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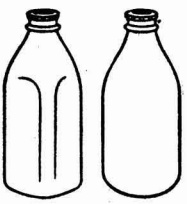

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A MODIFIED PHOSPHATASE TEST FOR CHEESE[1]

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In a recent paper (5) the authors presented a new principle and technic for the elimination of interfering substances in the Kay-Graham phosphatase test on Cheddar cheese. Additional research indicated that the trichloracetic acid technic could be simplified and improved further, and made into a phosphatase method for cheese possessing some distinct advantages over present methods. For example, the use of sodium barbitol was found unsatisfactory for cheese, as the optimum pH for phenol production could not be attained. As a result, a sodium carbonate-bicarbonate buffer (6) was substituted with highly satisfactory results. Other changes included the use of small phenol extraction flasks having a lower chamber capacity of 5 ml. and the combining of the trichloracetic acid and hydrochloric acid as the precipitating agent. A significant observation was that the BQC color reagent could be used interchangeably with the Folin-Ciocalteu color reagent in this new method.

With these considerations in mind, a more detailed account of this modification as a quantitative method is being presented, as well as the results from a series of experiments on Cheddar cheese showing the precision with which this method distinguishes raw from pasteurized milk cheeses.

PROCEDURE

Reagents[2]

1. *Carbonate-bicarbonate buffer substrate.* Weigh 11.5 g. C.P. sodium carbonate, anhydrous; 10.15 g. C.P. sodium bicarbonate, anhydrous; and 1.09 g. pure disodium phenyl phosphate, dissolve in distilled water and make up to 1.1. The pH will be 9.80. When not used fresh, add 10 ml. U.S.P. chloroform. This buffer should be tested after storage to assure freedom from phenol.

2. *Trichloracetic-hydrochloric acid precipitant (12.5 per cent trichloracetic acid plus 18 per cent HCl).* Make 25 g. C.P. trichloracetic acid (crystal) to 50 ml. with distilled water, add 50 ml. concentrated C.P. HCl (approximately 36

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1 This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Catherine Verwoert Work and Mr. Allan Levanthal for their aid in making many of the chemical analyses, and to Professor W. E. Ayres for his aid in the manufacture of several lots of cheese.

2 Keep all reagent bottles and color standards tightly stoppered and in a cool place.

per cent) and stir well. The resulting liquid is slightly yellow and can be held for a number of days, but fresh solution is preferable.

3. Ether. U.S.P. grade.

4. Sodium carbonate solution (4 per cent). Dissolve 40 g. C.P. anhydrous sodium carbonate in distilled water and make up to 1l. It is best to make this solution fresh, but it may be held well-stoppered in the refrigerator for a day or two.

5. Folin-Ciocalteu reagent.[3] It is prepared as stock solution according to A.O.A.C. (1) procedure for use with milk. The stock reagent should be diluted with two parts of water just before using.

6. Permanent Kay-Graham color standards and colorimeter. For producing permanent color standards refer to A.O.A.C. (1) or to Gilcreas and Davis (3). The color standards are the same as for milk.

Luximeter (no. 5) can be purchased from General Electric Co., Schenectady, New York.

The Method. One-half g. of a representative sample of ground cheese was placed in a 25 × 150 mm. test tube and mashed well with a glass rod. Following this, 1 ml. of warm (40° C.) carbonate-bicarbonate buffer substrate was added and the cheese was stirred into a paste. Then 9 ml. more of the buffer substrate and four drops of U.S.P. chloroform were stirred into the tube. A piece of parchment paper was fitted over the rod and tube and held in place by a rubber band. The tube next was incubated at 32 to 37° C. for 18 to 24 hours. After incubation, 1 ml. of the trichloracetic-hydrochloric acid precipitant was added gently to the tube. The pipet may be filled by immersion rather than by suction. The resulting precipitate was filtered off through Whatman no. 42 paper (11 cm.)

Five ml. of the clear filtrate then were pipetted into a small-sized Mojonnier type extraction flask (fig. 1). Next, 15 ml. of ethyl ether at 10 to 20° C. were added and the flask stoppered.[4] The flask then was inverted slowly ten times. For this purpose a special combined holder and shaker may be used (fig. 1). A clear ether layer extending to the neck of the flask developed after about 10 seconds of standing and was poured off into a 25 × 150 mm. test tube containing 7 ml. of 4 per cent Na_2CO_3 solution. The ether then was boiled off, in about 4 minutes by placing the tube in a beaker of hot water (150 to 160° F.) or on a steam-heated water bath.

After the ether was removed completely, 2 ml. of diluted Folin-Ciocalteu reagent (two parts water to one part stock reagent) were added and shaken. The mixture was placed in boiling water for 5 minutes, cooled to room temperature and filtered. The colored filtrate was compared to color standards or read in a Luximeter. Tentatively, values greater than 0.02 mg. phenol per 0.5 g. cheese indicate cheese made from underpasteurized or raw milk.

Use of 2-6-dibromoquinone-chloramide (BQC) instead of Folin-Ciocalteu reagent. Four drops of BQC solutions can be added in place of the Folin-Ciocalteu

[3] May also be purchased from Will Corporation, Rochester, N. Y. as stock solution.

[4] Extraction flasks can be purchased with either plain necks or ground glass stoppers. If cork stoppers are used they should be covered with clean tin foil.

reagent with very satisfactory results. The color is allowed to develop for 15 minutes and then compared against suitable carbonate-bicarbonate color standards. The ability to use BQC or Folin-Ciocalteu reagent gives greater flexibility to the method in that it would be easy to get checks on the results by splitting the carbonate solution containing the phenol and adding one of these indicators to each portion. In addition, using BQC in this method provides some advantages over other methods using BQC. Apparently very little or no protein or protein products are present in the final carbonate solution, so there would be no interfering yellow color-producing compounds. For this same reason, butyl alcohol may be used for extraction without encountering an emulsion after shaking, thus doing away with all centrifuging which is required at this point using other methods. Add 5 ml. of N-butyl alcohol, shaking the tube ten times, wait for 1 minute, and read colors at eye level or in a colorimeter.

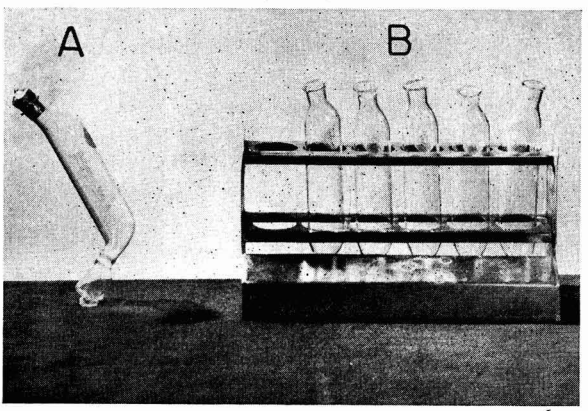

FIG. 1. A—Small phenol extraction flask. Manufactured by Will Corporation, Rochester, N. Y., and Mojonnier Bros. Co., Chicago, Ill. Extraction flasks with ground glass necks and stoppers also obtainable from Will Corporation, Rochester, N. Y. B—Extraction flask holder (use optional). Manufactured by Mojonnier Bros. Co., Chicago, Ill. Holder also can be made in laboratory using either sheet metal or wood.

Sensitivity of method with Folin-Ciocalteu and BQC reagent. Three lots of fresh raw milk were obtained on three different days from the Cornell University herd. One lot was divided into 350-lb. portions, heated to 143 or 145° F. in glass-lined vats and different portions held at this temperature for varying holding periods. Another lot of milk was divided into portions which were heated to various temperatures in the vicinity of 143° F., but all portions were held for 30 minutes. A third lot of milk was pasteurized properly at 143° F. for 30 minutes, but to separate portions were added different amounts of raw milk. The milks were tested by the standard laboratory Kay-Graham test and gave values corresponding to those expected from the heat treatment of the milk.

A total of 16 American Cheddar cheeses, weighing about 35 lb. each, were made from the above milks and were ripened at 50° F. for 6 months. These cheeses then were tested for phosphatase activity and sensitivity by the modified method using both the Folin-Ciocalteu and the BQC reagents.

Results using the Folin-Ciocalteu reagent are presented in table 1. When the milk was pasteurized properly the phosphatase values on the cheeses were less than 0.01 mg. phenol per 0.5 g. cheese. At no time in the analyses of a large

TABLE 1

The sensitivity of the modified phosphatase test on cheddar cheese ripened 6 mo. at 50° C. (incubated 20 hr. at 35° C.)

Heat treatment of cheese milk		Phosphatase values of cheese	
		Using the Folin-Ciocalteu color reagent[a]	Using BQC color reagent[b]
(° *C.*)	(*min.*)	(*mg. phenol/0.5 g. cheese*)	(γ *phenol/0.5 g. cheese*)
145	30	0.003	0.5
143	30	0.006	1.0
143	25	0.034	12.0
143	20	0.033	14.0
143	10	0.128	> 40.0
143	0	0.654	> 40.0
143	30	0.007	1.0
141	30	0.107	> 40.0
139	30	0.227	> 40.0
137	30	0.654	> 40.0
143 – 30 + No Raw		0.003	1.0
143 – 30 + 0.1% Raw		0.032	9.0
143 – 30 + 0.2% Raw		0.048	18.0
143 – 30 + 0.4% Raw		0.064	> 40.0
143 – 30 + 0.7% Raw		0.115	> 40.0
143 – 30 + 1.0% Raw		0.183	> 40.0

[a] A reading of 0.05 mg. phenol/0.5 g. cheese indicates a very deep blue color using the Folin-Ciocalteu reagent, as the units (mg.) are 1,000 times greater than the units (γ) expressed when using the BQC color reagent.

[b] 2-6-dibromoquinone-chloramide. Dissolved 50 mg. in 10 ml. of methyl or ethyl alcohol.

number of Cheddar cheeses made from properly pasteurized milk have the phosphatase values extended beyond 0.02 mg. phenol per 0.5 g. cheese. On the other hand, a change of from 30 to 25 minutes of holding the cheese milk at 143° F. produced a phosphatase value on the cheese greater than 0.02 mg. phenol per 0.5 g. cheese. Similarly, when the temperature of heating the cheese milk was reduced from 143 to 141° F. and held for 30 minutes, a phosphatase value of 0.11 mg. phenol per 0.5 g. cheese was recorded. In the case of raw milk contamination, and addition of 0.1 per cent raw milk manifested itself as a phosphatase test greater than 0.03 mg. phenol per 0.5 g. cheese.

Data showing how well results obtained on Cheddar cheeses over one year old compared to those obtained by the method of Sanders and Sager (7) are shown in table 2.

TABLE 2

The phosphate test of cheddar cheese aged over 1 yr. and made from pasteurized milk to which raw milk had been added

Milk	Extraction procedure Kosikowsky-Dahlberg[a]		Sanders-Sager[b]			
			Direct reading[c]		Butyl alcohol reading[d]	
	(*mg. phenol/0.5 g.*)	(*interpretation*)	(*γ phenol/0.25 g.*)	(*interpretation*)	(*γ phenol/0.25 g.*)	(*interpretation*)
Pasteurized (143° F., 30 min.)	0.008	Pasteurized	0.0	Pasteurized	0.3	Pasteurized
Pasteurized + 0.1% raw	0.018	Pasteurized	0.0	Pasteurized	0.3	Pasteurized
Pasteurized + 0.2% raw	0.031	Underpasteurized	0.5	Pasteurized	0.8	Pasteurized
Pasteurized + 0.3% raw	0.050	Underpasteurized	1.1	Pasteurized	2.0	Pasteurized
Pasteurized + 0.7% raw	0.086	Underpasteurized	2.2	Pasteurized	3.0	Pasteurized
Pasteurized + 1.0% raw	0.131	Underpasteurized	3.8	Underpasteurized	4.1	Underpasteurized

[a] More than 0.02 mg. indicates underpasteurization (Folin-Ciocalteu reagent).
[b] More than 3 γ indicates underpasteurization.
[c] Color in blanks, pinkish-yellow; in test, yellowish-blue; blank reading 0.
[d] Color in blanks, pinkish-yellow; in test, bluish-green; blank reading of 0.3 subtracted from results on cheese.

