monthly, **26**: 7, 60. 1937.
(181) RUSSELL, H. L. Wisc. Agr. Expt. Sta. Cir. Inform. 32. 1912.
(182) SANMANN, F. P., AND OVERMANN, O. R. The Creamery and Milk Plant Monthly, **15**: 7, 39–40. 1926.
(183) SANMANN, F. P., AND OVERMANN, O. R. The Creamery and Milk Plant Monthly, **15**: 6, 39–41. 1926.
(184) SANMANN, F. P., AND OVERMANN, O. R. The Creamery and Milk Plant Monthly, **15**: 6, 42–43. 1926.
(185) SCHMID, W. Ztschr. f. Analyt. Chem., **27**: 464. 1888.
(186) SCHMIDT, F. Jour. f. Landw., **26**: 361–400. 1878.
(187) SCHMIDT, F., AND TOLLENS, B. Centbl. f. Agr. Chem., **12**: 226–229. 1878.
(188) SCHMOEGER, M. Jour. f. Landw., **29**: 129–143. 1881.
(189) SCHRODT, M., AND HENZOLD, O. Milch Ztg. (Bremen), **16**: 554–557. 1887.
(190) SCHROTT-FIECHTL, H. Milch Ztg., **25**: 183–185; 199–201; 217–220. 1896.
(191) SCHROTT-FIECHTL, H. Milch Ztg., **26**: 830. 1897.
(192) SEBELIEN, J. Die Landw. Versuchs-Stationen, **33**: 393–410. 1887.
(193) SEBELIEN, J., AND STOREN, K. Norsk Landmandsblad, **13**: 325–329; 332–334. 1894.
(194) SIEGFELD, M. Molk. Ztg., **13**: 51. 1899.
(195) SIEGMUND, H. B., AND CRAIG, R. S. JOUR. DAIRY SCI., **4**: 32–38. 1921.
(196) SHIVER, F. S. S. C. Agr. Expt. Sta. Bul. 33. 1898.
(197) SHORT, F. G. Wisc. Agr. Expt. Sta. Bul. 16. 1888.
(198) SHUTT, F. T. The Analyst, **17**: 227. 1892.
(199) SHUTT, F. T. Canada Exptl. Farm Rpt., 135–137. 1893.

(200) SHUTT, F. T. Canada Exptl. Farm Rpt., 149–151. 1893.
(201) SJÖSTRÖM, A. Tidskr. f. Landtmän, **11**: 552–556. 1889. Bied. Centbl. f. Agr. Chem., **19**: 855–856. 1890. Original paper not examined.
(202) SMITH, P. H. Mass. Agr. Expt. Sta. 22nd Ann. Rpt., Part 1, Being Part 3 of the 47th Ann. Rpt. of Mass. Agr. Coll., 142–147. 1910.
(203) SNYDER, H. N. Y. (Cornell) Agr. Expt. Sta. Bul. 25. 1890.
(204) SNYDER, H. N. Y. (Cornell) Agr. Expt. Sta. Bul. 29. 1891.
(205) SOXHLET, F. Rept. d. Anal. Chem., **7**: 250. Ztschr. f. Analyt. Chem., **27**: 92. 1888. Original paper not examined.
(206) SPILLMAN, W. J. Wash. Agr. Expt. Sta. Bul. 18. 1895.
(207) SPILLMAN, W. J. Wash. Agr. Expt. Sta. Bul. 32. 1897.
(208) SPITZER, G., AND EPPLE, W. F. JOUR. DAIRY SCI., **7**: 131–137. 1924.
(209) SPROULE, W. H. The Babcock Test. Cited by Webster (224). 1938.
(210) STANDARD METHODS OF MILK ANALYSIS. Amer. Pub. Health Assoc., New York City. 1923, 1925, 1934, 1939*.
(211) STOKES, A. W. Chem. News, 60: 214–215. 1889.
(212) SWOPE, W. D. Pa. Agr. Expt. Sta. Cir. 129. 1929.
(213) THEOPHILUS, D. R., AND BARNETT, D. C. Proc. 24th Ann. Meet. West. Div. Amer. Dairy Sci. Assoc., 76–79. 1938.
(214) THOMPSON, R. T. Glasgow City Soc. Rpts., p. 5. 1895.
(215) TOLLENS, B., AND GROTE, F. Jour. f. Landw., **27**: 145–152. 1879.
(216) TOLSTRUP, M. R., AND MORTENSEN, M. Iowa Agr. Expt. Sta. Cir. 32. 1916.
(217) TRACY, P. H. 28th Ann. Conv. Internatl. Assoc. Milk Dealers, Plant Sec. Proc., 39–66. 1935.
(218) TRACY, P. H., AND TUCKEY, S. L. Ill. Agr. Expt. Sta. Bul. 459. 1939.
(219) TROUT, G. M. 26th Ann. Conv. Internatl. Assoc. Milk Dealers, Lab. Sec. Proc., 199–200. 1933.
(220) TROY, H. C., AND SHARP, P. F. JOUR. DAIRY SCI., **20**: 77–81. 1937.
(221) UNITED STATES BUREAU OF STANDARDS. U. S. Bur. of Stand. Cir. 9: 15–23. 1916.
(222) VAN SLYKE, L. L. Modern Methods of Milk Testing. Orange Judd Co., New York. *1906, 1907, 1913.*
(223) WEBSTER, E. H. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 58. 1904.
(224) WEBSTER, H. G. Ontario Milk Producer, **14**: 6, 122. 1938.
(225) WELD, I. C. N. H. Agr. Expt. Sta. Bul. 126. 1905.
(226) WHITE, W. B. N. Y. Ann. Rpt. Agr. and Markets. 1930.
(227) WHITMAN, R. C. Jour. Amer. Med. Assoc., **47**: 204–205. 1906.
(228) WILEY, H. W. Principles and Practice of Agricultural Analysis. Chemical Publishing Co., Easton, Pa., **3**: 499. 1897.
(229) WILSTER, G. H., AND ROBICHAUX, R. P. Ore. Agr. Expt. Sta. Bul. 383. 1940.
(230) WINDISCH, K. Milchw. Zentbl., **4**: 97–108. 1908.
(231) WINTON, A. L. Conn. (New Haven) Agr. Expt. Sta. Bul. 106. 1891.
(232) WINTON, A. L. Conn. (New Haven) Agr. Expt. Sta. Bul. 108. 1891.
(233) WINTON, A. L. Conn. (New Haven) Agr. Expt. Sta. Ann. Rpt., 107–123. 1891 (1892).
(234) WINTON, A. L. Conn. (New Haven) Agr. Expt. Sta. Bul. 117. 1894.
(235) WINTON, A. L., AND OGDEN, A. W. Conn. (New Haven) Agr. Expt. Sta. Bull. 119. 1894.
(236) WINTON, A. L., AND OGDEN, A. W. Conn. (New Haven) Agr. Expt. Sta. 28th Ann. Rpt., 209–244. 1894.
(237) WOLL, F. W. Wisc. Agr. Expt. Sta. 17th Ann. Rpt., 76–81. 1900.
(238) ZEHENTER, J. Chem. Centbl., **65**: 458–459. 4 Folge, 6 Jahrg. Band 2. 1894.
(239) ZIEGLER, P. R. Private communication.

* Title has been changed, slightly.

American Dairy Science Association Announcement

March 9, 1942

To the Members of the
American Dairy Science Association:

I am happy to report to you that our committees have nearly completed their plans for the meeting of the Association here at Michigan State College June 22–26. Our dairy staff as well as the numerous other members of the Association located in Michigan extend you the invitation to be with us.

Those of us here are fully aware that unusual conditions confront our members this year as they contemplate any trip. We are even reconciled to a possibility that some may not be able to come to East Lansing. Nevertheless, our committees are proceeding in a belief that an especial obligation devolves upon us this year, more than in normal times, to provide a profitable and memorable meeting.

The May number of the JOURNAL OF DAIRY SCIENCE will furnish you the detailed program for the meeting. You will note from the program that first attention centers on those questions of especial import to dairy workers today. The role our industry plays in our national emergency makes it imperative that we dairy workers keep apace. Some persons express a conviction that never before in our experience has there been a more urgent need for dairy workers to meet for conferences on technical topics and for discussion and dissemination of research results pertaining to our industry. And all of us are involved whether we be engaged in research, in instructional work either resident or extension, in plant operation or engaged in regulatory activities.

Shortly you will receive from our publicity committee some useful material pertaining to the meeting and Michigan. This will be of interest to your families also. In due time our Registration Committee will communicate with you. Your cooperation is solicited in complying with the requests this committee will make of you.

Sincerely,

EARL WEAVER,
Head Dairy Department,
Michigan State College

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SPECIAL PUBLICATIONS

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The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

212. The Curd Number Test. A Method of Testing the Curdling Qualities of Milk. BERNHARD SPUR AND IRVING J. WOLMAN, Milk Research Laboratory, Children's Hospital, Philadelphia, Pa.

The present paper records a fresh approach to the investigation of coagulation of milk in the human stomach and describes: 1. A device for reproducing more closely the actual happenings which take place within the human stomach following the taking of milk; 2. A standard procedure for producing, preserving and measuring the curds which form; and 3. An empirically derived scale for indicating the curd size distribution. The method utilizes the apparatus developed by Chambers and Wolman but with a routine of operation considerably altered from the initial report.

When a specimen of milk is made to coagulate within an artificial curdling device under rigidly controlled conditions, the curds which form manifest a size distribution which appears to be a constant physical characteristic of the milk undergoing test. After hardening, drying, sieving and weighing the masses of curds thus obtained and then applying to the weight data a so-called "a-b-c" formula empirically derived, it is possible to arrive at a "curd number" which epitomizes the milk's curdling qualities. This proposed technique for curd number has been subjected to critical analysis and found to be experimentally useful in problems dealing with the coagulating properties of cow's milk preparations as related to human digestion. Curd number has been found in general to run parallel to curd tension, but its approach is broader in scope and of greater applicability to research.

213. A Study of the Coliform Group in Ice Cream. H. J. FOURNELLE AND H. MACY, University of Minnesota.

The coliform content of commercial ice cream and other frozen desserts was determined. The most probable numbers ranged from 0 to 9,180 per milliliter in factory-packed samples and from 0 to 101,000 per milliliter in scoop samples from retail stores. Brilliant green-bile broth proved to be a satisfactory presumptive medium. The following species were isolated: *Escherichia coli, E. coli* var. *acidilactici, E. coli* var. *neapolitana, E. coli* var. *communior, E. freundii, Aerobacter aerogens,* and *A. cloacae.* The last-named species was most prevalent.

214. Curd Strength of Evaporated Milk. J. C. MARQUARDT AND D. W. DENNISTON, New York (Geneva) Agr. Expt. Sta.

A study has been made with properly selected samples which defines re-

constituted evaporated milk in terms of its curd strength and creaming power.

Comparisons between the curd strength standards established by Hill and those of the American Medical Association and actual commercial homogenized milk and re-constituted evaporated milk have been made.