When BQC color reagent was used in place of the Folin-Ciocalteu color reagent the sensitivity was equally as good and the value of phenol which apparently divided the cheeses made from properly pasteurized milk from those made from improperly pasteurized milks was about 5.0 γ phenol per 0.5 g. cheese.

Amount of phenol obtained from a second extraction. Phenol is somewhat soluble in water at 20° C., although in ether it is extremely soluble. Possibly an appreciable amount of free phenol never was extracted from the aqueous acid solution in one extraction. That this is not the case was verified experimentally. A number of incubated cheese-buffer substrate solutions, after precipitation with trichloracetic-hydrochloric acid, were extracted with two 15 ml. portions of ethyl ether. About 92 to 96 per cent of the free phenol was extracted the first time. The amounts obtained from a second extraction would not affect materially the initial values, as they were very small and would, on the whole, remain fairly constant for pasteurized milk cheese.

Effectiveness of the trichloracetic-hydrochloric acid precipitating agent. Cheddar cheeses ranging in age from 2 days to 11 years, as well as 14 other varieties of cheese of varying age, were mixed in 0.5 g. lots with 10-ml. quantities of carbonate-bicarbonate buffer substrate, well-emulsified, and then 1 ml. of trichloracetic-hydrochloric acid reagent was added to each tube. This precipitating agent would precipitate very satisfactorily cheeses of all ages and varieties without any further adjustment. This was shown by the clear filtrates which were produced.

DISCUSSION

A technic to eliminate interfering substances in the Kay-Graham phosphatase procedure for ripened cheese as proposed earlier by Kosikowsky and Dahlberg (5) now is presented in more detail and in simplified form as a laboratory phosphatase method for all cheese. This method possesses advantages. It produces clear filtrates devoid of interfering substances which give clear blue colors matching standards exactly, thus providing for greater accuracy. It has great flexibility, as either the Folin-Ciocalteu or the BQC color reagent may be used simply by substituting one for the other in the last step. The sodium carbonate-sodium bicarbonate buffer and the trichloracetic-hydrohcloracetic acid precipitating agent in this method, although more study may be necessary, may be used for any variety of natural or process cheese or cheese product of any age without adjusting either the buffer or the precipitating agent to care for differences in the buffer capacities of the cheeses. The buffer substrate and cheese need not be heated after incubation to destroy the phosphatase. Therefore, there is less tendency to decompose the disodium phenyl phosphate. When using butyl alcohol for BQC color extraction purposes, one can shake the tube vigorously without forming a permanent emulsion. The phosphates and citrates in process cheese will not affect the final results when using the carbonate-bicarbonate buffer substrate, as shown by Kosikowsky and Dahlberg (6). Finally, few reagents are required and a number of these, including the Folin-Ciocalteu reagent, may be purchased as stock solutions from chemical firms.

Although this method no longer closely resembles that of the standard Kay-Graham method for milk (4), it nevertheless possesses some of the elements and the basic principles. An inclusion of this test for cheese would make the Kay-Graham test (4) more universally applicable. This is important, as the standard Kay-Graham test is a proved basic test for milk and other dairy products. Preliminary studies also indicate this modified method will work well on chocolate milks and ice creams.

The fact that an 18- to 24-hour incubation period is recommended should not prove to be any disadvantage for a laboratory test dealing with cheese. The longer incubation period would allow a more orderly and efficient use of laboratory time, as a large number of samples could be placed in the incubator in the afternoon and then could be run the next morning. This idea has been substantiated by an informal survey of men in charge of research for cheese companies and in charge of state testing laboratories. If speed is essential, an extraction method using shorter incubation periods of 1 or 4 hours at 37° C., using BQC color indicator, might be developed.

Actually, using the method in its modified form does not entail much more operating time than that involved in the standard Kay-Graham laboratory method for milk (4) nor is it more expensive. As each step can be organized to fit in well with the next without having to consider any time element beyond which the test would not function properly, a large number of samples may be run during the day. From the time of adding the precipitating agent after incubation, two analysts completed over 25 individual samples in 1 hour.

When the small phenol extraction flasks are not available, equally good results can be obtained with standard-size Mojonnier fat extraction flasks. Add 5 ml. of filtrate to the flask and enough distilled water (25° C.) to bring to bottom of neck. Add 25 ml. of ethyl ether and shake ten times. Proceed thereafter as with regular modified method. Where extraction flasks are unavailable, small separatory funnels may be substituted as a temporary measure. With these funnels 5 ml. of filtrate can be shaken ten times with 15 ml. of ethyl ether; the filtrate then is taken off through the bottom and the ether is poured off through the top.

The results have been expressed emperically as mg. phenol per 0.5 g. of cheese to agree approximately with the Kay-Graham method (4). The data may be calculated readily to exact quantitative expression of results to agree with the expression used by Sanders and Sager (7). As 0.5 g. of cheese was diluted to 11.5 ml., from which 5 ml. of filtrate were used, the results obtained as mg. phenol could be multiplied by 1.15 to give quantitatively the mg. phenol per 0.25 g. cheese. If then multiplied by 1,000, the results are in γ phenol per 0.25 g. cheese.

Two factors favor greater accuracy by this modified procedure than by the Sanders and Sager method (7), which is the only other method applicable to cheese. The color in the present procedure has no interfering off-colors and the pH, the buffer and the longer time of incubation induce maximum enzyme activity. The greater sensitivity is indicated by the standard or criterion for

pasteurization. In the Sanders and Sager method (7) pasteurization is indicated by 3 γ or less of phenol per 0.25 g. cheese. In the procedure herein presented using Folin's reagent, 0.02 mg. phenol per 0.5 g. cheese or less represents pasteurization. This is 23 γ phenol per 0.25 g. cheese, or about eight times the quantity of phosphatase activity shown by the Sanders and Sager method (7). The greater sensitivity also is shown by the effect of known amounts of raw milk upon the results. In collaborative tests on cheese, Gilcreas (2) found it was difficult to detect 0.3 per cent of raw milk added to pasteurized milk using the Sanders-Sager method as nine of the fifteen collaborators found such cheese to be made from pasteurized milk. The values varied from 1 to 7 and averaged 3.11 γ phenol per 0.25 g. cheese, which is only 0.1 γ above the criterion for pasteurization. The modified Kay-Graham extraction method in the present study gave results over twice the criterion for pasteurization with only 0.2 per cent of raw milk. Again Gilcreas (2) reported data for the 15 collaborators on cheese made from pasteurized milk containing 1 per cent of raw milk which varied from 4 to 11 γ phenol per 0.25 g. cheese, and the average value was 2.5 times the critical value for pasteurization using the Sanders-Sager method. The data herein presented for the same raw milk contamination show values in cheese of 9 times the critical value for pasteurization.

PRECAUTIONS

Precautions involved in the use of this method should be pointed out. In rare cases a temporary emulsion may be formed in the upper chamber of the extraction flask during extraction (the narrow emulsified layer which may be formed between the ether and aqueous layer in the neck of the flask is considered normal). This condition may be caused by very excessive agitation or the use of too porous filter paper, causing turbid filtrates. To remove this temporary emulsion, place the extraction flask, with the cork loosely stoppered, under the hot water tap (140 to 160°·F.) for 10 to 20 seconds, shake the flask briefly and then cool quickly under the cold water tap.

In boiling off the ether, fire safety rules should be followed. The ether should be boiled off completely to make a satisfactory test. This can be seen easily by visual inspection or by carefully shaking the tube to detect foaming. In some cases the ether reagent may show a slight trace of phenol. This can be detected and compensated for by conducting a reagent blank test on all reagents and going through all the steps in the process, including incubation.

Although control samples on the cheese consistently have given very low values, control tests are advisable where it is suspected phenol may have formed during ripening. To do this heat the sample of cheese to 170° F. for 5 minutes in a test tube and cool, take 0.5 g. of the cooled sample, mix it with 10 ml. of carbonate-bicarbonate buffer substrate and conduct a regular test on it.

The extraction flasks are rinsed out easily between samples with lukewarm tap water. These flasks need not be dry, but as the smaller extraction flasks are calibrated for 5 ml. there should not be too much free water in the lower chamber.

SUMMARY

A phosphatase test for cheese is presented in detail with data showing its sensitivity. This method possesses a number of advantages. It is very accurate, as the final filtrates produced in this modification were clear and devoid of interfering substances. Conditions of incubation promote maximum phosphatase activity. The Folin-Ciocalteu color reagent and the BQC color reagent can be used interchangeably with proper color standards. Apparently, any natural or process cheese or cheese product can be tested without making adjustments in the buffer substrate or precipitating agent.

In the new method the cheese sample is incubated with a sodium carbonate-bicarbonate buffer substrate at pH 9.55 for 18 to 24 hours at 32 to 37° C. This solution then is precipitated by a trichloracetic-hydrochloric acid reagent and some of the clear filtrate is placed in either a standard size or a small Mojonnier type extraction flask. Ethyl ether is used as the extracting agent but later is boiled off from an alkaline solution leaving the phenol behind. The phenol in this alkali solution then is determined colorimetrically by adding either the Folin-Ciocalteu or the BQC color reagent and comparing the developed blue colors against proper color standards.

The sensitivity of this method is high. Tentatively, any value above 0.02 mg. phenol per 0.5 g. cheese, using the Folin-Ciocalteu reagent, or any value over 5.0 γ phenol per 0.5 g. cheese, using the BQC reagent, is considered to indicate cheese made from raw milk, improperly pasteurized milk or pasteurized milk contaminated with raw milk.

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APPLICATION OF A SODIUM CARBONATE–BICARBONATE BUFFER IN THE PHOSPHATASE TEST FOR CHEESE[1]

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In the past a number of different buffers have been used in the buffer substrate when conducting the phosphatase test. Kay and Graham (6) have successfully used sodium barbitol in their test for milk, Aschaffenburg and Neave (1) have suggested the use of sodium carbonate, whereas Scharer (10) has used a borate buffer.

As far as cheese was concerned, Caulfield and Martin (2), Lambert (8) and Sanders and Sager (9) all have stressed the importance of a proper buffer as the cheese, because of its different buffering capacity, exerts a decided effect on the pH of the buffer substrate. If a buffer were unable to maintain the proper pH for optimum phenol production low values would be obtained. Although Sanders and Sager (9) appear to use a very good modified borate buffer, it still is necessary in their test to make adjustments for different aged cheeses or cheeses of different varieties. During a recent study (7) on the elimination of interfering substances in the phosphatase test, it was noted that the sodium barbitol buffer used was affected greatly by cheeses of different age or variety and that additions of cheese invariably would carry the pH of the cheese-buffer substrate solution much lower than that recommended by earlier investigators for optimum phenol production by the alkaline phosphatases. As a result, it was decided to abandon the sodium barbitol buffer and substitute one which would maintain the pH near its optimum for phenol production.

A study of several buffers pointed to a sodium carbonate-sodium bicarbonate combination as showing the most promise. This buffer had been studied earlier on the alkaline phosphatase systems by Fischer (4), Schwarz and Fischer (11), Horwitz (5) and Delory and King (3). The latter investigators stated that the carbonate-bicarbonate buffer can be held for as long as 6 months over a pH range of from 8.8 to 10.6 at 37° C. in well-stoppered wax bottles without any demonstrable change in pH. As this buffer covered a region of higher pH than did the sodium barbitol buffer, a study was made to determine the optimum pH for phenol production with this buffer and also its effectiveness in maintaining the optimum pH in the presence of cheese. The concentration of carbonate in the buffer used for this experimental work was much greater than that used in the carbonate buffer of Delory and Kling (3) and others (4, 11).

METHODS

To study phenol production at varying pH values, the trichloracetic acid technic for the Kay-Graham test recently presented by Kosikowsky and Dahlberg (7) was used with the exception that the sodium carbonate-bicarbonate buffer was

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substituted for sodium barbitol in the buffer substrate. A series of buffer substrates was used with a wide range of pH. These differences were obtained by varying the relative proportion of sodium carbonate to sodium bicarbonate while keeping the carbonate concentration constant in each buffer substrate solution. One-half g. portions of a sample of Cheddar cheese were incubated for 20 hours at 35° C. in 10 ml. portions of the different buffer substrates. The following day quantitative phenol determinations were made.