215. A Statistical Study of the Influence of Moisture and Acidity on the Palatability and Fermentation Losses of Ensiled Hay Crops. T. E. WOODWARD AND J. B. SHEPHERD, U. S. Dept. Agr. Bureau of Dairy Industry.

Silage was made in different kinds of silos and from different kinds of grasses and legumes at the Beltsville Research Center. Comparisons were made of silages with high and low contents of moisture and with high and low acidities. In some of the silages the acidity was increased by molasses; in others, by hydrochloric and sulphuric acids. The effects of moisture and acidity were measured by the quality of silage as judged by the odor and by the quantity of dry matter cows would consume; they were also measured by the losses in the silo of dry matter, protein, and carotene. The odor of silage made from high moisture legumes was improved by all three treatments—a reduction of the moisture content, the addition of molasses, and the addition of acids. The quantity of dry matter that cows would consume was increased significantly by a reduction in the moisture content, and less significantly by the addition of molasses; while the addition of the acids greatly reduced the consumption of dry matter. The losses of carotene were significantly increased by a reduction in the moisture content.

216. The Nutritive Value of Alfalfa Hay. I. Cystine as a Supplement to an all Alfalfa Hay Ration for Milk Production. C. F. HUFFMAN AND C. W. DUNCAN, Mich. Agr. Expt. Sta., East Lansing.

Five lactating cows which had received alfalfa hay as their sole ration since calving and which had declined in milk production to the point where they were consuming larger amounts of total digestible nutrients than were required by a liberal standard were used to study the supplementary value of l-cystine on milk production. The addition of 20 gm. of cystine per day to the ration of each of four cows appeared to check the rapid decline in milk and fat production obtained on an all alfalfa hay ration but did not increase milk production significantly. The addition of 40 gm. of cystine per day to the ration of one cow resulted in a marked decrease in milk and fat production. Body weight increased but the consumption of hay decreased. The replacement of part of the alfalfa hay with isocaloric amounts of corn or barley resulted in significant increases in milk and fat production over the initial alfalfa feeding period and the subsequent cystine feeding period.

The results of this experiment indicate that cystine is not the first deficiency of an all alfalfa hay ration or of an alfalfa and corn starch ration.

217. Seventy Years of Selection for Conformation in Dairy Cattle. A. A. LEWIS, Department of Dairy Husbandry, University of Missouri.

A study is reported of 5180 Commended and Highly Commended Island Jersey cattle and their parents. The data were tabulated in five period groups from 1866, including practically all calves registered during each period.

It was found that the proportion of the sires of Island Jersey cattle which were classified as Highly Commended increased from 64 per cent in 1866–82 to 82 per cent in 1890–3, and rose to the surprising proportion of 90 per cent in 1930–5. Only 29 per cent of the dams were HC in 1866–83, but over $\frac{2}{3}$ were so classified in three of the following periods. There appeared to be a greater tendency to select HC bulls than heifers from HC sires and dams.

The mating of Commended sires to Commended dams declined from 27 per cent of the total matings in 1866–82 to 2.8 per cent in 1930–5. HC males $\times$ C dams declined from 43.6 per cent to 17.5 per cent while HC $\times$ HC matings increased from 21 per cent to 73 per cent in the same periods. There appeared to be assortive mating among the parents of registered bull calves in the 1866–82 and 1930–5 periods. The proportion of HC progeny to HC parents shows an increase, from 23 per cent to 43 per cent, between the first and second periods and thereafter considerable fluctuation but no consistent increase.

When the dams were only Commended about half as many calves were rated HC as when the dams were HC. Whether the sires were HC or C made little difference. Whether this was due to a greater genetic influence of the dam on her offspring or was a result of the selection practiced was undetermined.

218. The Relationship of Errors in the Babcock Test to Losses in Cream Plants. J. L. HILEMAN, K. K. RUSH, AND CLARENCE MOSS, Dairymen's League Co-operative Association, Inc., Syracuse, N. Y.

The Babcock test gives a result that is too high in the case of both milk and cream. Because the error is proportionately greater in milk than in cream, a loss results. This loss increases with increasing fat content of the milk skimmed, and decreases with increasing fat content of the cream produced. It may vary from about 0.35 per cent where cream testing 50 per cent fat is made from milk testing 3.35 per cent fat, up to about 3 per cent where cream testing 20 per cent fat is made from milk testing 5 per cent fat.

The Babcock test for fat in skim milk shows only about one-seventh of the fat actually present according to the Mojonnier test. If this fat in the skim milk is ignored on butterfat accounting, it will be impossible to explain the loss of fat which it represents. This loss amounts to between 1.5 per cent and 2.0 per cent.

219. Vitamin A and Carotene Requirements for the Maintenance of Adequate Blood Plasma Vitamin A in the Dairy Calf. P. D. BOYER, P. H. PHILLIPS, N. S. LUNDQUIST, C. W. JENSEN AND I. W. RUPEL, University of Wisconsin, Madison.

Studies have been made to determine the blood plasma concentrations and the intakes of carotene and vitamin A necessary for the growing calf.

The data obtained showed that the blood plasma vitamin A was a more delicate measure of the state of vitamin A nutrition in the calf than either growth or blood carotene. A blood plasma vitamin A level of 10γ or more per 100 cc. was found to be necessary for adequate vitamin A nutrition of the growing calf. Blood plasma vitamin A levels of 7–8γ per 100 cc. were borderline levels while values below this were definitely inadequate.

Daily intakes of vitamin A which would maintain deficient, borderline, and adequate concentrations of blood plasma vitamin A were found to be approximately 6, 12 and 18γ per kg. of body weight respectively. The daily carotene requirements necessary to maintain an adequate plasma vitamin A and prevent deficiency symptoms were 75γ per kg. for Holstein yearlings and 125γ per kg. for Guernsey yearlings.

The blood plasma carotene levels which would maintain an adequate blood vitamin A were 50–70γ of carotene per 100 cc. for Holsteins and 110–140γ of carotene per 100 cc. for Guernseys.

220. Comparative Palatability of Some Cereal Pastures. A. O. SHAW AND F. W. ATKESON, Kansas State College, Manhattan.

To study the relative palatability of some cereal crops used for pasture, a five acre field was planted in four strips to Reno barley, Turkey wheat, common rye, and Balbo rye on October 19, 1939. Grazing observations were taken on six consecutive days, March 19–24, 1940, when the plants averaged four to six inches high. Two Holsteins, two Ayrshires and two Jerseys were used. Since the cows were fed grain, silage and hay at night, it was thought differences in palatability might be reflected more truly than if the cows were hungry. The production plane varied from two dry cows to one producing 70 pounds of fat monthly. The cows were started grazing on different strips each day. Length of time spent grazing by each cow on each strip was recorded at one minute intervals until all cows ceased grazing. The cows spent an average of 45 minutes or 52 per cent of the grazing time

on Balbo rye; 21 minutes or 24 per cent on common rye; 15 minutes or 18 per cent on wheat; and 5 minutes or 6 per cent on barley. A pronounced preference for Balbo rye and an evident dislike for barley were uniform for all cows. The average grazing time averaged about an hour and a half, and was quite uniform regardless of plane of production. The evident dislike for barley would be an important factor in considering its value in comparison with other cereals as a pasture crop.

BOOK REVIEW

221. Industrial Instruments for Measurement and Control. THOMAS J. RHODES, The Procter & Gamble Co., McGraw-Hill Book Co., Inc., 1941.

This book, intended as a textbook for the formal study of the subject of instruments and automatic control in engineering schools, may well be used as a practical reference book for those concerned with instrument and control problems in industry.

The material contained in this book is broad in scope and comprehensive in treatment, and aside from one chapter (IV) on High-Temperature Pyrometry, it outlines the underlying theories upon which the operations of control instruments employed in the dairy and food industries are based, and quite thoroughly deals with operational applications. The chapter listings are as follows: Standards; Pressure and Vacuum Gauges; Indicating and Recording Thermometers; High-Temperature Pyrometry; Theory of Differential-Pressure Flow Meter Primary Measuring Instruments; Differential-Pressure Flow Meter Secondary Measuring, Recording, and Integrating Elements. Miscellaneous Inferential and Volumetric Flow Meters; Liquid-Level Measurement; Telemetering; Automatic-Control Theory; Automatic-Control Mechanisms; Miscellaneous Industrial Instruments. From this it can be seen that attention is given to methods of measurement and control under industrial requirements of four physical quantities, Temperature, Pressure, Flow, and Liquid Level. L.M.D.

BACTERIOLOGY

222. Variability in Streptococci of Group B. J. M. SHERMAN, ELIZABETH C. CHASE AND C. F. NIVEN, JR., Cornell Univ., Ithaca, N. Y. Jour. Bact., *41*, No. 1: 101. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Streptococci of Lancefield's group B are clearly defined physiologically and serologically, although diverse reactions occur in hemolytic power and ability to ferment lactose and salicin. The authors question whether strains are variants within the species, *Streptococcus mastitidis,* or should be given

separate recognition. The latter view is supported by the suggested specific names: *Str. mastitidis* (hemolysis +, lactose +, salicin +); *Str. agalactiae* (hemolysis –, lactose +, salicin +); *Str. opportunus* (hemolysis +, lactose –, salicin +); *Str. asalignus* (hemolysis +, lactose +, salicin –).

Pure cultures were plated and hundreds of daughter cultures were reisolated in intermittently studying stock cultures. Hemolytic and nonhemolytic strains were obtained from the same culture. Salicin fermenting and non-fermenting daughter cultures were obtained from both originally fermenting and non-fermenting strains. Lactose-non-fermenting strains were isolated from bovine sources and several stock cultures lost their lactose-fermenting ability. Most cultures were stable with respect to lactose but positive and negative strains were obtained from three positive and one negative culture.

All variants were otherwise physiologically typical and were serologically identified as members of Group B. D.P.G.

223. **The Value of Certain Tests in the Differentiation of *Lactobacillus bulgaricus* from *Lactobacillus acidophilus*.** J. M. SHERMAN AND H. M. HODGE, Cornell Univ., Ithaca, N. Y. Jour. Bact., *40*, No. 1: 11. 1940.

Here are presented differential tests applied for more than twenty years. Recent experiments confirm the validity of earlier methods and these tests correlate with differential methods applied by others.

The authors summarize the differentiation thus: "*Lactobacillus bulgaricus* is unable to make repeated growth in a lactose-peptone-yeast extract broth, is unable to grow in media containing 2.5 per cent sodium chloride, and cannot grow in broth with a reaction of pH 7.8. *Lactobacillus acidophilus,* on the other hand, is not inhibited in such mildly alkaline or saline media, and grows well through ten successive transfers in the relatively simple broth.

"*Lactobacillus bulgaricus* rarely grows at 15° C. whereas *Lactobacillus acidophilus* usually grows at this temperature. At 50° C., *Lactobacillus acidophilus* apparently never grows; newly isolated strains of *Lactobacillus bulgaricus* nearly always grow, though old laboratory cultures frequently fail."

Emphasis is given to the statement that no claims are made for the tests if applied to other closely related species. D.P.G.

224. **Physiological Characteristics of Lactic Acid Bacteria Near the Maximum Growth Temperature. I. Growth and Acid Production.** ROBERT M. STERN AND W. C. FRAZIER, Univ. of Wisconsin. Jour. Bact., *42*, No. 4: 497. 1941.

In some commercial processes, as in the manufacture of Swiss cheese,

bacteria must not only survive high temperatures but must grow at near-maximum temperatures. For *Lactobacillus bulgaricus,* 37° C. and 49.5° C. were chosen as optimum and near-maximum temperatures. Growth was observed by turbidity measured by an Evelyn photoelectric colorimeter with a 7,200 Å filter.

With *L. bulgaricus* at 37° C. there was close relationship between the rate of lactic acid production and the rate of growth, but at 49.5° C., as much acid was produced after reproduction had ceased as was produced during growth.

Differences resulting from variations in the size of inoculum of *L. bulgaricus* were apparent only during the early stages of growth at 37° C. An increase in the number of organisms present in the inoculum tended to shorten the lag phase, but had no apparent effect on the maximum amount of growth obtained. At 49.5° C., when larger inocula were employed, the increase in growth was more rapid than when smaller inocula were used, and the maximum population obtained depended on the number of cells added at the start of the experiment.

With *L. bulgaricus inocula* of various ages there was an increase in the length of the lag phase coincident with an increase in the age of the inoculum but the total amount of acid produced at the end of 24 hours was the same, although less growth was observed in the medium inoculated with older cultures.

When *Streptococcus thermophilus* and *L. bulgaricus* were grown at near-maximum temperatures, the production of volatile acids was affected only slightly. *L. bulgaricus* produced more volatile acid at 49.5° C. than at 37° C. When these two organisms were grown at near-maximum temperatures, there was no difference in the distribution of the two isomers of lactic acid from that found at optimum temperatures. D.P.G.

225. Physiological Characteristics of Lactic Acid Bacteria Near the Maximum Growth Temperature. II. Studies on Respiration. ROBERT M. STERN AND W. C. FRAZIER, Univ. of Wisconsin. Jour. Bact., *42,* No. 4: 501. 1941.

The rate of respiration is stimulated and respiratory enzymes are soon inactivated at temperatures near the maximum for growth. Differences were found in the rate of oxygen uptake of resting cells of two strains of *Streptococcus thermophilus.*

The growth curve and the oxygen uptake curve of *Lactobacillus bulgaricus* were similar during the period of active growth at 37° C., but respiration continued at a rapid rate after reproduction had ceased. At 49.5° C. the rate of oxygen uptake was rapid during the early stages of growth, but after several hours there was a marked inactivation of the respiratory mechanisms, followed by a decrease in the growth rate. D.P.G.

226. The Lactic Acid Fermentation of Various Kinds of Streptococci. PAUL A. SMITH AND J. M. SHERMAN, Cornell Univ., Ithaca, N. Y. Jour. Bact., *41,* No. 1: 101. 1941. (Abstract of paper presented at annual meeting of S. A. B.)

In a study of the production of lactic acid from glucose, 147 cultures representing the better-known groups and varieties of streptococci were used. The average percentages of lactic acid produced from glucose by four groups of the streptococci were: pyogenic streptococci, 82.1 to 88.4; viridans streptococci, 89.9 to 93.6; enterococci, 91.2 to 96.0; lactic streptococci, 93.7 and 96.6.

"The slightly lower average efficiency of the pyogenic streptococci is of possible significance, although the overlapping of individual cultures makes this conclusion doubtful." D.P.G.

227. Stimulation of the Growth of Lactobacilli by Extracts of Streptococcus lactis and Streptococcus cremoris. P. ARNE HANSEN. Biotech. Chem. Lab., Copenhagen, Denmark. Jour. Bact., *41,* No. 1: 41. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Extracts of autolyzed cells of several strains of *Streptococcus lactis* and *Str. cremoris* were prepared. When added to milk these extracts raised the endpoint of fermentation for *Lactobacillus casei.* "This effect may be of significance in explaining the predominance of this type of lactobacilli in cheese, a medium in which lactic streptococci develop abundantly at first and then die and disintegrate." D.P.G.

228. The Activity of Vitamin B_6 Analogues for Lactic Acid Bacteria. NESTOR BOHONOS, BRIAN L. HUTCHINGS AND W. H. PETERSON. Univ. of Wis., Madison. Jour. Bact., *41,* No. 1: 40. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Using a synthetic basal medium it was possible to determine the vitamin B_6 requirements of various species of lactic acid bacteria as well as the biological activity of various analogues of vitamin B_6.

Vitamin B_6 was found to be essential for growth and acid production by some lactobacilli, stimulatory for one and non-essential for others. It was shown that three species of *Lactobacilli* synthesize vitamin B_6. D.P.G.

229. The Production of Bactericidal Substances by Aerobic Sporulating Bacilli. RENE J. DUBOS AND ROLLIN D. HOTCHKISS, Hospital of the Rockefeller Inst. for Med. Res. Jour. Expt. Med., *73,* No. 5: 629. 1941.

In this paper further reports are given on the nature and action of the bactericidal substances produced by *B. brevis, B. subtilis* and other aerobic

sporulating bacilli. Cultures isolated from soil, manure, sewage and cheese were found to be endowed with properties antagonistic to *Staphylococcus aureus* and *Escherichia coli.* Material suspected of containing such antagonistic bacteria was heated to 70° C. for 30 minutes to destroy the non-sporulating forms present. This heated material was then inoculated into suspensions of living cells of *E. coli* and *S. aureus.* The activity of the antagonistic flora was determined by frequent microscopical and cultural tests.

The antagonistic cultures of aerobic sporulating bacilli all yielded an alcohol-soluble, water-insoluble fraction endowed with bactericidal activity. The name tyrothricin has been proposed for this fraction. Tyrothricin has yielded two crystalline products. One of these substances has been called gramicidin because of its selective bacteriostatic and bactericidal effect against gram-positive microorganisms. Gram-negative bacteria are resistant to gramicidin. Intraperitoneal injection of gramicidin exerts a protective action against infection of mice with pneumococci and streptococci. Gramicidin, when applied locally at the site of the infection, retains *in vivo* a striking activity against gram-positive microorganisms. Little or no hemolytic effect was observed with gramicidin. The other crystalline product yielded from tyrothricin has been called tyrocidine, so named partly because the substance is rich in tyrosine. Tyrocidine, when tested in buffer solution in the absence of broth, affects both gram-positive and gram-negative species. Tyrocidine causes immediate hemolysis of washed red cells of the rabbit. Tyrocidine is essentially ineffective *in vivo.* Crystalline preparations can exert a definite protective action against pneumococcus infections in mice, but all attempts to obtain a protective effect with tyrocidine against gram-negative infections have so far failed. C.N.S.

230. The Probable Identity of Diphtheroids Isolated from Aseptically-drawn Milk with Corynebacterium bovis and Bacterium Lipolyticum. L. A. BLACK, Univ. of Maryland, College Park, Maryland. Jour. Bact., *41,* No. 1: 99. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Seventy-one cultures of diphtheroid bacilli were isolated from milk and other animal sources, including 7 different species of *Corynebacterium.* All cultures from milk were alike and appeared to be identical to *Corynebacterium bovis* (Manual of Determinative Bacteriology, Fifth Edition). Representative cultures of the diphtheroids from milk caused rancidity in cream. D.P.G.

BREEDING

231. Influence of Cell Concentration on Respiration Rate of Sperm. C. F. WINCHESTER AND FRED F. MCKENZIE, Dept. Animal Husbandry,

Univ. Missouri, U.S.D.A. cooperating. Soc. Expt. Biol. and Med., Proc., *48:* 648. 1941.

Respiration rate of ram and boar semen was measured by means of a modified Barcroft-Warburg respirometer. Sperm concentration was determined with a hemocytometer. An increase in concentration was found accompanied by a decrease in sperm respiration rate at sperm concentrations from 1 to 6 billion cells per cc. This effect was not invariably observed at concentrations of 0.2 to 1 billion sperm per cc. The effect of sperm concentration on sperm respiration rate did not appear to be due to changes in pH, diminished supply of O_2 to individual cells, an enzyme, or metabolic products. R.P.R.

232. Influence of Hydrogen Ion Concentration on Respiration Rate of Sperm. C. F. WINCHESTER AND FRED F. MCKENZIE, Dept. Animal Husbandry, Univ. Missouri, U.S.D.A. cooperating. Soc. Expt. Biol. and Med., Proc., *48:* 654. 1941.

It was found that the hydrogen ion concentration of the media in which ram and boar sperm were suspended definitely influenced respiration rate. The optimum pH for respiration rate of boar semen was 7.2 to 7.3 and for ram semen it was 7.0 to 7.2. As the pH was raised or lowered from the optimum the respiration rate progressively declined. Unit changes in pH had significantly less influence on respiration rate of ram sperm than on that of boar semen. R.P.R.

BUTTER

233. Value of Producer Interviews in Reducing Mold Mycelia in Butter. GAIL SMITH AND WALTER L. SLATTER, Ohio State Univ., Columbus. Natl. Butter and Cheese Jour., *33*, No. 2: 12. 1942.

Sixty-two small cream producers on one cream route were divided into two groups, only one of which from April 23 to August 15 was interviewed and taught by personal visits at monthly intervals to use better methods of production. The cream from each group was collected and examined at weekly intervals and was churned separately. The interviewed group produced a higher percentage of first grade cream with an average Parson's test of 1.91, as compared with 2.37 for the other patrons. There was no significant difference in butter score and little difference in mold mycelia in the butter. It is not easy to improve quality of cream coming from small shippers. W.V.P.

234. Wapping and Packing Butter. E. G. HOOD, Dept. Agr., Ottawa, Canada. Natl. Butter and Cheese Jour., *32*, No. 12: 8. 1941.

The quantity of butter degraded for surface defects, although not large, is of economic importance. Double-lining boxes with 40-pound or heavier

parchments minimizes but does not wholly overcome woody and other surface flavors; better results are obtained by using the double lining along with a casein-formalin treated box and a parchment head piece on the top surface. The casein-formalin treatment consists in applying two solutions simultaneously with a double-nozzle spray gun to the butter box or shook. Solution A is made in the proportions of 50 grams casein (from self-soured milk), 7.5 grams borax and 300 cc. water; solution B is 1.5 volumes of 45% formalin and 10 volumes of water. The two solutions are delivered simultaneously in the correct proportions over the whole treated surface. About one pound of casein solution (2 ounces of casein) and one-eighth pint of formalin solution are used per box. A new, improved one-piece liner made in part of aluminum has been developed but will not be available immediately; it can be sterilized with hot water. Surface bleach, bleached top or freezer scald can be prevented by treating parchment wraps with a 15% or stronger brine solution, rewetting the box liner, if necessary, with cold brine and by taking the butter leveler and finishing roller from a cold brine solution. High surface color caused by evaporation of moisture from surface layers can be cured by using a wrapper which gives a tight seal.