RESULTS

Optimum pH for phenol production. Data obtained using the new sodium carbonate-bicarbonate buffer are shown in table 1. The optimum pH for the

TABLE 1

The optimum pH for phosphatase activity on cheese and milk using the carbonate-bicarbonate buffer substrate over 18-hour incubation at 32–35° C.

pH of buffer substrate plus milk	Phenol values (*mg. phenol/0.5 g. milk*)	pH buffer substrate plus cheese	Phenol values (*mg. phenol/0.5 g. cheese*)
11.04	0.007	10.55	0.005
10.32	0.009	10.25	0.016
9.97	0.035	10.05	0.028
9.83	0.045	9.95	0.049
9.71	0.056	9.79	0.073
9.64	0.062	9.63	0.115
9.47	0.051	9.47	0.111
9.22	0.042	9.32	0.068
8.95	0.023	9.05	0.062
		8.68	0.052
		7.93	0.019

production of phenol with cheese was approximately 9.55, while the optimum range roughly extended from about pH 9.40 to 9.70. Optimum pH data obtained with a sample of milk were very similar.

Effectiveness of the carbonate-bicarbonate buffer in maintaining the optimum pH range. The carbonate-bicarbonate buffer substrate used in this portion of the study was the same as that which was found to produce the optimum pH. This solution contained 11.5 g. anhydrous sodium carbonate, 10.15 g. sodium bicarbonate and 1.09 g. disodium phenyl phosphate made up to 11. with water. This solution had a pH of 9.8 measured at 25° C.

Cheddar cheeses ranging in age from 2 days to 11 years, as well as samples from 14 other varieties of cheese of varying age, were mixed in 0.5 g. lots with 10-ml. quantities of the carbonate-bicarbonate buffer substrate. In addition, fluid milk and fluid milk products, using 1 ml. per 10 ml. buffer, also were tested. Measurement of pH at 25° C. was conducted a few minutes after mixing and again after incubation for 24 hours at 35° C. Data obtained are shown in table 2.

Addition of 0.5 g. portions of cheese reduced the pH, on the average, to about 9.55 or a drop of 0.25 pH unit. For all the cheeses tested, the pH change in the buffer substrate did not extend beyond the optimum pH range of 9.45 to 9.70

TABLE 2

Effectiveness of carbonate-bicarbonate buffer in controlling the pH of cheese and other dairy products
(optimum pH at 35° C. using carbonate-bicarbonate buffer = 9.55 ± .15)

Dairy Product	Age	pH Range[c]
12 Cheddar cheeses	1 to 132 mos.	9.40–9.68
19 Other cheese varieties[a]	Unknown	9.49–9.65
Other dairy products[b]	Fresh	9.61–9.71

[a] Process Limburger, process gruyre, cream, pimento cream, Swiss, Limburger, Liederkrantz, Chantelle, Snappy club, Kaukauna club, Sharp process, Munske, d'Oka, Edam, Blue, Romana, Cottage.

[b] Milk, Heavy cream, Chocolate milk, Bottle milk, Sour cream, Vanilla ice cream mix, Chocolate ice cream mix.

[c] All pH measurements in this study made with Beckman pH meter, laboratory model G.

after incubation for 24 hours at 35° C. Where milk and other fresh dairy products were tested the pH ranged from 9.5 to 9.7.

Effect of phosphates and citrates upon phosphatase activity in the presence of the carbonate-bicarbonate buffer substrate. Sanders and Sager (9) have stated that excess phosphates and citrates, as normally found in process cheese, may inhibit the action of phosphatases but that in the presence of a borate buffer this inhibiting effect does not occur. With a view towards applying the carbonate-bicarbonate buffer substrate to eventual use on process cheese, an investigation was made of the significance of excess citrate and phosphate emulsifying salts in natural Cheddar cheese. To a number of Cheddar cheeses, 3 per cent quantities of disodium phosphate or sodium citrate were added and the trichloracetic acid technic, using a 20-hour incubation period, at 35° C. was conducted on the cheese. Table 3 shows that excess amounts of phosphates and citrates exert no effect upon

TABLE 3

The phosphatase values of cheddar cheese with and without added stabilizing salts, using the trichloracetic technic on the Kay-Graham method with a sodium carbonate-bicarbonate buffer

Cheese	Phosphatase values of cheese		
	Natural cheddar cheese no added salt	Natural cheddar cheese plus 3% NaH_2PO_4	Natural cheddar cheese plus 3% sodium citrate
		(mg. phenol/0.5 g. cheese)	
1	0.003	0.009	0.006
2	0.003	0.010	0.013
3	0.032	0.038	0.038
4	0.048	0.048	0.048
5	0.064	0.083	0.093
6	0.115	0.118	0.135
7	0.183	0.131	0.158
8	0.654	0.676	0.632

phenol production in the presence of carbonate buffer substrate. This would mean that this technic could be used with process cheese.

DISCUSSION

The use of a sodium carbonate-bicarbonate buffer in the modified trichloracetic acid phosphatase test for cheese appears to show promise. This buffer was used in greater concentration than by previous investigators. It has the advantages of being very inexpensive, very soluble and easily obtainable. At the concentration and pH at which it will be employed it is stable, according to Delory and King (3). This recently was verified in this work. In addition, this buffer is strikingly effective in maintaining optimum pH with cheeses of wide buffer capacities and in its presence excess amounts of phosphate and citrate salts exert no noticeable effect upon phosphatase activity. This buffer shows no noticeable inhibition of phosphatase activity in the 18- to 24-hour test at around pH 9.6, using the trichloracetic technic of the Kay-Graham method.

SUMMARY

A sodium carbonate-bicarbonate buffer solution containing 11.50 g. sodium carbonate and 10.15 g. sodium bicarbonate per liter of solution has been found to be highly effective in maintaining optimum pH for phosphatase activity in the trichloracetic acid Kay-Graham 24-hour phosphatase test for cheese.

Optimum pH for phosphatase activity was maintained with the single sodium carbonate-bicarbonate buffer upon the addition of a great variety of dairy products, including very aged hard cheeses.

The optimum pH for phosphatase activity during incubation for 24 hours at 35° C. with a carbonate-bicarbonate buffer substrate was 9.55 ± 0.15.

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THE UTILIZATION OF FETAL STORES OF VITAMIN A BY THE NEWBORN CALF[1]

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The liver storage of vitamin A in the newborn calf can be increased by dietary means (6, 10, 16, 18). Whether this increased liver storage and other fetal stores of vitamin A can be utilized by the neonatal calf has not been demonstrated (14, 16).

The purpose of this study was to determine whether the fetal storage of vitamin A could be utilized in the young dairy calf, as indicated by blood plasma levels and liver storage of vitamin A when a vitamin A-free ration is fed.

EXPERIMENTAL

Nine male calves from Ayrshire, Guernsey and Holstein dams born in the University of Connecticut herd from February to June, 1948, were used in this experiment. The dams of these calves received the same basal ration fed on the basis of liveweight for 8 weeks prior to the calculated parturition date. This consisted, per 100 lb. liveweight, of 1 lb. of U. S. no. 2 alfalfa hay, 3 lb. of well-matured corn silage and 1 lb. of grain mixture consisting largely of cereal grains and containing approximately 13.5 per cent crude protein. The hay, silage and grain contained on an average of 3.87, 1.06 and 0.15 mg. of carotene per lb., respectively, as determined by the method of Moore and Ely (9) as modified by Nelson *et al.* (12). Six of the dams of these calves received daily 1 million U.S.P. units of vitamin A in the form of shark liver oil containing 25 per cent by weight of crude soybean lecithin[2] for 30 days prior to the calculated parturition date. This oil contained 54,440 U.S.P. units of vitamin A per gram, as assayed spectrophotometrically against the U.S.P. vitamin A reference standard (vitamin A acetate in cottonseed oil).

The newborn calves were not allowed to nurse but were removed immediately to a separate portion of the barn. There they received 3 lb. of reconstituted skim milk two times daily. The skim milk fed contained no detectable carotene or vitamin A, as determined by the method of Boyer *et al.* (1).

Venous blood samples were drawn from the jugular vein each day between 7 and 8 A.M., citrated, cooled to 4° C., and centrifuged; the carotene and the vitamin A contents of the plasma were determined immediately. On the tenth morning, the calves were slaughtered. The entire liver was removed and cooled at 4° C. The whole liver was macerated in a Waring blendor and analyses run on aliquot samples within 48 hours. In several cases the livers were frozen and held at −18° C. for a period not exceeding 2 weeks until analyses could be made.

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[2] This oil generously was supplied by Melvin Hochberg of the Nopco Chemical Co., Harrison N. J.

The carotene and vitamin A contents of the plasma were determined by the method of Kimble (8) and that of the liver by the method of Davies (2). Both determinations were made with an Evelyn photoelectric macrocolorimeter which had been standardized previously with crystalline β-carotene in petroleum ether (b.p. 30–60° C.) and crystalline vitamin A alcohol in chloroform.

The data were treated statistically by analysis of variance essentially as outlined by Snedecor (15).

RESULTS

Data on the carotene and vitamin A content of plasma for individual calves are given in table 1. The carotene content of the plasma of calves from dams re-

TABLE 1

The effect of prepartum supplementation of vitamin A on blood plasma levels of carotene and vitamin A in newborn calves fed reconstituted skim milk

Calf no.	Birth	Age (d.)								
		2	3	4	5	6	7	8	9	10
		(γ *carotene/100 ml.*)								
Basal dams										
1	3	2	2	2	1	0	0	2	2	1
2	2	1	2	6	1	2	2	2	2	3
3	3	3	2	2	2	2	2	2	3	3
$\bar{X}$	2.7	2.0	2.0	3.3	1.3	1.3	1.3	2.0	2.3	2.3
Basal + vitamin A dams										
4	1	0	0	0	1	1	0	1	0	0
5	1	0	0	0	1	1	0	1	1	1
6	1	2	0	0	0	0	1	1	1	1
7	1	1	1	1	0	1	1	1	2	1
8	2	2	1	2	2	2	2	2	2	1
9	3	2	2	1	1	1	1	1	0	2
$\bar{X}$	1.5	1.2	0.7	0.7	0.8	1.0	0.8	1.2	1.0	1.0
		(γ *vitamin A/100 ml.*)								
Basal dams										
1	4.9	3.8	5.3	2.4	4.9	1.8	1.4	1.4	1.3	1.4
2	4.4	8.2	1.4	2.5	1.4	2.1	4.4	4.3	3.8	2.6
3	4.2	3.0	0.8	1.7	0.5	0.8	0.8	0.6	0.5	0.8
$\bar{X}$	4.50	5.00	2.50	2.20	2.27	1.57	2.20	2.10	1.87	1.60
Basal + vitamin A dams										
4	5.2	6.8	9.7	9.2	9.9	11.1	6.2	8.8	7.0	7.0
5	7.0	5.7	8.8	8.8	8.3	9.0	5.3	7.8	6.5	7.5
6	13.5	9.2	8.3	7.5	9.9	12.3	12.8	13.5	14.6	12.6
7	9.9	11.3	10.2	9.6	11.6	13.8	14.3	6.2	17.3	17.0
8	11.1	10.1	9.6	6.0	6.0	11.1	7.7	4.8	6.6	2.3
9	6.0	10.7	7.1	11.3	9.0	7.5	9.8	6.6	6.2	5.7
$\bar{X}$	8.78	8.97	8.95	8.73	9.12	10.80	9.35	7.95	9.70	8.68

ceiving the vitamin A supplement tended to be lower ($P < 0.05$) than of the calves from basal dams. However, the vitamin A levels in the plasma of calves from dams receiving the basal ration plus vitamin A were significantly higher

($P < 0.001$) than those of the calves from dams receiving the basal ration alone. The plasma vitamin A was significantly higher ($P < 0.01$) on each individual day except at 2 and 10 days of age in the calves from dams receiving vitamin A.