While improved methods of wrapping and packaging will assist in maintaining quality, high quality cream, careful methods of manufacture, controlled sanitary conditions, lack of delay between churn and storage, and low, uniform storage temperature are also necessary for keeping quality. Surface defects can be minimized by controlling the acidity of the cream to produce a pH of 6.8 to 7.2 in the butter; by reducing contamination of butter with heavy metals, especially copper; by avoiding exposure to light during manufacturing or packing; and by placing butter in storage at 0° F. as soon as possible after packing. W.V.P.

235. Distribution of Pseudomonas putrefaciens. H. F. Long and B. W. Hammer, Iowa Agr. Expt. Sta., Ames, Iowa. Jour. Bact., *41*, No. 1: 100. 1941. (Abs. of paper presented at annual meeting of S. A. B.)

Pseudomonas putrefaciens causes a putrid or cheesy condition of salted butter. Materials were examined for the presence of the organism by direct smears on a special gelatin agar and by enrichment in litmus milk at 3° C. followed by smearing on the medium.

Ps. putrefaciens was isolated from raw, sweet milk and cream and from normal and putrid salted butter, but not from sour cream or highly ripened, unsalted butter, "where the sensitivity of the organism to acid may have resulted in its destruction." It was obtained from a sample of moist soil, but not from three samples of dry soil; from stream, lake and roadside water collected in various states; from creamery water supplies and from the

floors and sewers in dairy plants, particularly from sites that tended to remain moist.

In studies of dairy equipment, *Ps. putrefaciens* was obtained from parts of a butter printer in a plant that was having difficulty with putrid butter; from bolt heads, between staves and from the junction of staves and ends from three of four churns; and from the lining of a leaky milk vat immediately after the vat was taken out of service.

(See also JOUR. DAIRY SCI., Vol. 24: 921–924, 1941.) D.P.G.

CHEESE

236. Italian Cheese Varieties. J. C. MARQUARDT, N. Y. Agr. Expt. Sta., Geneva, N. Y. Natl. Butter and Cheese Jour., *33*, No. 1: 10. 1942.

Much time and effort are required to produce and merchandise a foreign variety of cheese. The first requirement is to employ a qualified cheesemaker who knows how. Through the cooperation of Raphael Giolletti, Geneva, N. Y., and Hugo Bonetti, Caracas, Venezuela, translations have been made of procedures for making Caciocavallo, Provolone, Provole, and Percorino Romano. Some of these formulae have not been tried in the Geneva laboratory but are given as a guide to the best procedures outlined in Italian references.

Procedures for making American Provolone and Romano types are described briefly. W.V.P.

237. A New Method for Making Cheese. C. C. FLORA, Virginia Polytechnic Inst., Blacksburg, Natl. Butter & Cheese Jour., *33*, No. 1: 16. 1942.

Marketable cheese can be made more quickly by adding 5% starter immediately before the rennet, by diluting the whey with water to prevent whey acidity from exceeding 0.175% and by shortening all steps in processing. Water may be added after cutting the curd until the acidity is near 0.12%. W.V.P.

238. Manufacture of American Cheese from Pasteurized Milk. H. L. WILSON, U. S. Dept. Agr., Washington, D. C., Natl. Butter and Cheese Jour., *33*, No. 2: 18. 1942.

Experiences in laboratory and factory practice indicate that: the curing of pasteurized milk cheese may be inhibited by excessive acid development during making; pasteurization of milk gives a better and more uniform cheese; low grade milk injures the quality of pasteurized milk cheese; pasteurizing simplifies making and increases yield; and rate of acid development must be controlled. The schedule of manufacture is described. This type of cheese has proved well adapted to packaging in valved cans.

W.V.P.

239. **Observations on the Composition of Cheddar Cheeses.** J. C. MARQUARDT AND M. W. YALE, N. Y. Agr. Expt. Sta., Geneva, N. Y. Natl. Butter and Cheese Jour., *32,* No. 12: 16. 1941.

Analyses were made of cheese exhibited at the New York State fairs in 1940 and 1941. In 1940 some entries contained less salt than desirable; salt in the washed curd class was especially variable. In 1941 salt percentages were more uniform but generally tending too close to the lower limit. In 1941 about 7% of the samples contained more than the legal limit of moisture, but most lots had between 35.5 to 40.0%. A total of 319 samples of cheese selected at random in New York State and including cheese made in other states showed 22% with 36 to 38% moisture, 15.5% with less than 36% moisture, 32.5% with from 38 to 40% moisture, and 30% above the legal moisture limit; the average was well below 40%. Using the same 319 samples it was found that 50% contained less than the desirable minimum of 30% fat, about 30% had over 32% while the rest had from 30 to 32% fat. W.V.P.

240. **A Preliminary Study of the Effects of Varying Pitching Consistency and Rate of Scald on the Physical and Chemical Properties of Cheddar Cheese and on the Firmness of the Cheese as Judged by Cheese-makers, Bakers and Others.** G. W. SCOTT BLAIR, F. M. V. COPPEN AND D. V. DEARDEN. Natl. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., *12,* No. 2: 170–177. 1941.

A preliminary experiment is described in which six cheese of the Cheddar type were made under conditions as similar as possible, except that the consistency of the curd at pitching, and in two cases the rate of scald, were varied. Chemical and rheological analyses, as well as measurements of moisture content and vapor pressure, were made on the cheese and also judgments of firmness under various conditions were given by cheesemakers, bakers and non-experts.

The firmness of the cheese appears to have been influenced by the consistency of the curd at the pitching point and by the rate of scald.

S.T.C.

241. **Volatile Acids of Cheese III. Application of the Extraction Method.** E. R. HISCOX, J. HARRISON AND J. Z. WOLF. Natl. Inst. for Res. in Dairying, Univ. of Reading, England. Jour. Dairy Res., *12,* No. 2: 155–169. 1941.

Details are given of an extraction method for the estimation of the volatile acids in cheese. Twenty grams of the cheese are shaken with 100 ml. of CO_2 free water at about 40° C. The mixture is centrifuged and cooled

to harden the fat which is then transferred to an ether solution. The supernatant liquid is poured into a flask and the residue washed twice more. The combined water washings are acidified to pH 2 with H_2SO_4 and subjected to the usual steam distillation. The ether solutions of the fat were neutralized with NaOH, then washed six times with 50 ml. quantities of N/10 NaOH solution. After driving off the ether the combined washings are acidified to pH 2 and steam distilled. The water insoluble fraction was titrated separately from both distillations.

The total volatile acid as estimated by this extraction method was three to five times greater in Stilton and Gorgonzola cheese than estimated by direct steam distillation; and about twice as great in other cheese studied. For Stilton and Gorgonzola cheese the proportion of volatile acids in the distillate from the fat fraction was higher than that from the water fraction. The reverse was found in white cheeses such as cheddar and in blue cheese of the Roquefort type. This is taken to indicate that in the white cheeses such as cheddar, there is very little lipolysis and that water-soluble acetic acid (not derived from fat) constitutes the bulk of the volatile acids.

S.T.C.

CHEMISTRY

242. Photochemical Studies of Rancidity: The Chlorophyll Value in Relation to Autoxidation. MAYNE R. COE, Agr. Chem. Res. Div. Bur. Agr. Chem. and Engin., U.S.D.A. Oil and Soap, *18*, No. 11: 227. 1941.

Fresh oils fluoresce when placed under an ultraviolet lamp and the intensity of this fluorescence decreases with increased oxidation indicating that a loss of the reacting substance has taken place. When the acceptor molecules, or reacting substances, are progressively oxidized, or when an oil becomes rancid, they lose the property of quenching the fluorescence of chlorophyll. Advantage has been taken of this property of oils toward chlorophyll in following rancidity autoxidation, by titrating a given amount of chlorophyll with the oil under examination. The number of cubic centimeters of oil necessary to quench the red chlorophyll fluorescence is called the "Chlorophyll Value."

The chlorophyll value of an oil remains essentially the same as long as the oil is organoleptically sweet. The chlorophyll value test parallels very closely the results obtained by organoleptic methods used to evaluate the development of rancidity. The chlorophyll value test appears to enable one to detect and follow the very earliest stages in the process of rancidification, to obtain a quantitative knowledge of the state of oxidation and to make a comparison of the potential keeping qualities of any two or more oils or fats. Details of the method for determining the chlorophyll value of fats are given.

V.C.S.

243. The Fluorometric Determination of Riboflavin in Urine and Other Biological Fluids. VICTOR A. NAJJAR, Dept. Ped., Johns Hopkins Univ. School of Med., Baltimore. Jour. Biol. Chem., *141*, No. 2: 355. 1941.

A method for determining riboflavin in urine by measuring the fluorescence photometrically is given in detail. This procedure may be used for the determination of riboflavin in milk. The fat is first separated by high speed centrifugation and the proteins precipitated with trichloroacetic acid (2 cc. of skim milk plus 8 cc. of 10 per cent trichloroacetic acid). Five cubic centimeters of the filtrate are assayed for riboflavin by the direct method used for urine. V.C.S.

244. The Estimation of Lactose in Milk. A. K. R. MCDOWELL. Dairy Res. Inst. (N. Z.), Palmerston North, New Zealand. Jour. Dairy Res., *12*, No. 2: 131–138. 1941.

The values for lactose content of milk as estimated by the following methods were found to show good agreement, and the author considers that they may be accepted as the true milk sugar content: 1. Direct volumetric copper reduction method using either unclarified milk or milk clarified and decalcified. Removal of the protein only resulted in low lactose values. 2. Polarimetric method using milk clarified with either zinc hydroxide or cadmium hydroxide. Clarification with acid mercuric nitrate or phosphotungstic acid gave high results. 3. Iodometric method using milk clarified with zinc hydroxide or cadmium hydroxide. Clarification with dialysed iron or phosphotungstic acid gave high results. 4. Chloramine-T method using milk clarified with dialysed iron, phosphotungstic acid, cadmium hydroxide or zinc hydroxide. S.T.C.

245. Studies on Ass's Milk. Composition. C. P. ANANTAKRISHMAN. Dept. of Biochem., Indian Inst. of Science, Bangalore. Jour. Dairy Res., *12*, No. 2: 119–130. 1941.

The author presents the following summary of his analysis of ass's milk:

The total solids of samples of ass's milk ranged from 7.14 to 8.50, and the fat from 0.54 to 0.71%.

The nitrogen distribution in ass's milk is: casein 39.5, albumin, 35.0, globulin 2.7 and non-protein nitrogen 22.8% of the total nitrogen. Ass's milk contains: casein 0.70, albumin 0.62 and globulin 0.07%. The total protein content is 1.39%. Ass's milk is therefore characterized by a low casein, a low globulin and a high albumin content.

The non-protein nitrogen consists of amino nitrogen 8.1, urea nitrogen 24.3 and uric acid 0.7 mg./100 ml. of milk. The urea content is twice that present in cow's milk.

The mean chloride and lactose contents of the milk samples are 0.037 and 6.1% respectively.

The average calcium and phosphorus contents of ass's milk are 0.081 and 0.059% respectively. Half the calcium is ionic, and half is in colloidal form.

The phosphorus distribution is: total acid soluble 84.0, acid soluble organic 38.5, easily hydrolysable ester 27.4, inorganic 46.0, and colloidal inorganic 23.0% of the total phosphorus. The ratio of $CaO : P_2O_5$ is 1:1; 46% of the total phosphorus is in ester form; this is high when compared with only 12% in cow's milk; most of the phosphoric ester forms soluble barium salts, which is a distinguishing feature of ass's milk.

The total sulphur content is 15.8 mg./100 ml.

The fat has a penetrating odor and is colored orange-yellow. It has an iodine value of about 86, which is much higher than for human milk fat. The Reichert (9.5) and Kirschner values (5.7) are low.

In general, the composition of ass's milk resembles that of human rather than of cow's milk. S.T.C.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

246. The Baking Industry—a Market for Dry Milk. L. W. NOLTE, American Dry Milk Inst., Chicago, Ill. Natl. Butter and Cheese Jour., *32,* No. 12: 14. 1941.

The baking industry is using three times as much milk solids today as 15 years ago; it uses more milk than any other single industry. Research in developing the use of dry milk in bread and in better methods of manufacture has made this possible. W.V.P.

257. The Keeping Quality of Milk Powders. Part I. Addition of Antioxidants. R. WAITE. The Hannah Dairy Res. Inst., Kirkhill, Ayr. Jour. Dairy Res., *12,* No. 2: 178–183. 1941.

Hydroquinone in a concentration equivalent to 0.05% of the weight of fat (0.12% on the weight of powder), was found to be an effective antioxidant for the butterfat of spray dried whole milk powder, but produced an objectionable "metallic" flavor in the powder and reconstituted milk. Oat flour was much less efficient as an antioxidant but 0.25% added to the milk, preferably before condensing, increased the resistance of the resultant powder to the development of tallowiness by the equivalent of about 4 months at normal temperatures. Raising the concentration of oat flour to 0.5% doubled this increase but imparted a noticeable oat flavor to the product. S.T.C.

248. The Effects of Additions of Dried Skim Milk and Dried Whey on the Baking Quality and Nutritive Properties of White Bread.

K. M. HENRY, J. HOUSTON, S. K. KON AND J. POWELL, Natl. Inst. for Res. in Dairying, Univ. of Reading, Reading, England; and R. H. CARTER AND P. HALTON, Res. Assoc. of British Flour-Millers, St. Albans. Jour. Dairy Res. *12*, No. 2: 184–212. 1941.

The authors present the following summary of their extensive work:

Experiments were carried out to study the effects on the quality and nutritive value of bread of the addition to white flour of roller-dried skim milk and roller-dried whey. Both samples were typical commercial products.

Additions of 2% of the dried milk could be made without any marked effect on loaf quality or on flavor. The addition of 4% or more definitely lowered the quality of the bread, the volume being smaller and the crumb more rubbery. As the content of milk was increased above 2% the flavor of the bread became increasingly distinctive and departed from the normal neutral flavor of water bread.

Up to 5% of dried whey could be added to the flour without any marked deterioration in the crumb of the bread, although with one flour this quantity decreased the volume by 16%. At this level, however, the whey imparted a distinct cheesy flavor to the bread.

Attention is drawn to the fact that the effects produced by the addition of dried milk or whey to bread can only be considered in relation to the particular sample used, since other workers have found that modifications in the method of manufacture considerably alter the value of the product as far as its use in bread is concerned. For this reason improved types of dried milk or whey might well lead to their greater use by the baking industry.

The addition of 2% milk solids doubled, 6% whey trebled and 6% milk quadrupled the calcium content of white bread. In hard-water districts a large part of the calcium of white bread is derived from tap water. When white bread was the sole source of calcium for young growing rats, their calcium intake was grossly subnormal. Owing to unavoidable metabolic losses only 60% of the ingested calcium was retained. The bread calcium was, however, well utilized and is probably as available as that of calcium acid phosphate and only slightly less so than milk calcium. Addition of dried skim milk or dried whey to the bread increased the percentage retention of calcium. This added calcium was almost completely retained.

A comparison of the biological values and true digestibilities of white bread, 2% milk bread and 6% milk bread by the method of Mitchell gave the following respective values: 44.7 and 90.9; 47.6 and 89.6; 49.7 and 88.9. A separate comparison of white bread, 2% white bread and 6% whey bread yielded similarly 47.4 and 92.9; 45.9 and 91.9; 47.4 and 88.9. At most, 15% of the bread proteins were derived from milk and no supplementary relation was detected at such low levels. Addition of 6% milk solids increased the "protein value" of bread by 25%.

Fluorimetric tests showed that the vitamin B_1 content rose from 1.0 μg./g. dry matter in white bread to 1.3 μg./g. in the 6% milk or whey loaves.

Riboflavin was similarly increased from 36 to 86–100 μg./g. It is possible that the full extent of the increase was not measured by the fluorimetric test.

Several experiments on rats in which the breads were fed as an exclusive diet showed that the marked beneficial effects of milk or whey additions were due not only to the increase in calcium but also to a large extent to the increase in riboflavin and other members of the vitamin B_2 complex.

S.T.C.

DISEASE

249. Culture Media for Brucella. GRACE P. KERBY AND ROYALL M. CALDER. Brucellosis Laboratory, Clayton Foundation for Research, Petroleum Building, Houston, Texas. Jour. Bact., *40*, No. 5: 637. 1940.

In an attempt to secure a culture medium that is reliable for clinical use a large number of materials were investigated for their effect as growth-promoting factors for *Brucella* when added to the basic medium, Bacto-Tryptose broth. Ratings were made on the basis of a standard comparison of each series with tryptose broth, as prepared in uniform lots by "Difco."

Milk medium containing 2 per cent Bacto-Tryptose, 0.5 per cent sodium chloride, 1 : 700,000 crystal violet, made up with fresh, whole, Grade A pasteurized milk and sterilized by tyndallization, showed earlier and more abundant growth than that obtained in a tryptose broth control medium. Also, growth was positive in the milk medium when the inoculum had been reduced beyond the point where *Brucella* could be recovered from tryptose broth. The use of skimmed milk, homogenized milk, powdered milk, certified raw milk, or heavy cream as a base in this medium was not satisfactory. Incorporation of a 50 per cent liver-infusion base into the milk medium seemed to have a slightly favorable effect, but the results were not convincing. Media made with peptonized or trypsinized milk base were inferior to tryptose broth. The pasteurized whole milk medium was not dependable due to variation in the lots of milk used.

Various sodium caseinate media were studied in the series. The one most effective in growing *Br. abortus* 456 consisted of 2 per cent sodium caseinate, 2 per cent Bacto-Tryptose, 0.1 per cent Bacto agar, 0.5 per cent sodium chloride, and 1 : 700,000 crystal violet. Preparation of sodium caseinate medium can be expected to result in uniform lots of media but the growth of only *Br. abortus* 456 was enhanced in a series including 1 recently isolated *Br. melitensis* strain, 4 old *Br. melitensis* strains, 1 old porcine strain, 2 recently isolated *Br. abortus* strains and 4 old *Br. abortus* strains.

The value of sodium caseinate medium in routine culture work is questionable.

Addition of cod liver oil (16 per cent) resulted in inhibition of growth in milk media and in tryptose broth. Various tomato juice media failed to support growth of the organism. Addition of carrot extracts to tryptose broth resulted in slight inhibition of growth.

Attempts to enrich tryptose media by addition of vitamins A, B_1 and B_2 in the form of Embo products were unsuccessful, actually inhibitory, as were Hormodin A, and anterior pituitary extract. Spleen-infusion agar did not prove superior to tryptose agar. Glutathione yeast extract, gelatin, whey and *Bacillus subtilis* extracts did not improve tryptose media. Slight inhibition was noted in media containing Bacto Asparagine, Bacto Malt Extract, bone meal extracts, bone marrow extracts, purified corn phosphatid, and soy bean extracts. Sodium sulfite, 0.05 per cent in tryptose broth, slightly inhibited *Brucella.* Also unsatisfactory were liver-infusion, the Burky modification of Huntoon's hormone medium, and Brewer's sodium thioglycollate medium. D.P.G.

250. The Relationship of Methods of Bacteriological Examination to the Eradication and Control of Mastitis (*Stretococcus Agalactiae*). I. Use of Enrichment Technique in Revealing Streptococcal Infections of the Cow's Udder. II. *Streptococcus agalactiae* Infections in Heifers. A. T. R. MATTICK, P. M. F. SHATTOCK AND M. MOREIRA JACOB. Natl. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. Jour. Dairy Res., *12,* No. 2: 139–154. 1941.

In the course of attempts on a field scale to eradicate or control *Str. agalactiae* infections, comparisons of the efficiency of several bacteriological methods were made. An enrichment method in which 9.5 ml. of milk plus 0.5 ml. of an alcohol water solution of brom-cresolpurple to give a final concentration of 0.025 per cent was incubated at 37° C. (98.6° F.) for 24 hours, prior to streaking on aesculin crystal violet blood agar plates, was found to detect many lightly infected cases which were reported as negative using sodium azide broth or the plating on Edward's medium of the centrifuged deposit. Increasing the frequency of examination was shown to increase materially the number of positive cases discovered.

Streptococcus agalactiae was found to be present in 21 per cent of the animals in their first lactation and 4.5 per cent of heifers were found to harbor this organism while in isolation before joining the herd. The authors suggest the probability that if more stringent methods were devised and examinations at very short intervals made, the organism could be demonstrated in the milk of a high proportion of milking cattle. The "carrier" state may thus be widespread. S.T.C.

FEEDS AND FEEDING

251. Magnesium Studies in Calves. II. The Effect of Magnesium Salts and Various Natural Feeds upon the Magnesium Content of the Blood. C. F. HUFFMAN, C. L. CONLEY, E. C. LIGHTFOOT AND C. W. DUNCAN, Dairy Husbandry Dept. and Agr. Expt. Sta., Michigan State College, East Lansing, Mich. Jour. Nutr., *22:* 609–620. 1941.

Calves fed a basal ration consisting of whole milk supplemented with iron, copper, and manganese, with or without the addition of starch or other similar carbohydrates always resulted in subnormal blood plasma magnesium values. Normal plasma magnesium levels resulted by the addition of magnesium in sufficient quantities to bring the intake up to 30 to 40 mg. of magnesium per kilogram of body weight. When either corn or alfalfa hay was fed as supplements to the basal ration, a total intake of 12 to 15 mg. of magnesium per kilogram of body weight was sufficient to maintain normal levels. When sufficient magnesium oxide was fed with corn gluten meal so that the magnesium intake was equal to that when corn was fed 12 to 15 mg. of magnesium per kilogram of body weight also was sufficient to maintain normal blood plasma magnesium values. The magnesium in alfalfa ash was not used efficiently. These data indicate the utilization of magnesium by growing calves is more efficient when magnesium is furnished by natural feed than when supplied by magnesium salts.

C.F.H.

252. The Utilization by Calves of the Energy Contained in Balanced Rations Composed of Combinations of Different Feeds. H. H. MITCHELL AND T. S. HAMILTON, Anim. Nutr. Div., Univ. Illinois, Urbana, Ill. Jour. Nutr., *22:* 541–552. 1941.