The carotene and vitamin A storage in the liver at 10 days of age is shown in table 2. The liver storage of carotene and vitamin A in calves from dams fed

TABLE 2

The effect of prepartum supplementation of vitamin A on the liver storage of carotene and vitamin A at 10 days of age in calves fed reconstituted skim milk

Calf no.	Carotene		Vitamin A	
	γ/g.	Total γ	γ/g.	Total γ
		Basal dams		
1	0.24	148	0.29	178
2	0.50	361	0.21	152
3	0.71	432	0.15	91
X	0.483	313.7	0.217	140.3
		Basal + vitamin A dams		
4	0.20	114	1.28	727
5	0.14	83	1.00	593
6	.....	.....	.....	.....
7	0.16	98	7.80	4789
8	0.44	314	2.45	1747
9	0.55	459	5.19	4334
$\bar{X}$	0.298	213.6	3.544	2438.0

supplementary vitamin A paralleled the blood plasma levels; that is, the liver storage of carotene was reduced and that of vitamin A was increased as compared with calves from dams on the basal ration alone. The difference in vitamin A was statistically significant ($P < 0.005$), but the difference in carotene was not.

DISCUSSION

These data indicate that the vitamin A stored by the fetus can be utilized by the young calf and should be considered in the nutrition of the young calf in addition to colostrum.

Previous work (4, 17) has shown that the feeding of supplementary vitamin A during the prepartum period has raised the levels of vitamin A in the plasma during the neonatal period in both calves and lambs. One source of this vitamin A is believed to be fetal storage. This emphasizes the importance in calf studies of evaluating critically the nutritional history of the dam.

Other workers have reported the apparent depression of the carotene level when supplementary vitamin A is fed. Wise *et al.* (19) recently have reviewed this decrease in respect to the lactating cow, and other workers (3, 11) have reported a similar finding when vitamin A is fed directly to the calf. This experiment corroborates the finding of Esh *et al.* (5) that intrauterine nutritional influences seemed to depress the plasma carotene.

The possibility that reconstituted skim milk may stimulate the mobilization of storage depots of vitamin A should not be overlooked. Several reports (3, 7) of such an effect can be found in the literature. It would seem desirable, therefore, to eliminate this factor by feeding a whole milk with a uniform, relatively low content of vitamin A. Preliminary work (13) has indicated that fetal storage of vitamin A contributes to the higher blood plasma levels in young calves, especially after 2 weeks of age, when a standardized colostrum and whole milk is fed.

SUMMARY

One million U.S.P. units of vitamin A were fed daily to the dam for 30 days prior to the calculated parturition date and the effect of this supplement on blood plasma levels and liver storage of carotene and vitamin A was measured in six young male dairy calves fed only reconstituted skim milk. Parallel measurements were made on three control calves. These data indicate that the fetal storage of vitamin A can be utilized by the newborn calf. This is shown by the decrease in blood plasma levels of carotene, the increase in blood plasma levels of vitamin A, and the greater liver storage of vitamin A at 10 days of age of calves from dams fed supplementary vitamin A.

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THE EFFECT OF ADDED AMINO ACIDS ON THE FLAVOR OF CHEDDAR CHEESE MADE FROM PASTEURIZED MILK[1]

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The protein in cheese curd has been shown to undergo a series of chemical changes during the ripening process, until amino acids and ammonia are present in some quantities, in addition to the more complicated degradation products. Whether certain protein decomposition products, materials produced by action upon butterfat or products resulting from changes in other components of the cheese curd are responsible for the characteristic Cheddar flavor has not been established satisfactorily, partly because of the complexity of the system in which the changes are occurring.

Davies *et al.* (2) added, at the time of salting, various chemical compounds which might affect the growth and metabolism of bacteria or the activity of rennet enzymes. Cystine was added in concentrations of 0.01 and 0.02 per cent, but it had no significant effect on the rate of ripening, even though the flavor score of the cheese made with cystine was slightly higher than that of the control cheese. No differences were noted in the amounts of the various nitrogen fractions. After the present investigation had been completed, Harper and Swanson (4) indicated that mixtures of amino acids may contribute to the flavor of Cheddar cheese.

The purpose of the present study was to test the possibility that the addition of various amino acids to cheese curd made from pasteurized milk might contribute to production of the typical flavor and aroma characteristic of a fine raw-milk Cheddar cheese ripened properly, either directly or as substrata upon which microorganisms or enzymes might act.

METHODS

Manufacture of Cheddar cheese. The milk used in all cheese was mixed herd milk with a fat content varying from 3.1 to 3.8 per cent. It was pasteurized at 143° F. for 30 minutes in a spray-vat pasteurizer. For lots 1 through 8, 170 lb. of milk were used and for lots 9 through 12, 125 lb. of milk were used. In all lots the method of manufacture was essentially that of Wilson (7), with the modification that after the curd was salted, it was weighed into separate 2.5-lb. quantities. Lots 1 through 8 required six separate quantities from each quantity of curd, while lots 9 through 12 required five quantities. The amino acid-salt mixture described later then was incorporated into the 2.5-lb. quantities of curd and allowed to dissolve for approximately 10 minutes before the curd was placed in small wooden hoops. The hoops were used to produce a cheese 5 inches in diameter and 2.5 inches thick, weighing approximately 2.5 lb. The pressure was applied slowly in the press to minimize the amount of whey expressed from the cheese until the

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amino acids had been in contact with the curd for some time. The cheeses were ripened at 60° F.

After curing for 3 days, samples of cheese were obtained and determinations were made of the fat, moisture and total chlorides. The various cheeses were sufficiently similar in composition, both within a lot made at one time with different amino acids and between the various lots within a series, that differences in flavor could not be attributed to variations in gross composition. Therefore, the data on composition are not presented.

Organoleptic examination. Lots 1 through 8 were examined every 4 weeks for a period of 24 weeks. Lots 9 through 12 were examined at 2-week intervals for a period of 12 weeks, and again at the age of 24 weeks. The cheeses in each lot were compared with the control to determine any difference in flavor characteristics, as the emphasis was upon possible increase in characteristic Cheddar flavor, rather than on a score based upon commercial scoring practices. Each lot was ranked from 1 to 6 or 1 to 5, as determined by the number of cheeses in the lot, with 1 being the most desirable cheese and 6 or 5 being the least desirable. In case no differences were noted between certain cheeses in a lot, an average of the rankings covered by the undifferentiated cheeses was assigned to each of the cheeses thus grouped.

Preparation of amino acids. For this study DL-alanine, L-arginine, DL-aspartic acid, L-cystine, DL-glutamic acid, glycine, L-histidine, L-hydroxyproline, DL-isoleucine, DL-leucine, L-lysine, DL-methionine, DL-phenylalanine, L-proline, DL-serine, DL-threonine, L-tryptophan, L-tyrosine and DL-valine were used. In lots 1 through 8, 0.5 g. (0.04 per cent of the weight of the cheese curd) of the pure amino acid was weighed out and combined with 5 g. of cheese salt, the resulting mixture being added to 2.5 lb. of curd. This mixture was ground thoroughly in a mortar and pestle, to obtain a finely divided material. The amino acids chosen for lots 9 through 12 were those which had given what was apparently the most desirable cheese in previous trials and included glycine, methionine, tyrosine, serine, glutamic acid, arginine, aspartic acid and valine. These eight amino acids were added to separate cheese in amounts of 0.5 g. (0.04 per cent) and 1.5 g. (0.13 per cent). In each case the amino acid was ground with 5 g. of salt.

The amount of amino acid originally chosen, 0.5 g., was based on the assumption that it was a large enough amount to produce any probable beneficial effects, as it would represent the freeing of a considerable fraction of the acid in question from the proteins. In lots 9 through 12, the use of 1.5 g. of each amino acid chosen was to determine if the larger amount would have any effect upon the flavor produced or upon the rate of bacterial growth.

Microbiological examination. Microbiological examination of the cheese in lots 9 through 12 was thought advisable to determine any correlation between the bacterial counts and the flavor of the cheese, or any effect of the amino acids upon bacterial development. The sampling and plating procedures were those proposed by the American Public Health Association (1). Tomato juice agar (5) was the plating medium, and the plates were incubated at room temperature for 5 days. Typical colonies were picked from the plates and examined for morphological

TABLE 1

Ranks according to flavor within lots of cheese in which 0.5 g. of amino acid had been incorporated at salting

Lot	Amino acids used	Rank at 4 wk.	8 wk.	12 wk.	16 wk.	20 wk.	24 wk.	Total rank
				First series				
	Control	6	6	6	4.5	6	6	34.5
	Valine	4	4.5	4	4.5	4	4	25
	Leucine	3	4.5	5	4.5	5	5	27
1	Glutamic acid	2	2	2	1	3	3	13
	Serine	1	1	2	2	1	1	8
	Arginine	5	3	2	4.5	2	2	18.5
	Control	5	3	5	1	2	2	18
	Aspartic acid	3	2	3.5	3	2	2	15.5
	Proline	1	5	1	5	4	4	20
2	Phenylalanine	2	4	3.5	3	5	5	22.5
	Tyrosine	4	1	2	3	2	2	14
	Histidine	6	6	6	6	6	6	36
	Control	3.5	5	5	5	5	4	27.5
	Threonine	3.5	3.5	3	4	4	4	22
	Methionine	3.5	1.5	1.5	2	2	1	11.5
3	Lysine	3.5	6	1.5	2	2	4	19
	Tryptophan	3.5	3.5	4	6	6	4	27
	Isoleucine	3.5	1.5	6	2	2	4	19
	Control	3	4	6	5	6	3.5	27.5
	Alanine	6	4	3.5	2	4	3.5	23
4	Glycine	3	4	1	1	1	1	11
	Hydroxyproline	3	6	3.5	6	5	3.5	27
	Cystine	5	2	3.5	3	2	6	21.5
				Second series				
	Control	4	5	6	4	4.5	3.5	27
	Valine	2	2	2	1	2	1	10
5	Aspartic acid	3	2	1	3	4.5	3.5	17
	Threonine	1	2	3	6	4.5	3.5	20
	Alanine	5	4	5	5	4.5	6	29.5
	Control	4	5	6	6	4.5	4.5	30
	Leucine	5	1	4	5	4.5	4.5	24
	Proline	1	6	5	3	4.5	4.5	24
6	Methionine	2	3.5	1	2	2	2	12.5
	Glycine	3	3.5	2	1	1	1	11.5
	Isoleucine	6	2	3	4	4.5	4.5	24
	Control	5	5	5	5	4	4	28
	Cystine	1	3	3	2.5	2	2	13.5
	Tyrosine	3	2	1	2.5	2	2	12.5
7	Glutamic acid	3	1	2	2.5	2	2	12.5
	Histidine	6	6	6	6	6	6	36
	Lysine	3	4	4	2.5	5	5	23.5
	Control	4	2	4	5	6	5	26
	Arginine	4	6	3	1.5	1	2	17.5
	Phenylalanine	4	5	6	6	5	6	32
8	Hydroxyproline	1	4	5	4	4	3	21
	Tryptophan	4	3	2	3	3	4	19
	Serine	4	1	1	1.5	2	1	10.5

characteristics. For the direct microscopic count, a 0.01 ml. sample was pipetted directly from the 1:10 dilution, spread in a film over an area of 1 cm.^{2}, dried, treated with xylol and stained with the Newman-Lampert stain.

RESULTS

The data from the first two series of cheese, in which 0.5 g. of amino acid was added to 2.5 lb. of curd at the time of salting, are presented in table 1. The only difference between the two series is the association within lots within the series. Since the differences in flavor between the cheeses in one lot commonly were small, the error of ranking undoubtedly is considerable.

Histidine consistently contributed a very objectionable and characteristic flavor and was placed last in each comparison. An aqueous solution of histidine had this same characteristic flavor, indicating that the amino acid as such imparted the undesirable flavor to the cheese. Cheese to which other amino acids had been added showed a slight tendency in some cases or no tendency in other cases toward improvement over the controls. On the basis of these results, eight amino acids which showed some possibility of affecting the flavor favorably were chosen for further trials in a third series.