Eight Shorthorn calves were fed four different rations containing similar proportions of the various classes of nutrients distinguished by approximate chemical analysis, and to be adequate in all of essential nutrients at two different levels of nutrition. The metabolism and respiration experiments on these steers indicated that the metabolizable energy of the various rations is equally utilized for maintenance and for body increase.

The percentage net availability of the metabolizable energy was quite similar for the four experimental rations at the lower plane of nutrition and also on the higher plane of nutrition. C.F.H.

253. Biennial Reviews of the Progress of Dairy Science. Section A. Physiology of Dairy Cattle. II. Nutrition. ANONYMOUS. Sec-F. Milk-Borne Diseases. Jour. Dairy Res., *12,* No. 2: 213–240. 1941.

Recent literature on dairy cattle nutrition is reviewed under the follow-

ing headings: Feeding standards, the feeding of calves, utilization of non protein nitrogen, mineral metabolism and vitamins. 152 references. Literature on milk borne diseases is reviewed under the headings, epidemiology, streptococcal infections, staphylococcal food poisoning, tuberculosis, salmonella infections, dysentery, brucella infections, virus infections and miscellaneous infections. There are 71 references. S.T.C.

FOOD VALUE AND DAIRY PRODUCTS

254. Vitamin A and C in Cow's Milk, with a Note on the Synthesis of Vitamin C in Bovines. S. N. RAY, KARAM CHAND AND K. GOVIND RAU. Imperial Vet. Res. Inst., Mucktesar, India. Jour. Dairy Res., *12*, No. 2: 109–118. 1941.

The mean carotene and vitamin A content of 16 samples of milk from different breeds of cows was 71 (± 32.03) Moore's yellow units and 115.6 (± 35.46) Moore's blue units, respectively per 100 ml. milk. The mean value for reduced ascorbic acid in the milk from 13 cows was 1.94 (± 0.35). No great individual variation in ascorbic acid was found.

A vitamin C free ration was prepared from a grain mixture and wheat straw in the following manner: The concentrate and roughage mixtures were made alkaline by moistening with caustic soda solution and autoclaved at 120° C. for two hours. The autoclaved mass was kept over night, then dried in a hot-air oven at 120° C. and then crushed in a mill. Analysis showed a reduction in the ascorbic acid content of the grain mixture from 1.65 mg. to 0.45 mg./100 mg., and in the wheat straw from 0.85 to 0.28 mg./100 g. Since these values could not be decreased by further heating, it was assumed the residual reducing substances were not ascorbic acid. The concentrations of ascorbic acid in the blood plasma of calves fed on the heated ration was not lower than that of calves fed on the unheated ration, showing synthesis of vitamin C in the animal body.

Unsuccessful attempts were made to produce vitamin C in vitro by growing bacteria isolated from various parts of the alimentary canal on media prepared from ingesta taken from the regions from which they were isolated. S.T.C.

ICE CREAM

255. Some Newer Ice Cream Stabilizers and Their Functions. ALLAN LEIGHTON, Div. Dairy Res. Labs., U. S. Bur. Dairy Indus., Washington, D. C. Ice Cream Trade Jour., *37*, No. 12: 12. 1941.

A vegetable stabilizer of the alginate type and a mixture of monoglyceride and gelatin were compared with gelatin as stabilizers for ice cream. The newer type stabilizers shortened the whipping time and increased the maximum amount of overrun obtainable. Evidence was pre-

sented to show that more water is present in the form of ice in the ice cream stabilized with alginate and monoglyceride gelatin alone, a condition which is associated with small ice crystals and a smooth texture. It was concluded that these new type stabilizers were capable of producing ice cream of excellent quality and also capable of obviating difficulties encountered in the whipping process incident to freezing. W.H.M.

256. Reducing Power Costs. C. T. BAKER, Atlanta, Ga. Ice Cream Rev., *24*, No. 8: 43. 1941.

The cause for high power bills should be traced and corrections made. The situation found in one ice cream plant is discussed. J.H.E.

257. Variegated Ice Cream. ANONYMOUS. Ice Cream Rev., *24*, No. 8: 42. 1941.

The variegated or ribbon type of ice cream is apparently here to stay, the most successful flavors being chocolate, strawberry, raspberry and butterscotch. A manufacturing difficulty is the settling of heavy flavors to the bottom of the can. Prevention lies in freezing the ice cream stiffer and using less sugar in the syrup portion. Iciness can be overcome by the use of more pectin and chilling the syrup down to 30° F. before adding. J.H.E.

258. Superheated Condensed Milk in Ice Cream. J. H. ERB, Ohio State Univ., Columbus, O. Ice Cream Rev., *24*, No. 8: 56. 1941.

The "superheated" process applied to ordinary whole or skim condensed milk is a method of obtaining greater water binding power from milk proteins. Ice cream made from superheated milk is more resistant in body and smoother in texture. Two methods for making superheated condensed milk are given, as well as factors influencing time required to superheat. J.H.E.

259. Ice Cream Tarts. VINCENT M. RABUFFO, Editor. Ice Cream Trade Jour., *38*, No. 1: 10. 1942.

Many ice cream manufacturers are stimulating winter sales by offering a new item known as ice cream tarts.

Each tart contains $3\frac{3}{4}$ to 4 fluid ounces of ice cream and about an ounce of fruit, or about 4 to the pint or 32 to the gallon of ice cream. The paper tart dishes are $3\frac{5}{8}$″ in diameter at the top and $1\frac{3}{8}$″ deep. Fruits used for filling are chilled to about the same temperature as the ice cream, and after placing in the dish it is decorated with a ring of whipped cream.

Raspberries, cherries, pineapple, strawberries, and peaches may be used in tarts. A box of 4 retails at 35 to 38 cents, or they may be sold individually from 10 to 15 cents. W.H.M.

260. Research in Ice Cream During 1941. W. J. CORBETT, Univ. Illinois, Urbana, Ill. Ice Cream Trade Jour., *37*, No. 12: 14. 1941.

Important research in the field of ice cream making was reviewed and attention directed to the following findings:

1. The flavor and body and texture of ice cream were not affected by type of frozen condensed milk used nor the length of time it was held frozen prior to using.

2. Heating cream to 170° F. and the addition of 2% oat flour (on fat basis) prevented or reduced the development of oxidized flavor during the storage period. The addition of 10% sugar to cream before freezing did not prevent development of off flavor but it did aid in defrosting and reduced oiling off. Acid reduction to 0.10% in mixes containing frozen cream increased the whipping ability.

3. A method for making and using soluble casein in ice cream indicated that up to 40% of the serum solids of the mix could be supplied from this source. Higher amounts resulted in a curd-like flavor and gummy body. A marked increase in whipping ability was observed when the mixes contained the casein-gel.

4. Work by several investigators working with the various sweeteners made from corn indicate that 25 to 33% of the sucrose on a sweetness basis can be replaced by the corn sugars without any injury to the quality of the ice cream. In some instances an improvement in body and texture resulted from the substitutions.

5. Improvement in whipping properties of the mix and in the body and texture of the ice cream resulted when 0.4% of a homogenous mixture of 0.06% monoglyceride and 0.30% gelatin was used as a stabilizer.

6. The addition of calcium and magnesium alkalies to ice cream mixes seems to have some merit in controlling mix acidity.

7. Vacreation method of pasteurization of ice cream mixes resulted in some improvement in flavor. The mixes were more resistant to the development of oxidized flavor and less flavor was required in the ice cream. This method was also efficient in reducing bacterial counts.

8. Sodium alginate and locust bean gum exerted a more noticeable protective action than gelatin and sugar in retarding bacterial destruction when mixes were pasteurized at 143° F.

9. Ice cream comparable to that from a mix homogenized at 1000 to 1500 pounds pressure was produced from a mix homogenized at 500 pounds on a rotary machine, but it was inferior to that produced when 2000 to 3000 pounds pressure was used.

10. The higher the homogenization pressure and the oftener a mix is homogenized the wetter the ice cream appears when drawn from the freezer. Higher pressures resulted in a more rapid and smoother melt-down and some improvement in body.

11. A suitable syrup to use in the manufacture of variegated ice cream should have a desirable color, contain a satisfactory stabilizer (1% pectin), have a sugar content of approximately 40%, and have a proper acidity (about 1%).

12. A simplified tester and a procedure for determination of total solids in ice cream mix has been developed by Harmon and Renner at the Texas Technological Institute, Lubbock, Texas.

13. A method has also been developed to detect the presence of sodium alginate in dairy products by Shroeder and Racidot (Journal Indus. and Engin. Chem., Analyt. Ed., *13*, No. 3: 165. 1941). W.H.M.

261. Fancy Form "Fotos." ANONYMOUS. Ice Cream Trade Jour., *38*, No. 1: 14. 1942.

C. Everett Sells, fancy form technician for Moores and Ross, Columbus, Ohio, has developed a method of transferring photographs or emblems to individual slices of ice cream.

It is done by printing the picture or emblem on a piece of sensitized silk, then spraying certified food color through this screen. Bricks of ice cream are cut into eight portions and then dipped in a mixture of whipped cream and soft ice cream which is thinned to about the consistency of chocolate coating to give a smooth surface on which to stencil the image.

The cost of making the stencil is about double the price of an individual mould. W.H.M.

262. Mix Stabilizers. P. H. TRACY, Univ. Illinois, Urbana, Ill. Ice Cream Rev., *24*, No. 8: 44. 1941.

This article is a review of the common ice cream stabilizers and the effect of each on mix and ice cream properties. Acid-type gelatines were found superior to alkaline gelatines. High Bloom test (275 grams) gelatines were found to give better results than low. One tenth to 0.30% of locust bean was found to have a desirable effect upon the body of ice cream. However, the ice cream showed poor melting characteristics with this stabilizer. A combination of locust bean with gelatin gave improved melt-down characteristics.

Addition of pectin to the mix will produce some stabilizing results but it is desirable to use it in combination with gelatin.

When comparing 0.30% sodium alginate and 0.35% gelatin the body scores of the ice cream were comparable and except in counter freezers there was little difference in whipping qualities.

As little as 0.10% Irish moss produced body results practically comparable with those produced with 0.35% gelatin (215 Bloom). The body was slightly more crumbly than gelatine stabilized ice cream.

Gum tragacanth and gum karaya are used successfully in ices and sherbets. J.H.E.

263. Diabetic Ice Cream. ANONYMOUS. Ice Cream Rev., *24,* No. 8: 36. 1941.

The experience of a company producing an ice cream suitable for people suffering from diabetes is cited. The use of 0.025% saccharin is used in place of 16% sugar. The mix should have about 38% total solids. Since milk concentrates cannot be used because of their high protein content, glycerol can be used to build up solids.

The fact that glycerol and saccharin are used should be printed on the containers in which the ice cream is sold. Diabetic patients are prescribed a diet low in carbohydrates, low in proteins and high in fats. J.H.E.

MILK

264. The Effect of the Two Quart Bottle and Gallon Jug on Plant Operations. GEORGE NINOW, The Akron Pure Milk Co., Akron, Ohio. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34:* 121–124. 1941.