TABLE 2

Ranks according to flavor within lots of cheese in which 0.5 g. and 1.5 g. amino acid had been incorporated at salting

Lot	Amino acids used	Amt.	Rank at 2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.	24 wk.	Total rank
			Third series							
		(*g.*)								
	Glycine	0.5	3.5	2	3	3	3	3.5	3	21
	Glycine	1.5	5	2	3	3	3	3.5	3	22.5
9	Control	.....	3.5	2	3	3	3	3.5	3	21
	Methionine	0.5	1	4.5	3	3	3	3.5	3	21
	Methionine	1.5	2	4.5	3	3	3	1	3	19.5
	Tyrosine	0.5	1.5	1	3	3	3.5	5	3	20
	Tyrosine	1.5	5	3.5	3	3	3.5	3	3	24
10	Control	.....	3.5	3.5	3	3	5	3	3	24
	Serine	0.5	3.5	3.5	3	3	2	3	3	21
	Serine	1.5	1.5	3.5	3	3	1	1	3	16
	Glutamic acid	0.5	2.5	3	3	3	5	3	3	22.5
	Glutamic acid	1.5	5	3	3	3	3	3	3	23
11	Control	.....	2.5	3	3	3	3	3	3	20.5
	Arginine	0.5	2.5	3	3	3	3	3	3	20.5
	Arginine	1.5	2.5	3	3	3	1	3	3	18.5
	Aspartic acid	0.5	4.5	3	3	3	1	3	2.5	20
	Aspartic acid	1.5	2	3	3	3	4.5	3	5	23.5
12	Control	.....	2	3	3	3	4.5	3	2.5	21
	Valine	0.5	2	3	3	3	2.5	3	2.5	19
	Valine	1.5	4.5	3	3	3	2.5	3	2.5	21.5

The data on the rankings of the third series of cheese are presented in table 2. This series consisted of cheese made with 0.5 g. and 1.5 g. amino acid per 2.5 lb.

of curd. There was no appreciable difference in the level of flavor development, as indicated by the total ranks for the 24-week curing period. Serine added at the rate of 1.5 g. per 2.5 lb. of curd showed a possibility of some desirable effect at the 12-week ranking, but no difference was noted at the 24-week ranking. Data for the other additions were too inconsistent to indicate any potential desirable effect of these compounds upon flavor production in Cheddar cheese.

Little effect of the added amino acids upon the microbial population could be demonstrated. Generally the types varied as would be anticipated for normal cheese, with the greatest number of lactic acid streptococci present early in the curing period, followed by a brief period when yeasts were evident and this in turn usually followed by an increase in the numbers of gram-positive non-sporulating, long and short rods. The gram-positive rods varied from a negligible number in the case of the use of 1.5 g. of methionine to the predominant type during the latter part of the ripening period of those cheese made with added serine, but the results seemed to be conditioned more by the lot of milk used than by the added amino acid.

DISCUSSION

The addition of individual amino acids to cheese curd made from pasteurized milk appeared to have little consistent effect upon the flavor of the resultant Cheddar cheese. One exception is that the addition of histidine resulted in a very definitely undesirable flavor defect, and another exception is the possible slight beneficial influence of serine. Other amino acids either gave no improvement or results which were so inconsistent as to prevent the drawing of definite conclusions that the flavor was improved as a result of their addition. These results are in accord with previous reports, such as those of Van Slyke *et al.* (6) and Freeman and Dahle (3), that additional proteolysis, particularly that resulting from the use of quantities of rennet greater than normally employed, did not result in additional or accelerated flavor development. However, these earlier studies did not determine definitely that breakdown to amino acids was involved.

Serine possibly increased the numbers of lactobacilli present in the cheese; this is of interest because the cheese to which 1.5 g. of serine had been added was the only one of series 3 which showed possibly significant evidence of improved flavor. The relatively small numbers of gram-positive rods in other lots of the third series of cheese, both among the control cheese and those cheese to which amino acids were added may have been a factor in the lack of flavor development.

Although a known amount of each amino acid was added to the curd, no studies of the amounts of amino acids retained in the cheese were made, and the data should be interpreted with that limitation in mind. The varying solubilities of different amino acids may have affected the amount of each which was retained in the cheese, although the lots in which increased amounts were used without difference in effect would indicate that variations in retention are not an important factor.

Another factor which should be considered is that, although the addition of most amino acids to Cheddar cheese made from pasteurized milk failed to produce consistent improvement in flavor, the use of amino acids in cheese made from raw milk might produce different results because of the different bacteria and

enzyme systems which presumably are factors in the ripening of such cheese. Possibly some of the inconsistencies noted in the present study could be attributed to differences in the bacterial flora of the various lots of cheese. The testing of some of the potentially favorable amino acids by addition to cheese containing selected strains of bacteria might offer some interesting possibilities.

The preliminary report of Harper and Swanson (4), which appeared after the present study had been completed, indicates that amino acid mixtures may have a greater favorable influence on flavor development in Cheddar cheese than do the various amino acids added singly. This is an aspect of the problem which was not explored in the present investigations.

SUMMARY AND CONCLUSIONS

Nineteen of the amino acids normally present in casein were added in 0.5 g. quantities to 2.5 lb. of curd made from pasteurized milk, as a possible means of improving the flavor of the resulting cheese, either directly or as substrata for enzyme or microbial activity.

Histidine contributed a definitely undesirable flavor. Addition of the other amino acids resulted in cheeses indistinguishable from the controls in most cases and slightly but inconsistently better in a few cases. The eight amino acids which the first two series indicated might possibly contribute something to the flavor were used in 0.5 g. and 1.5 g. amounts per 2.5 lb. of curd in a third series of cheese to determine any effect of the increased amino acid content upon the typical flavor of Cheddar cheese. These eight amino acids had little or no effect on the flavor ranking and bacterial development except that serine possibly had a favorable effect on both flavor and bacteria.

The addition of amino acids to Cheddar cheese made from pasteurized milk had no consistent desirable effects upon flavor development under the conditions of this study.

The assistance of F. J. Babel in judging a number of the lots of cheese is acknowledged.

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THE PHYSICAL PROPERTIES OF EVAPORATED MILK WITH RESPECT TO SURFACE TENSION, GRAIN FORMATION AND COLOR

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The sterilization processes used in the manufacture of evaporated milk are designed not only for sterility but for those physical characteristics which improve the quality of the milk. As a rule, most manufacturers of evaporated milk grade the sterilized milk in terms of viscosity, grain and color; the relative importance assigned to these properties varies, depending on the point of view of the manufacturer.

In the quest for improved sterilization processes, the lack of organization of processing data has been an impediment to a comprehensive understanding of the sterilization process. A knowledge of the relationship existing between the color, viscosity, grain formation and surface tension for processes in general would assist greatly in predicting the effects of contemplated changes in processing.

Considerable attention has been given to the problems arising in the sterilization of milk. Bell *et al.* (1) found that high temperature-short time sterilized evaporated milk thickened on storage, but if the milk was filled into cans after the high temperature-short time sterilization process and the color increased by further heating, the gelation tendency was reduced greatly, if not eliminated. Holm *et al.* (3) determined the relationship between the cooking time and the temperature necessary for coagulation. Later, the logarithmic nature of the heat coagulation was noted by Webb and Holm (5) in test tube experiments. While many of the phenomena associated with the processing of evaporated milk are recorded in the literature, little if any attention has been given to the character of the heat coagulation or its relationship to color, viscosity and surface tension.

The purpose of this paper is to examine the results obtained in various sterilization processes with reference to grain formation, surface tension, viscosity and color and to show the relationship of these measurements to one another.

METHODS AND APPARATUS

The apparatus consisted of a thermostatically controlled oil bath, 75 mm. $\times$ 10 mm. test tubes, a wire tray for holding the tubes, a cold water bath for cooling the tubes quickly after heating, a Beckman spectrophotometer for reflectance measurements, a Du Noüy tensiometer, a Mojonnier-Doolittle Universal viscosimeter, a pilot sterilizer and a Brown recording potentiometer.

The method adopted for the analysis of sterilization processes is based on a comparison of the effects of time and temperature on standard evaporated milk in test tube experiments with the effects of time and temperature on similar milk processed in commercial or like equipment.

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EXPERIMENTAL PROCEDURE

A 50-lb. lot of raw milk was heated in a jacketed hot well to 203° F. and held at this temperature for 10 minutes. The time required for the milk to reach 203° F. was 10 to 12 minutes. Following this hot well treatment, the milk was drawn into a pilot evaporator where evaporation of water from the milk was conducted under vacuum at a temperature of 125° F. The average time for the evaporation of a 50-lb. lot of milk was 15 minutes. After evaporation, the milk was heated to 140° F. and homogenized at 2,500 lb. per square inch. Since more water was evaporated from the milk than is required for standard milk, it was standardized to 7.9 per cent fat and 26.5 per cent total solids by the addition of water. Finally, 2 ml. of this milk were inserted carefully into the small test tubes with a hypodermic needle. The tubes were sealed over a small flame, the hot tip of each tube being drawn into a loop so that the tubes could be suspended on a wire and placed in a tray.

Four lots of samples, each consisting of ten prepared tubes of milk at room temperature, were processed in the oil bath at the temperatures given in table 1. After processing, tubes were withdrawn at predetermined intervals, cooled in the water bath, dried, numbered and later the contents were analyzed for color and surface tension.

The relative color change was recorded in terms of the reflective index as used by Nelson (4). Surface tension determinations were made on the Du Noüy tensiometer after standardizing the instrument with weights, but the value in dynes per centimeter is relative, since the minor adjustments and corrections were not observed in the manipulation of the instrument. The essential part of these data is given in table 1.

TABLE 1

The effect of time and temperature on the color and surface tension of evaporated milk heated in small sealed test tubes

233.5° F.				244.5° F			
Sample	Time	Reflectance 520 mμ	Surface tension[b]	Sample	Time	Reflectance 520 mμ	Surface tension
	(*min.*)	(%)	(*dynes/cm.*)		(*min.*)	(%)	(*dynes/cm.*)
1	40	55.3	54.7	1	28	50.0	54.1
2	45	52.8	56.9	[a]2	31	47.8	56.0
[a]3	50	50.0	57.6	3	34	45.5	56.0
4	55	47.5	57.7	4	37	43.5	56.0
250.5° F.				257° F.			
1	20	50.3	54.5	1	13	54.9	52.3
[a]2	22	47.3	56.0	[a]2	16	48.8	55.4
3	24	44.5	56.0	3	18	45 8	55.4
4	26	42.0	56.9	4	20	43.3	55.8

[a] Curve 1, fig. 1 constructed from these data after correction for temperature lag.
[b] Surface tension of unsterilized milk was 50.3 dynes/cm. at 75° F. Reflectance of unsterilized evaporated milk at 520 mμ was 80.3%.

Preparation of a surface tension curve. A preliminary curve representing points of equivalent surface tensions with respect to temperature and time was

drawn from the data given in table 1. For greater accuracy, it was thought desirable to evaluate the time required for the tubes of milk to approach within 1° F. of the bath temperature. The method used was essentially that of Bigelow *et al.* (2). Thermocouples were inserted into milk at 75° F. contained in the small tubes described previously and these tubes were immersed in a constant temperature oil bath maintained at 200° F. The average time-temperature data obtained plotted as a straight line on semi-log paper and it was assumed that this relationship continued within the limits of the processing temperatures studied. The slope of this line was expressed as the number of minutes required to cross one log cycle, in this case 1.8 minutes. This slope was used for the four processes studied.

The time calculated for the milk in the tubes to reach the desired bath temperature varied from 3 minutes to attain the bath temperature of 233° F. to 3.5 minutes to attain the bath temperature of 256° F. This lag time was almost entirely without effect on the milk, since approximately 2.5 to 3.0 minutes were required to attain 200° F. and temperatures below 200° F. have relatively little effect on milk when compared with temperatures above 200° F. Because the time of processing was relatively long, there was little object in converting the 0.5 minute period above 200° F. into effective time at bath temperature. Therefore, a lag time of approximately 3 minutes was subtracted from the observed time given in table 1 and the preliminary curve based on the observed time of table 1 was replaced by curve 1, figure 1. This latter curve has a somewhat greater slope than the preliminary curve.

It was observed that the surface tension of evaporated milk, taken after heat processing, increased generally as the apparent viscosity increased. This increase in surface tension is in conformity with the well-known effect of proteins on the surface tension of a liquid; that is, a reduction in protein activity at a surface increases the surface tension. Therefore, in the preparation of a curve representing surface tension at the temperatures and time of processing given, for example, in table 1, the surface tension provides an indirect means of estimating the probable true extent of heat coagulation with greater precision than by judging from the amount of grain formation.

It was not found convenient to control the room temperature at the time of analysis; therefore, the surface tension values chosen for equivalence were taken at the time of abrupt change in the rate of rise, which generally was near the maximum value attainable. In general, most of the rise in surface tension occurred during the final third of the processing period. This period of increasing surface tension was marked by an initial rapid rise, then a period of little change and finally by a small further rise to a maximum value.

Preparation of evaporated milk samples. The pilot sterilizer used in obtaining the data on coagulation and color of milk sterilized in cans was designed so that the processed milk obtained would be comparable to evaporated milk obtained from a commercial Anderson-Barngrover sterilizer when similar processes were used. The pilot sterilizer used is a single-unit, cylindrical cooker, approximately 20 inches in length and 30 inches in diameter, in which a nine pocket reel is installed. An entrance valve is located on top, so that cans may be entered in a

manner similar to that in a commercial A-B sterilizer. A flexible metal belt is installed under the reel so that the cans may be given additional rolling to approximate more closely the conditions in the A-B sterilizer. Removal of a plate on the side of the sterilizer gave access to the cans after processing.

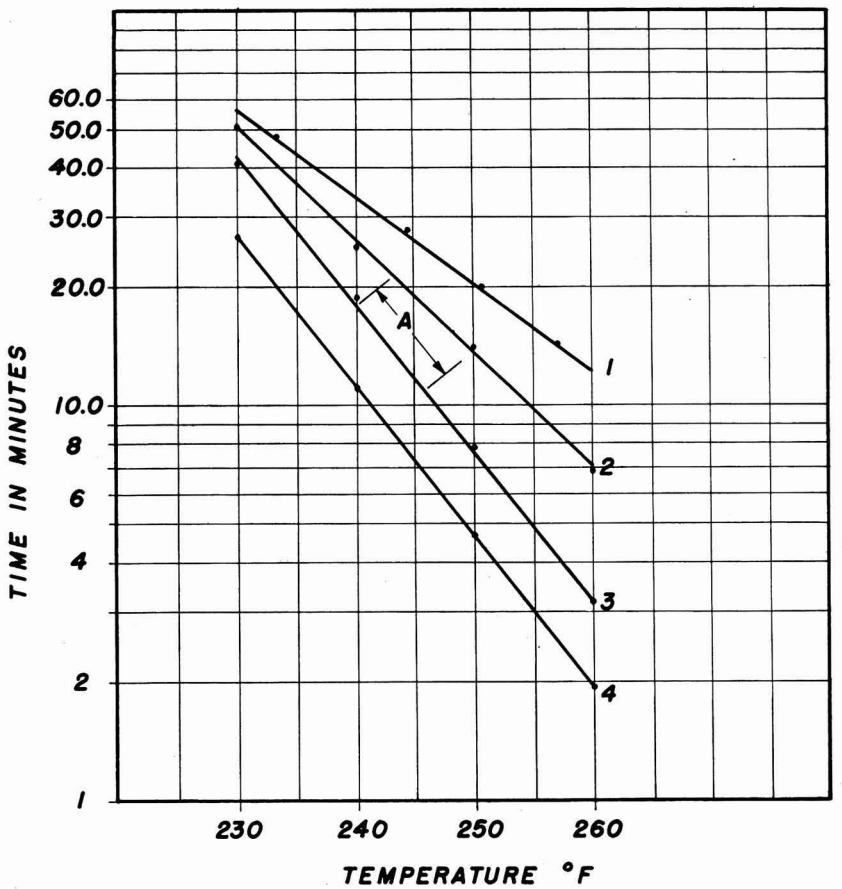

FIG. 1. The relationship between time and temperature of heating as it affects grain, surface tension and color of heat processed evaporated milk.

Curve 1. Represents points of equal color and surface tension as determined in test tube trials. Curves 2, 3 and 4 represent equivalent grain points in processed evaporated milk for raw milk forewarmed as follows: For 2-milk forewarmed to 210° F., held 10 minutes, followed by heating to 250° F. for 10 seconds; for 3-milk forewarmed to 210° F. and held 10 minutes; for 4-milk forewarmed to 195° F. without holding time. Commercial sterilization processes are indicated by Zone A.

Samples of standard evaporated milk in 14.5 oz. commercial milk cans were entered through a valve into the cooker maintained at 227° F. The time of pretreatment was 9 minutes. At the expiration of the pretreatment period, the temperature of the cooker was raised quickly (10–15 seconds) to the desired temperature and held at this temperature until the desired degree of grain formation was obtained. At the end of this processing period, the milk was cooled quickly by the addition of cold water to the cooker. Continuous rotation of the reel was maintained throughout the process. Grain formation in the milk was judged by the amount of coagulation noted in a film of milk formed by means of a wire loop or a similar method. The degree of graining was noted, in order of increasing severity, as very slight film, slight film, film and heavy film. Grain formation seldom was developed to the point of visibility on the back of a spoon. Data typical of these pilot runs are recorded in table 2. Curves 2, 3 and 4 of figure 1 were derived from these data.

TABLE 2

The effect of heat on the physical properties of evaporated milk

Group	Sample	Viscosity Mojonnier	% Reflectance 520 mμ	Grain	Surface tension	Cooker[a]	
						Time	Temp.
		(75° F.)	(%)		(dynes/cm.)	(min.)	(° F.)
2[b]	1a	30	62.8	sl. film	51.4	7.0	260
	2a	45	59.8	sl. film	54.2	13.5	250
	3a	67	57.5	film	55.7	23.5	240
	4a	125	54.8	hy. film	58.1	47.5	230
3[c]	1b	30	73.6	sl. film	53.2	3.5	260
	2b	40	70.3	film	54.1	7.0	250
	3b	51	65.3	film	54.3	17.0	240
	4b	57	60.0	film	55.0	37.0	230
4[d]	1c	30	81.2	sl. film	50.4	2.0	260
	2c	45	78.8	sl. film	51.8	4.0	250
	3c	48	75.5	sl. film	53.2	10.0	240
	4c	60	70.5	film	56.2	24.0	230

[a] Cooker time preceded by 9-min. process at 227° F.
[b] Forewarning process for group 2 was 210° F. for 10 min. followed by 10 sec. at 250° F.
[c] Group 3 was forewarmed to 210° F. and held 10 min.
[d] Group 4 was forewarmed to 195° F. but not held.

Conversion of process time into time at a given temperature. The evaluation process used herein is similar to that used by Bigelow *et al.* (2) for the conversion of the effect of time at one temperature into the effect of time at another temperature. The conversion is illustrated in figures 2 and 3. For example, figure 2 illustrates the heat penetration curve of a can of milk from the end of the pretreatment process at 227° F. through the 7-minute process at 250° F. and for 1.5 minutes of the cooling period. The slope of the heat penetration curve is 2.9 minutes per log cycle; this slope is used for all processes and was determined in this laboratory. The log cycle for the temperature range 249 to 250° F. is omitted for convenience in plotting and on account of the negligible effect on the calculated process time.

From the data of figure 2 a second curve is plotted (figure 3) in which the process time of figure 2 is plotted as the abscissa and the reciprocals of the time on curve 1, figure 1 corresponding to the process temperatures shown on figure 2 are plotted as ordinates. For example, after 2 minutes in the cooker the temperature of the milk (center of can) is 240° F. as shown on figure 2. The time for this temperature on curve 1 figure 1 is 34.5 minutes. The reciprocal of this time is

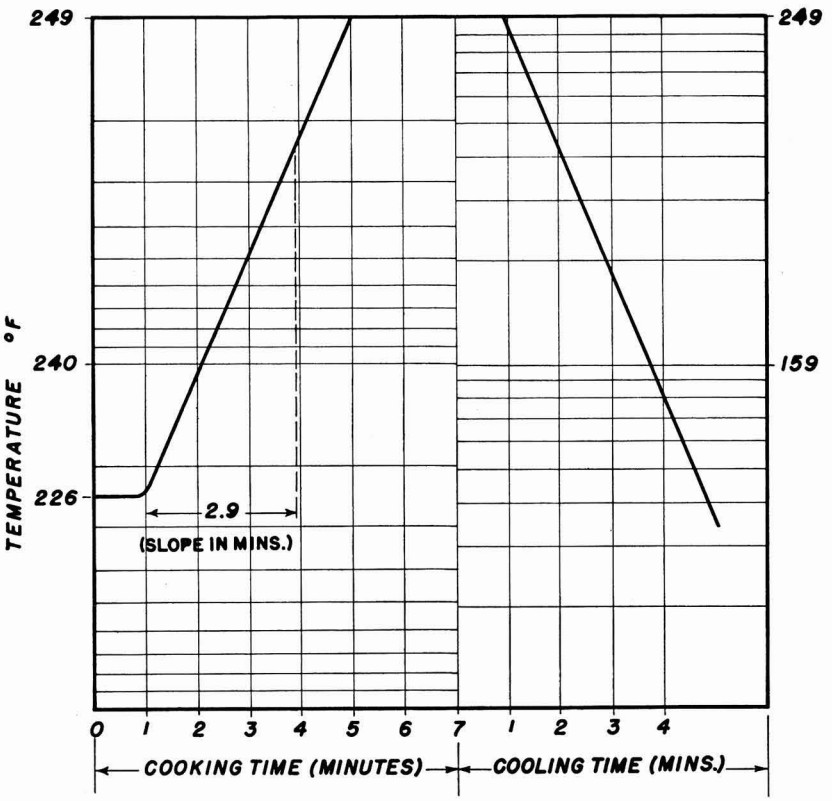

FIG. 2. Rate of heating and cooling of evaporated milk during processing.

plotted on figure 3 as ordinate of abscissa 2. The area under this curve (solid line) represents the part of the 8.5 minutes that is effective at 250° F., in this case 6.8 minutes. The pretreatment time (not shown but derived by similar methods) is equivalent to 1.2 minutes at 250° F. The total time plotted is 8 minutes as shown on curve 3, figure 1. The data used in the illustration are derived from the data for sample 2b, table 2.

Table 2 is a compilation of data for samples of evaporated milk heated as indicated to locate points of grain equivalence. Because the limited time and number

of samples available for this experiment were insufficient to permit determination of the rate of surface tension rise and grain formation, a second lot of evaporated milk was prepared for this purpose. In this second experiment the raw milk was forewarmed by the batch method to 205° F. and held 5 minutes at this tempera-

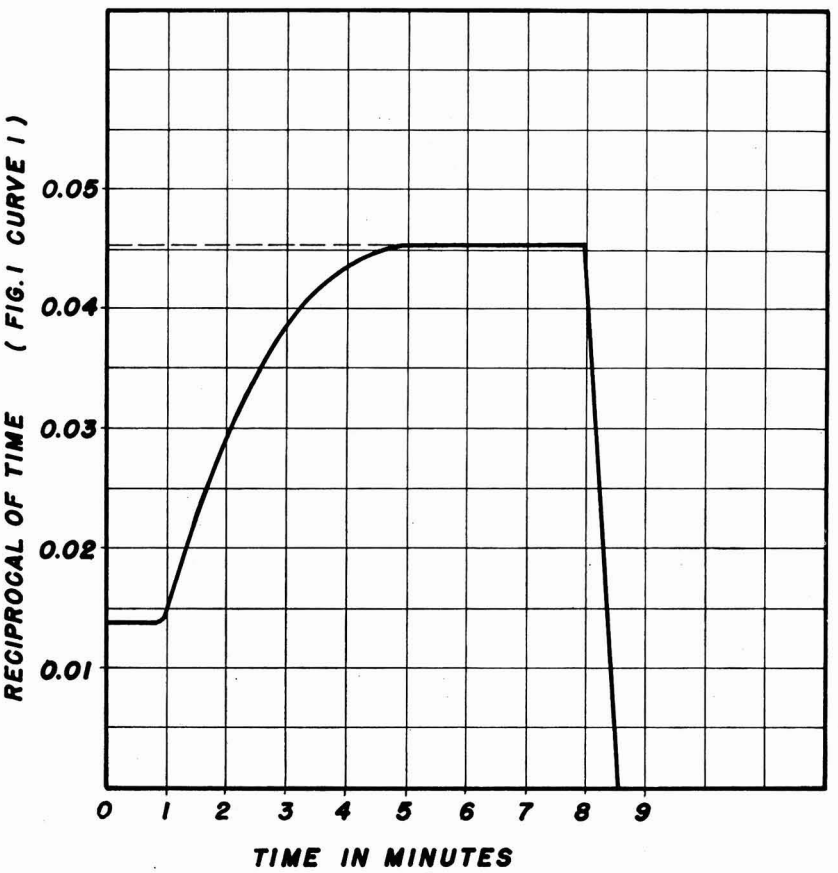

FIG. 3. Conversion of heating and cooling curve time into time at 250° F.

ture. Ten minutes were required to attain 205° F. Evaporation of the milk was conducted under vacuum at a temperature of 125° F., after which the milk was homogenized at 2,500-lb. per square inch and standardized to conform with Federal standard evaporated milk. Samples were filled into 14.5 oz. cans and processed to the point of grain formation. The samples were analyzed; the data are recorded in table 3.