Variations in the style of multiple quart containers are great. Capping presents problems and trouble has been expressed by some with corrosion of handles by strong alkali or chlorine. Much trouble has been due to the earlier equipment for filling and washing these containers not being suited to large scale operations. Due to greater value very few gallon containers find their way to rubbish piles. The decrease in efficiency due to a shorter run on small units, the unavoidable added labor costs due to another inventory item, and other considerations do not make it evident that there should be any large spread between the price of milk in quart and larger containers. E.F.G.

265. Plant Layout for Paper Container Equipment. J. H. FORSLEW, Bowman Dairy Co., Chicago, Ill. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34:* 117–120. 1941.

This discussion relates experience with the American Can Co. preformed milk container.

The handling of empty containers instead of being a simple operation is a technical and exacting operation not to be entered into without careful consideration and due regard for clean sanitary storage space and surroundings, proper handling and conveying equipment, and infinite care and control required at all times to maintain correct operating conditions. The superintendent should give his personal supervision to unloading and handling until the men have learned to properly handle the containers as the tendency is to treat them like glass bottles. Besides protection from con-

tamination and smoke it is important that containers do not get too warm as paraffin will soften and adhere to working parts of conveyor and filler. Filler and conveyor should be washed down every hour or two with hot water to remove paraffin accumulations. Chain conveyors are preferred for the incoming cases of empty bottles but belt conveyors are used to carry bottles to the filler. Inspection of both sides of the bottles by the operator is accomplished by means of properly placed mirrors. It is important that milk be as cold as possible as bottles are more rigid at the lower temperature. A metal canopy painted black inside with a tubular light beneath permits the operator to see how full the bottle is. Vans are packed solid with a cake of ice in each end. E.F.G.

266. Plant Problems in Connection with the 48 mm. Bottle. Ross J. Winning, Sheffield Farms Co., Inc., New York, New York. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34:* 114–116. 1941.

The larger roll at the top of the old 56 mm. bottle presented a greater illusion as to fullness than does the 48 mm. bottle with its smaller roll. Narrowness of neck on the latter bottle accentuates the lack of fullness. Incorporation of air in the milk and centering the bottle are more trouble problems in the case of the narrower neck bottle. The fill should be more precise with the new bottle. Besides difficulty in properly centering and seating the cap more trouble with milk seepage is experienced due to the smaller allowance for expansion as the milk becomes warmer. In the bottle washer centering with regard to jets must be more carefully done. The new bottle has some advantage in trippage. E.F.G.

267. Simplified Designs for Glass Milk Bottles, Standardization of Glass Milk Bottles. V. L. Hall, Glass Container Assoc., New York City. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34:* 107–113. 1941.

The first move toward standardization of milk bottles took place in 1926 and resulted in Simplified Practice Recommendation No. 10 issued by the Department of Commerce. In spite of this, by 1937 there were 33 distinct quart shapes. A great advantage of standardization of design is the simplification of many equipment problems. Standardization will also be a great aid in offsetting rising material and labor costs since the standardized container can be produced more cheaply.

It is recommended that: (1) Elimination of all shapes except the Economy (17¾ oz.) and the three standard shapes (22 oz.) for quarts and the Standard two-quart. (These are illustrated.) (2) Elimination of all but seven types and sizes of finishes. (These are named.) (3) Elimination of all but four dimensions of cap seats. (4) Avoidance of neck decorations. (5) Dairies using non-standard bottles be supplied but that an early change to a standard type be considered.

Adherance to the above program would provide 35 combinations of shapes with various diameter cap seats with or without lugs and bumper rolls for the quart and two quart sizes. This is about one-fourth of the combinations permitted by present standards and an even smaller fraction of the variety of bottle specifications now being ordered by dairymen. In the article drawings illustrate the various type bottles and finishes.

E.F.G.

268. Policy Considerations in the Administration of State Milk Control Acts. W. J. KUHRT, California State Dept. Agr., Sacramento, Calif. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34:* 143–151. 1941.

The first state control act was passed in 1935 and by 1941 more than 88% of the milk consumed in California came under state control. Emphasis in the control measures was placed upon correction of chronic unsatisfactory conditions and practices in the market rather than mere alleviation of the depression effects. The essential objectives of the control act are: to provide specific procedures for determining producer prices and distributor margins including the differential between fluid milk prices and manufacturing milk prices, and proper formulation of contracts. Authority to fix margins has resulted in more efficient delivery. Facts are obtained by detailed exhaustive surveys of both production and distribution and the results are available to all producers and distributors. Prompt modification of prices is provided for by an automatic change of 5 cents per pound of fat to the producer and ½ cent distributor price change as the cost of feed and the manufacturing milk price changes. A levy of 2 mills per pound of milk fat from both producers and distributors provides about $300,000 per year to cover expenditures divided about half for fact finding and half for enforcement.

The California Supreme Court upheld the constitutionality of the act in 1940. In California employees are under civil service regulations and their positions are secure from undue influences of pressure groups. No program is undertaken until a petition signed by at least 65% of the producers who produce at least 65% of the milk has been received. The law is administered "in the public interest" and it is especially important that this fact is kept constantly before the public if public confidence is to be retained.

Some of the results of the milk control act are establishment of 1 cent differential between store and home delivery, a half cent higher charge to the store for milk in paper containers, and the establishment of prices of milk sold to federal agencies. Consumption of milk is being encouraged through an educational program, through a lower multiple quart container price and by a special price on a 3.65% fat milk in a half gallon container.

E.F.G.

269. Fluid Milk Markets and the Lend-Lease Program. P. L. MILLER, U.S.D.A., Washington, D. C. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34:* 152–157. 1941.

The attempt to increase the production of butter and cheese by 30–40% and dry skim milk by 60% has resulted in a price for fat in milk used for these products usually above that paid for fat in farm separated cream. As the price for manufacturing milk increases, the question arises as to whether the price of fluid milk should be as high relative to manufacturing milk as it was before the lend-lease program. It is the view of the Department of Agriculture that increased supplies of dairy products are also essential for our own people in building up our national defense. Adequate supplies of fluid milk are needed and as the price structure has seemed to be inadequate to secure those supplies upward adjustments of fluid milk prices are in order. Requests for hearings on amendments to fluid milk marketing order have been acted upon as promptly as possible. Warranted price increases in fluid milk are not considered inflationary. E.F.G.

270. Seasonal Variations in Thermoduric Organisms and Methods of Control. H. MACY AND J. A. EREKSON. Internatl. Assoc. Milk Dealers, Assoc. Bul., *34:* 127–135. 1941.

Standard plate counts were made before and after laboratory pasteurization on 7,562 samples of milk from the St. Paul—Minneapolis milk shed during four seasons of the year. Field inspections were also made. Thermoduric organisms varied in number in relation to season and quality of raw milk. The thermo-tolerant organisms may be found in low as well as high count raw milk but a higher proportion of the poorer quality milk will be difficult to pasteurize satisfactorily regardless of the season. Percentage reduction is generally greater in high count milk although the number of thermoduric bacteria may be greater than in low count milk. In summer 40.5% of the samples showed less than 5,000 bacteria per ml. after pasteurization, while in winter the proportion below this figure increased to 80.1. These data indicate larger numbers of thermoduric bacteria in the summer milk usually due either to contamination of utensils or faulty cooling. Very high counts can usually be traced to improperly washed utensils or poor can washing. Laboratory pasteurization or individual producers' milk will locate the samples giving trouble and field inspection can usually eliminate the trouble. Careful producers will clean and sterilize a plant-washed can before using it if it has been allowed to stand some time under warm humid conditions. Milking machines are the most prolific single source of contamination; they were used on 41% of the farms having trouble and were found to be the responsible factor in every case. Wherever milkstone is present there will likely be trouble with thermodurics. Split seams, cracks,

crevices, broken solder or rusty spots or imperfections in rubber parts invite trouble. E.F.G.

271. **Put Your Taste Buds to Work on Your Flavor Problems.** K. G. WECKEL, Univ. Wisconsin, Madison, Wis. Internal. Assoc. Milk Dealers, Assoc. Bul., *34:* 136–140. 1941.

The bland flavor of milk probably accounts for the fact that it can be used with regularity in considerable quantities. However, if an unnatural flavor appears in the milk, many people quickly object to it and probably recognize such a flavor more readily than in most other foods. A small minority may dislike certain flavors such as oxidized, while the majority of milk users considers such flavors normal. Instead of waiting for flavor complaints by customers it is suggested that systematic flavor examinations of the product be made by one or two persons who have made an attempt to develop ability along this line. Education in detection and identification of flavors is proceeding in many consumer groups. Attention is called to work by Professor Wallenfeldt with agricultural and home economics teachers of Wisconsin to test ability to identify (a) by tasting solutions of salt, sugar, acid, and quinine and (b) by odor such substances as vanilla, orange, mint, lemon, maple, coffee, tobacco, fresh silage, etc. Men tend to excel in identifying solutions by taste but women excel in identifying odors.

E.F.G.

272. **The Effect of Pasteurization on Some Constituents and Properties of Goats' Milk.** H. S. HALLER, C. J. BABCOCK, N. R. ELLIS, U.S.D.A. Tech. Bul., 800. 1941.

The effect of pasteurization on the solubility of calcium and phosphorus, on the denaturation of proteins, and on the ascorbic acid content of goats' milk was found to be comparable to the effect of pasteurization upon the same constituents of cow's milk, as reported in the literature.

Curd tension was reduced considerably by the holder method of pasteurization but only slightly by high temperature pasteurization. Ascorbic acid was reduced 33 to 45% by the holder method but there was no effect from the high temperature method of pasteurizing.

The phosphatase enzyme in goats' milk was destroyed after 5 minutes heating at 143° F. making the phosphatase test as now conducted not applicable to goats' milk. P.H.T.

273. **Sour Cream—How to Prepare and Use It at Home.** Anonymous. U.S.D.A., Leaflet No. 213.

It is recommended that the cream be pasteurized before innoculating with commercial buttermilk. Directions for doing this in the home are

given. A number of recipes for the use of sour cream is included in this pamphlet. P.H.T.

PHYSIOLOGY

274. Uterine Distention and Lactation in the Rat. R. R. GREENE, Dept. Physiology and Pharmacology, Northwestern Univ. Endocrinology, *29:* 1026. 1941.

Ten to 15 cc. of paraffin were injected into the uteri of 15 rats within 24 hours of delivery. Within 48 hours 3 rats and their young were dead. Of the remaining 12 animals 6 successfully raised their litters although there was little or no weight gain of the young in the first 3 to 5 days. The young of the remaining 6 rats were dead within 24 hours to 6 days after the operation although a small amount of milk could be expressed from the mammary glands of the mothers. It was concluded that uterine distention *per se* is unable to prevent lactation. R.P.R.

275. Failure of Steroid Hormones to Induce Mammary Growth in Hypophysectomized Rats. SAMUEL L. LEONARD AND RALPH P. REECE, Dept. of Zoology and Dairy Husbandry, Rutgers Univ. Endocrinology, *30:* 32. 1942.