TABLE 3

The effect of cooker process variation on the physical properties of evaporated milk

Group	Sample	Viscosity Mojonnier	% Reflectance 520 mμ	Grain	Surface tension	Cooker process	
						Time	Temp.
		(*75° F.*)	(%)		(*dynes/cm.*)	(*min.*)	(*° F.*)
1	1	30	80.3	None	52.5	3.2	260
	2	32	80.0	Sl. film	53.5	3.4	260
	3	46	77.0	*[a]	55.5	3.6	260
	4	46	76.0	***	56.5	3.8	260
1	1	25	79.3	None	52.5	5.3	250
	2	31	78.1	None	55.5	6.0	250
	3	32	76.0	None	56.2	7.0	250
	4	45	73.8	F lm	56.5	8.0	250
	5	56	71.3	***	57.3	9.0	250
1	1	28	74.3	None	52.5	12.0	240
	2	36	70.5	None	54.0	15.0	240
	3	55	68.8	Sl. film	56.7	17.0	240
	4	72	64.8	***	57.0	19.5	240
1	1	23	77.8	None	53.0	15.0	230
	2	24	70.3	None	53.5	25.0	230
	3	47	64.5	None	55.5	35.0	230
	4	83	61.0	Film	57.5	40.0	230
	5	110	60.0	Hy. film	58.6	45.0	230
2	1	18	81.7	None	52	1	250
	2	22	80.3	None	52.2	3	250
	3	27	78.1	None	52.3	5	250
	4	30	77.1	None	52.4	6	250
	5	43	73.0	Trace	55.2	8	250
	6	52	70.5	Film	56.8	9	250
	7	70	69.0	***	57.8	10	250
2	1	17	81.4	None	52.2	1	260
	2	23	81.3	None	52.2	2	260
	3	24	79.3	None	52.5	3	260
	4	24	78.9	None	52.8	3.4	260
	5	30	77.5	None	53.8	3.8	260
	6	30	77.1	None	54.5	4.0	260
	7	39	75.3	Sl. Film	55.5	4.4	260
	8	48	74.4	***	56.0	4.8	260
	9	47	73.0	****	56.0	5.5	260
	10	70	67.0	*****	57.8	7.0	260
2	1	15	81.6	None	51.3	Pretreatment	
	2	10	81.0	None	50.3	Unsterilized	

[a] *, **, ***, **** and ****** indicate increasing severity of grain formation visible on the back of a spoon.

Attention should be called to the variation in time from the first appearance of grain to a severe grain formation at the various temperatures. At 260° F. the safe working range is not over 0.2 minutes, while at 230° F. it is about 5 minutes.

Samples comprising group 2 were added to show the change in surface tension and color at significant times and temperatures during a process. These data show that a rise in surface tension occurs during the pretreatment period and that more time is necessary to reach a maximum value than would be indicated by the rate of rise at the time of the first appearance of grain.

DISCUSSION

Four curves appear on figure 1. Curve 1 has been described as connecting points of equal surface tension or coagulation, but it is important to note from the

data of table 1 that this curve connects points of nearly equal color. Therefore, it will be found that the position of curves 2, 3 and 4 also indicate the relative color of milk processed as indicated by points along these curves. For example, the color index given in table 2 for the process at 260° F. on curve 3 is 73.6. If a line is drawn through this point parallel to curve 1, it will intersect the ordinates 250° F., 240° F. and 230° F. at 5.5, 9.4 and 16 minutes. The differences in time between these ordinates and the corresponding ones on curve 3 when multiplied by the rate of color formation at the respective temperatures and subtracted from 73.6 agree closely as color indices with the values obtained in the process. (The color index rate varies with concentration of the milk and seasonal factors but it is approximately 1.2 at 250° F., 0.7 at 240° F. and 0.4 at 230° F. for short time periods which are beyond the lag period.) This indicates that the derivation of curve 3 from the heat penetration data is essentially correct. Exception must be noted in those extreme cases involving short time-low temperature pretreatment of the raw milk, followed by a short time-high temperature cooking process. For example, sample 1c in table 2 has a reflectance value of 81.2 which is somewhat higher than the value usually obtained on unsterilized milk. It should be noted also that the very short cooking processes are completed during the lag phase of the color formation. In these cases, the color results are difficult to compute, since small changes in pretreatment of the raw milk, and perhaps other factors, result in somewhat unpredictable color values. This observation is supported by the data appearing in table 3 for processes at 260° F.

Since the surface tension curve based on data for the test tube process coincides approximately with the color curve, the time differences referred to in the preceding discussion of color also indicate the essential differences in coagulation observed for processes at points along curve 3. That is, while visible grain formation is essentially equal along the curves representing the various processes, the surface tension and viscosity are not. It will be noted by the data in tables 2 and 3 that the surface tension, viscosity and color index at the time of grain formation increase as the cooking temperature is decreased. The essential difference between heat coagulation in a static test tube and a can in a cooker is in the time of appearance of visible grain with respect to surface tension. In a test tube, visible grain formation, especially at the lower temperatures, does not appear until some time after the marked rise in surface tension has occurred. In the processing of a can of milk, agitation usually is sufficient to produce a visible grain before a high surface tension value can be reached. That is, there are two phenomena observable in the heat coagulation of evaporated milk—a compact flake or grain type of coagulation and the normal heat coagulation as shown by an increase in viscosity or surface tension. An example of these phenomena is evident in the data for processes at 230° F. and 260° F. (group 1, table 3). For milk heated at 230° F., the surface tension reaches a practical maximum before a grain visible on the back of a spoon forms, but for milk heated at 260° F., a grain forms early in the coagulation period and a severe hard grain forms before a high surface tension or viscosity can be obtained.

The problem commercially seems to resolve itself into finding some means of

decreasing the slope of the grain formation curve without, at the same time, so extending the time of coagulation as to render the milk too dark in color. For example, curve 2, figure 1 has a more favorable slope than curve 3, but the process time represented is too long. It has been the observation thus far that seasonal changes in milk composition have produced greater changes in the character of the coagulation than it has been possible to produce by variation in processing procedure.

In most commercial practice, pretreatment of the raw milk is regulated so that the sterilization processes generally fall within the process range represented by section A on curve 1 of figure 1. Since curve 3 happens to be near the location of the sterility curve and to have practically the same slope, variations in sterilization processes are indicated by curve 3. The physical properties of a given lot of evaporated milk are determined largely by the particular process it is given, represented by some point of the curve. The divergence of curve 3 from curve 1 increases with temperature; therefore, an increase in the temperature of processing is accompanied normally by a decrease in the color of the milk. Unfortunately the improvement in color and flavor are accompanied in general by a decrease in viscosity and fat stability. Until pretreatment procedures of the raw milk are devised to improve the fat stability of the processed milk, commercial evaporated milk processes which result in milk lighter than normal in color cannot be used without the risk of adverse results upon storage of the sterilized milk.

Attention should be called to the fact that if cooker times are plotted instead of the calculated process time, the slopes of the curves are, for most practical purposes, almost identical. This is not surprising, since the lag in the temperature rise largely is offset in the subsequent cooling .

SUMMARY

1. A method for estimating equivalent processes in terms of time and temperature, and with reference to grain point, changes in color and surface tension, has been applied to evaporated milk.

2. Surface tension, to the extent that it is affected by the proteins, is a measure of the degree of coagulation, while grain point is an observable coagulation of fat and protein generally occurring before the maximum surface tension value or coagulation has been attained.

3. For a given sample of milk the color index is a measure of the integrated effect of time and temperature of processing.

4. The curves representing points of equal color and surface tension, grain formation and sterility are logarithmic with respect to time. The differences in the physical properties of the evaporated milk studied are indicated by the location of the processes with respect to these curves.

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EFFECT OF THE QUICK AND COMPLETE ELIMINATION OF VITAMIN C ON THE DEVELOPMENT OF THE OXIDIZED FLAVORS IN HOMOGENIZED MILK, WITH SPECIAL REFERENCE TO THE ACTION OF DAYLIGHT

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Many instances occur of homogenized milk developing quickly an oxidized flavor and it generally is recognized that daylight is a greater factor in the development of the oxidized flavors in homogenized milk than in unhomogenized milk. In a review of oxidized flavors in dairy products, Brown and Thurston (1) recorded several studies on the effect of homogenization and on the action of daylight on the development of these flavors in milk and its products. Many investigators have reported that proper homogenization was one way of preventing the production of the oxidized flavors for at least a week or so and that daylight could cause serious deterioration of the flavor of dairy products, whether or not the milk or cream had been homogenized.

A variety of terms has been used to describe oxidized flavors. In the opinion of the authors, the term "oily" best describes the oxidized flavor caused by daylight or sunshine. Also, from the viewpoint of clearness it should be known that the vitamin C of milk is represented by both ascorbic and dehydroascorbic acids. The latter is the less stable form, being destroyed easily by heat. In the process of eliminating the vitamin C of milk, the ascorbic acid first is oxidized to dehydroascorbic acid, and, secondly, the dehydroascorbic acid is destroyed by the heat of pasteurization. The term "vitamin C" in this paper includes both ascorbic and dehydroascorbic acids. At the time of the publication of the review of Brown and Thurston (1), the elimination of all the vitamin C in milk as a means of maintaining freshness in dairy products was not known.

One of the summary statements of the first article of this series (2) by the authors is: "The reaction which produces the tallowy flavor could be inhibited by quick and complete photochemical or chemical oxidation of ascorbic acid in the milk to dehydroascorbic acid prior to its pasteurization and storage. Partial oxidation of ascorbic acid stimulates the development of the tallowy flavor." This paper reports a continuation of that study.

This research was concerned with the effect of rapid and complete oxidation of vitamin C and the partial oxidation of vitamin C prior to pasteurization and homogenization on the development of the oxidized flavors. Various factors, such as different homogenization pressures, the complete oxidation of vitamin C of milk by sunlight, a partial oxidation of vitamin C of milk by sunlight, the addition of vitamin C to milk from which the original vitamin C had been oxidized, and the catalytic action of copper were studied.

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PROCEDURE

The milk in each experiment of this study was fresh, mixed herd milk obtained from ten 10-gallon cans of morning's milk from the Cornell University herd. The milk was pasteurized by heating to 143° F. for 30 minutes. It was stored in brown glass bottles at 0 to 5° C. The tables in this paper represent many sets of similar data.

RESULTS

In the experiments represented in table 1, copper and ascorbic acid, either alone or together, were added to different portions of milk. The milk was exposed

TABLE 1

Effects of complete oxidation of ascorbic acid in milk by daylight prior to pasteurization and homogenization and of the subquently added ascorbic acid and copper on the development of the oxidized flavors in homogenized milk

Homogenization pressure	Storage period	Flavor criticisms of:		
		Milk depleted of it's total vitamin C (A)	Milk A + 20 mg. ascorbic acid/l.[a] (B)[a]	
		Cu added[b]	Control	Cu added[b]
(lb.)	*(d.)*			
Control	1	.....	.....	oxidized
	2	.....	.....	very oxidized
	7	.....	.....	very oxidized
No pressure	1	.....	oxidized	very oxidized
	2–7	.....	very oxidized	
500	1	.....	?	?
	2–7	.....	slightly oxidized to oxidized	
1,000–3,000	1–7	.....	.....	.....

[a] Commercial ascorbic acid was added within a few hours after the natural ascorbic acid was oxidized.