Desoxycorticosterone, testosterone and estrogen, either alone or in combination failed to induce new growth in the mammary glands of hypophysectomized rats. Testosterone seemed to slow the rate of mammary gland involution which normally follows hypophysectomy. The administration of estrogen directly on the skin over the mammary gland of hypophysectomized rats was without effect although in the normal or partially hypophysectomized rat new growth was stimulated. The results of the experiments were believed to further the concept that pituitary mammogen is essential for steroid hormone effects on the mammary glands of hypophysectomized rats. R.P.R.

276. Mammary Gland Development with Mammogen I in the Castrate and the Hypophysectomized Rat. A. A. LEWIS, E. T. GOMEZ AND C. W. TURNER, Dept. Dairy Husbandry, University of Missouri and Bureau Dairy Industry, U.S.D.A. Endocrinology, *30:* 37. 1942.

The injection of a dosage of 0.02 to 1.6 mouse units of a lipid mammogen I extract of pregnant-cattle anterior pituitary glands over a 6 day period produced no mammary growth in castrated male rats. Positive results, were obtained following either longer periods of treatment or increased dosage. Of sixteen 25- to 44-day-old male and female hypophysectomized rats treated with mammogen I for 7 to 10 days 15 showed evidence of mammary growth at autopsy. The weights of the thyroids, ovaries, and uteri of treated hypoph-

ysectomized female rats were similar to those of untreated hypophysectomized female rats. Adrenal weights of treated rats were significantly less than those of untreated rats. R.P.R.

277. **Mammary Growth in Hypophysectomized Male Mice Receiving Estrogen and Prolactin.** W. U. GARDNER AND ABRAHAM WHITE, Dept. Anatomy and Physiol. Chem., Yale Univ. Soc. Expt. Biol. and Med., Proc., *48:* 590. 1941.

Hybrid male mice were hypophysectomized at 4 to 8 weeks of age and from 3 to 47 days later received either the lactogenic hormone alone or in conjunction with estrogen. The lactogenic hormone was administered intraperitoneally and the estrogen subcutaneously on alternate days. Completeness of operation was checked by examination of serial sections of the sella. Of 11 mice which were completely hypophysectomized and which received a purified prolactin preparation 3 of them showed mammary growth. Of 13 completely hypophysectomized mice injected with the lactogenic hormone plus an estrogen all showed mammary growth. R.P.R.

278. **Effect of Various Dietary Supplements on Growth and Lactation in the Albino Mouse.** ZELDA B. BALL AND RICHARD H. BARNES, Dept. Physiol., Univ. Minnesota. Soc. Expt. Biol. and Med., Proc., *48:* 692. 1941.

It was found that purified diets containing 8% yeast were not adequate for lactation in mice. Dehydrated grass and wheat bran effectively increased lactation but the addition of these two substances to a basal purified diet, either alone or in combination, did not provide for as good lactation as did a stock commercial diet. The lactation-promoting effect of dehydrated grass and wheat bran was found not to be due to an increase of thiamin, riboflavin, pantothenic acid, factor W, or inositol in the diet. R.P.R.

279. **The Gonad-Stimulating Potency of the Pituitary of Hypothyroid Young Male Rats.** KATHYRN F. STEIN AND MARGARET LISLE, Dept. Zoology, Mt. Holyoke College. Endocrinology, *30:* 16. 1942.

Fifty-five 21- to 28-day-old male rats were thyroidectomized and after 21 to 50 days their pituitaries were injected into immature female mice 18 to 20 days of age. The recipients were given 1 to 3 pituitary glands and they were killed on either the 4th or 5th day after the first injection. Thyroidectomy invariably elicited an increase in pituitary weight and the presented data indicated a decrease in gonad-stimulating potency of the pituitary following thyroidectomy. R.P.R.

280. Gonadotropic Hormone in A P of Male and Female Rabbits during Growth. A. J. BERGMAN AND C. W. TURNER, Dept. of Dairy Husbandry, University of Missouri. Endocrinology, *30:* 11. 1942.

The amount of gonadotropic hormone in the pituitaries of groups of male and female New Zealand White Rabbits of increasing body weight was determined by injecting the pituitary tissue into male chicks and weighing their testes at the end of the injection period. There was a gradual rise in hormone concentration which reached a maximum in a group of females weighing 1500 gm. and in the males weighing 2500 gm. There was a notable decrease in hormone concentration of pituitaries collected from a group of non-breeding female rabbits weighing around 4500 gm. The hormone concentration in the AP of sexually mature males averaged about 70 per cent above that of females of comparable weight. R.P.R.

281. Influence of Environmental Temperature on Growth of Mammary Lobule-Alveolar System. JOHN P. MIXNER AND CHARLES W. TURNER. Soc. Exp. Biol. and Med., Proc., *48:* 443. 1941.

Ovariectomized virgin mice kept at a high environmental temperature had a decreased ability to respond to progesterone and estrone injections as judged by mammary lobule-alveolar growth. High temperature did not inhibit mammary response in mice injected with a pituitary extract containing the lobule-alveolar growth factor in conjunction with estrone. It was concluded that the anterior pituitary of animals kept at a high environmental temperature has a decreased ability to respond to the stimuli of progesterone and estrone as indicated by the secretion of the lobule-alveolar growth factor. R.P.R.

282. Influence of Lactogenic Preparations on Mammary Glands and Time of Vaginal Opening in Young Rats. WILLIAM R. LYONS, M. E. SIMPSON AND H. M. EVANS, Inst. Expt. Biol. and Div. Anat., Univ. California. Soc. Expt. Biol. and Med., Proc., *48:* 634. 1941.

The subcutaneous administration of lactogenic hormone in doses from 0.5 to 2.0 mg. daily to 21-day-old rats did not delay vaginal opening and in some cases may have advanced it. The continued injection of the same doses of hormone produced continuous vaginal mucification (two weeks) and lobule-alveolar development of the mammary glands. R.P.R.

283. Effect of Pseudopregnancy on Mammary Carcinoma Incidence in Mice of the A Stock. L. W. LAW, ROSCOE B. JACKSON, Memorial Lab., Bar Harbor, Maine. Soc. Expt. Biol. and Med., Proc., *48:* 486. 1941.

A definite increase in the mammary carcinoma incidence occurred in

female mice of a tumerous strain following pseudopregnancy induced by mating with vasectomized males. Eleven or 25.6% of 43 experimental mice developed breast adenocarcinomas whereas it had been previously shown that the incidence of carcinoma in virgin mice was only 4.9%. R.P.R.

284. Quantitative Study of the Effect of Inanition on Responsiveness of the Mammary Gland to Estrogen. J. J. TRENTIN AND C. W. TURNER, Dept. Dairy Husbandry, University of Missouri. Endocrinology, *29:* 984. 1941.

Male albino mice were selected to fall within a 15 to 20 gm. weight range at the start of the experiment. The normal daily food consumption per mouse was determined by the daily weight difference of a grain ration provided in a deep container that prevented wastage. This was slightly in excess of 3 gm. daily as determined by individual feeding and collective feeding in groups of 10. The amount of estradiol benzoate required to give unit response at the measured feed levels of 3.0, 2.5, 2.0 and 1.5 gm. per mouse per day was determined and expressed in terms of the amount required to give unit response on unlimited food. As the food intake level decreased the amount of estradiol benzoate required to produce a minimum duct growth response of the mammary glands was considerably and proportionately increased. R.P.R.

285. Effect of Stilbestrol on Lactogenic Content of Pituitary and Mammary Glands of Female Rats. A. A. LEWIS AND C. W. TURNER, Dept. of Dairy Husbandry, University of Missouri. Soc. Expt. Biol. and Med., Proc., *48:* 439. 1941.

Stilbestrol treatment of multiparous spayed rats for a period of 10 days caused serous or milk secretion in the partially developed lobule-alveolar mammary glands. The lactogen content of the pituitaries was increased as much as 210% and this was brought about by an increase in pituitary size and an increase in hormone concentration. R.P.R.

MISCELLANEOUS

286. Selection and Operation of Mechanical Refrigeration. L. C. THOMSEN, University of Wisconsin, Madison. Natl. Butter and Cheese Jour., *33*, No. 1: 14. 1942.

The author explains some of the technical aspects of mechanical refrigeration. A formula is given for calculating the rated capacity of an ammonia system. Abbreviated tables showing properties of common refrigerants are given. Dairy plants have a choice of five major systems of refrigeration: the direct expansion system which is used for room cooling, freezing ice

cream, and cooling milk and cream; the brine system, in which brine transmits the refrigeration; the "sweet water" system in which chilled water is used instead of brine; the sweet-water ice storage system which stores refrigeration and transmits it by chilled water; and the use of such substances as propylene glycol to transmit refrigeration. Advantages and disadvantages of each system are given.

The trend toward elimination of gauges is undesirable and decreases possible efficiency; examples are shown with calculations to prove this point. Tables show the proper refrigerant temperatures to maintain desired room temperatures. W.V.P.

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The dues are $5.00 a year, $3.00 of which is for a year's subscription to the *Journal of Dairy Science.* Correspondence regarding membership and dues should be addressed to R. B. Stoltz, Ohio State University, Columbus, Ohio.

The Third Annual Meeting of the Institute of Food Technologists is to be held at the University Farms, St. Paul, Minn., June 15 to 17, 1942, the week previous to the American Dairy Science Association meeting in East Lansing, Michigan.

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January 10th, 1942

Attention of Mr. H. J. Kerr

Gentlemen:

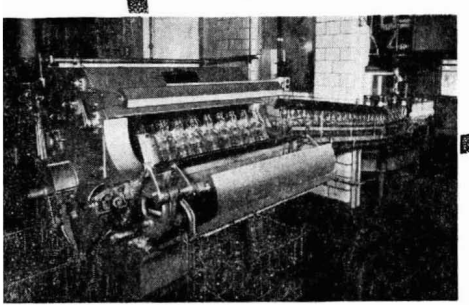
I wish at this time to take the opportunity to report upon the exceptional service which we have received from your No. 26 Super Roto-Matic Bottle Filler and Eight Wide CP Junior Bottle Washer.

The filler was installed about eight months ago and in the succeeding time we have handled through this ten valve machine an average of thirty-five thousand (35,000) bottles in a sixteen hour day. All this has been done with a minimum amount of upkeep. Compactness and simplicity of design with fewer moving parts are, in my estimation, mainly responsible for this excellent type of service.

For the past two months the eight wide CP Junior Bottle Washer has had the same long day of service and to date has shown no flaws of design or mechanical construction. It is delivering a clean and sterile bottle, using less steam, water, and alkali than the older type of machine that it replaced. The throw-outs are down to a minimum. This washer to date is showing some real savings in operation.

The service these two machines give and the number of units handled per day should be a guide in helping other dairymen in selecting this type of machinery.

Sincerely yours,
LOCKSHORE FARMS, INC.
R. E. Pretzer
Plant Supt.



View at left shows how the OP Junior Soaker Bottle Washer **and** OP Super Roto-Matic Bottler **are** teamed up in Lockshore **Farms'** modern **plant** to provide the **efficient** economical operation described in **Mr.** Pretzer's letter. There's **a** OP **Washer** and Bottler "hook-up" that **will** give **you maximum efficiency** . . . with minimum attention.

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