[b] 0.1, 0.2 and 0.3 mg. Cu added per l. of milk.

to sunlight for 25 minutes, pasteurized and then homogenized at the pasteurization temperature. The "no pressure" samples were taken from the milk that was forced through the homogenizer without pressure, whereas the "control" milk was a portion removed prior to homogenization. Certain parts of the homogenizer, particularly the valves, contain copper. The catalytic action of this copper may account for the differences in flavor in the "control" and "no pressure" samples in table 1, the control sample being the one without copper and the one without an oxidized flavor. Development of the oxidized flavors merely was retarded at 500 lb. pressure, whereas it was prevented completely at the higher pressures. The most important observation is that when the milk was depleted of all vitamin C, the oxidized flavors did not develop even when copper had been added, they quickly became intense in the unhomogenized milk, developed to a slight intensity

in milk homogenized at 500 lb., and did not develop in the milk homogenized at 1,000 lb. and above.

Likewise, a study was made of the quick-partial elimination of ascorbic acid in homogenized milk by sunlight on the development of the oxidized flavors. In table 2, the milks that were homogenized at pressures of 1,000 lb. or higher and

TABLE 2

Effect of a quick-partial oxidation of ascorbic acid in homogenized milk produced by sunlight on the development of oxidized flavors (14.3 mg. ascorbic acid/l. milk after homogenization)

Homogenization pressure	Storage period	Flavor criticisms of:			
		Milk unexposed to sunlight		Milk exposed to sunlight for 7 min.[a]	
		Control	Cu added[b]	Control	Cu added[b]
(*lb.*)	(*d.*)				
Control	1	.....	sl. oxidized	.....	very oxidized
	3–6	.....	very oxidized	very oxidized	very oxidized
No pressure	1	.....	oxidized	sl. oxidized	very oxidized
	3–6	oxidized to very oxidized		very oxidized	
500	1–6	slightly oxidized		oxidized to very oxidized	very oxidized
1,000 and 1,500	1–6	.....	.....	oxidized to very oxidized	very oxidized
2,000 and 3,000	1–6	.....	.....	very oxidized	

[a] At the end of exposure for 7 min. and after pasteuration, the milk contained 6 mg./l. ascorbic acid.

[b] 0.1 mg. copper/l. was added immediately after the milk was pasteurized, exposed to daylight, and put into the sample bottles.

were unexposed to sunlight did not develop the oxidized flavors. The data on the milk exposed to sunlight show that the oxidized flavors became pronounced even when only 1 day old, under all pressures.

In the experiments summarized in table 3 the milk was homogenized at 2,000 lb. pressure at the beginning of pasteurization. The pasteurization process was completed after homogenization. The exposures to sunlight in this experiment were 20 and 40 minutes, in contrast to 7 minutes in table 2. When milk containing 18 mg./l. of ascorbic acid was homogenized at 2,000 lb. pressure, the oxidized flavors were not produced. This was true of both normal milk and normal milk plus copper. When those milks that were homogenized at 2,000 lb. pressure were exposed to sunlight for 20 minutes, the metallic flavor soon became very pronounced. Exposure of the milk to sunlight for 20 minutes was sufficient to lower the ascorbic from 18 to 3 mg./l. Action of the sunlight for 40 minutes completely oxidized the ascorbic acid and there were no oxidized flavors. However, there was a slight "daylight" flavor which may have been due to over-exposure. When 19 mg./l. of ascorbic acid were added to a portion of milk from column C, the oxidized flavors appeared as in column B which contained the normal ascorbic acid of milk. In this experiment in which the pasteurization process was completed

TABLE 3

Effects of a quick-partial, and complete oxidation of ascorbic acid in homogenized milk by sunlight, and of the subsequently added copper on the development of oxidized flavors in homogenized milk[a]

	Unexposed to light A		Exposed to sunlight				Ascorbic acid added to C D	
			20 min. B		40 min. C			
	Ascorbic acid	Flavor criticisms	Ascorbic acid	Flavor criticisms	Ascorbic acid	Flavor criticisms	Ascorbic acid	Flavor criticisms
	(*mg./l.*)		(*mg./l.*)		(*mg./l.*)		(*mg./l.*)	
Prior to exposure	18.0	–	18.0	–	18.0	–	0.0	–
After exposure	–	–	3.0	–	0.0	–	19.0	–
After storage for:								
1 d.	15.3	–	0.0	sl. met.[b]	0.0	vsd	16.0	very met.
2 d.	12.2	–	0.0	very met.	0.0	?	13.0	met.
3 d.	7.0	–	0.0	very met.	0 0	?	8.4	sl. met.
7 d.	0.0	–	0.0	very met.	0 0	?	0.0	met.
1 d.[c]	8.0	–	0.0	very met.	0.0	?	7.3	very met.
2–7 d.[c]	0.0	–	0.0	very met.	0.0	?	0.0	very met.

[a] The milk was homogenized at 2,000 lb. pressure.
[b] Sl. = slightly; met. = metallic; vsd = very slightly daylight; – = good; ? = questionable.
[c] Copper added – 0.1 mg./l.

after homogenization, the results were similar to those of the first two experiments when pasteurization preceeded homogenization.

SUMMARY

1. Milk alone, or in the presence of copper, did not develop the oxidized flavors in 7 days when homogenized at pressures of 1,000 lb. or above.

2. Milk from which the vitamin C had been oxidized completely by sunlight, with or without copper, did not develop the oxidized flavors in 7 days in either the homogenized or unhomogenized samples.

3. The addition of commercial vitamin C to milk from which the original vitamin C had been eliminated resulted in oxidized flavors like those produced in the samples from which the original vitamin C had not been eliminated.

4. The development of the oxidized flavors as affected by the different factors in this study is the same when pasteurization follows homogenization as when it precedes homogenization.

5. Milk from which the vitamin C had been oxidized partially by sunlight, with or without copper, quickly developed a "very oxidized" flavor in both the homogenized and the unhomogenized samples.

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THE PASTEURIZATION OF AMERICAN CHEDDAR CHEESE BY RADIO-FREQUENCY HEAT

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Radio-frequency or electronic heating has found many applications in heavy industry. However, only in recent years has attention been directed to its application in the food industry. Experimental studies have shown that it is possible to cook meat (1), blanch vegetables (8) or heat bread (2) directly in the package. Each of these applications has shown promise, while at the same time presenting new problems.

According to Kinn (3) radio-frequency heating may be defined as the generation of heat in a material normally considered an electrical insulator when that material is placed in a varying electrostatic field. Heating may or may not take place very quickly and uniformly, depending upon the character of the material treated.

The material is inserted between two plates (or electrodes) and an alternating potential is applied. One electrode has a positive charge and the other a negative charge at any given instant and the electrostatic field between the plates causes a deformation of the molecular structure in the material. As a result of the high frequency alternation of the electric charge, the molecules are deformed repeatedly and a molecular stress or friction is set up. This friction becomes apparent in the formation of heat generated throughout the material. By increasing either the frequency or voltage, the movements of the molecular structure are increased and more heat is generated.

Milk has been pasteurized by Brown *et al.* (1) using radio-frequency heat, but, insofar as known, no attempt has been made to pasteurize cheese in this manner, though a preliminary report was presented on this subject by the authors in 1948 (7).

The purpose of this investigation was to pasteurize cheese to various temperatures using radio-frequency heat and to observe the effect of this heat treatment upon the physical nature of the cheese, upon the bacteria and phosphatase present and upon the curing qualities of the cheese.

EXPERIMENTAL METHODS

A number of batches of raw milk were made into American Cheddar cheese, which were pressed overnight in "20-lb." square hoops. These cheeses then were cut up into blocks ($1.5 \times 4 \times 5.25$ inches), packaged in Parakote and heated directly by placing between the two electrodes of an experimental R.C.A. radio-frequency oscillator having a possible power output of 750 watts at 150 megacycles. The time required for heating a 1.3-lb. block of cheese to the desired temperature ranged from 1.5 to 2.7 minutes. Temperatures to which cheeses were heated ranged from 117° to 155° F. After attaining the desired temperatures

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the cheeses were placed in cardboard boxes and left in the laboratory to cool. Mercury thermometers were used, being inserted in the centers and sides of the cheeses. The interior temperature of the cheese was maintained for about 30 minutes after removing from the machine.

Analytical methods included the standard plate count as earlier used on cheese by Kosikowsky and Dahlberg (4), the soluble protein by the method of Sharp (11) and the volatile fatty acids by the method of Kosikowsky and Dahlberg (5). In addition, the phosphatase results were obtained by the method of Sanders and Sager (9) and also by a new method based upon modification of the Kay and Graham method (6). Four experienced judges scored the cheeses.

Cheeses shown in this study do not represent the full number tested, for in the interest of simplicity only one representative group is shown.

EXPERIMENTAL RESULTS

Physical appearance of heated cheeses. Two-day-old raw milk cheeses held at 50° F. and heated to as high as 146° F. by the high frequency heater retained their physical form. There was no oiling off and, after cooling to 60° F., there was no notable difference apparent between the heated and unheated samples. However, if the raw milk cheese was held for 10 days at 50° F. and then heated by means of the radio frequency heater, the cheese could be heated to only about 135° F. before losing its physical shape. Above this point, the temperature of the surface continued to rise, while that of the center did not. Under these conditions the surface soon melted. This phenomenon was more evident in cheeses 6 months to a year old, where even lower heating temperatures were necessary. More uniform heating was produced by heating the cheese directly in the Parakote package than by heating the wrapped cheese in a cardboard box.

TABLE 1

The bacterial counts of American Cheddar cheese[a] heated to various temperatures with radio-frequency heat

Heat treatment of cheese	Bacterial count/g. 24 hr. after heating	% Destruction	Bacterial count/g. after 2 mo. at 60° F.	Bacterial count/g. after 6 mo. at 60° F.
(° *F.*)				
None	320,000,000	.....	51,000,000	1,800,000
126	120,000	99.96	9,000,000	3,200,000
132	5,000	99.99	7,800,000	1,800,000
134	13,000	99.99	9,000,000	880,000
138	3,500	99.99	6,000,000	1,200,000
140	2,000	99.99	6,300,000	6,600,000
146	1,500	99.99	3,100,000	6,600,000

[a] Moisture of control cheese = 36.0%; pH of raw cheese = 5.2.

Bacterial count and phosphatase values of heated cheeses. In table 1 it may be seen that heating the cheese by radio frequency heat to a temperature of only 132° F. reduced the total count by 99.99 per cent. However, 2 months later at 60° F. these same cheeses showed an increase in their bacterial count, indicating

that growth had taken place in the meantime. In 6 months (3 months at 60° F. and 3 months at 36° F.) this bacterial count still was maintained in some cases, but in others it had dropped. The types of bacteria growing in this cheese were not investigated.

TABLE 2

The phosphatase values of American Cheddar cheese heated to various temperatures with radio-frequency heat

Heat treatment of cheese	Phosphatase Values		Cheese negative or positive
	Sanders-Sager method[a]	Modified Kay-Graham method[b]	
(° F.)	(γ *phenol/ 0.25 g.*)	(*mg. phenol/ 0.5 g.*)	
None	40.0	0.624	+
126	9.0	0.063	+
132	1.0	0.006	−
134	2.0	0.012	−
138	1.0	0.009	−
140	1.0	0.006	−
146	1.0	0.000	−

[a] Value over 3.0 γ/0.25 g. cheese indicates underpasteurized or raw milk cheese.
[b] Value over 0.02 mg./0.5 g. cheese indicates underpasteurized or raw milk cheese. Data for this standard yet unpublished.

As may be seen by the results from the phosphatase tests (table 2), when the cheeses were heated to 132° F. and higher, negative results were obtained. This is in good agreement with the work of Sanders and Sager (10) who obtained a negative phosphatase test by heating Cheddar cheese to 130° F. for 13 minutes by conventional heating methods.

Ripening of heated cheeses. All the cheese heated by radio frequency heat ripened to some degree and in a measure dictated by the intensity of the heat treatment. None actually attained the degree of ripening of the raw milk control, but some were well broken down at the end of 6 months. The cheeses at the end of 6 months ranged from mild to medium in flavor intensity.

Some criteria of the degree of ripening can be ascertained by observing the increase in soluble protein and volatile fatty acids (table 3). The raw milk cheese in this table was highest in soluble protein and volatile fatty acids at the end of the ripening periods. However, curing had taken place in the heated cheese, as evidenced by the varying increases in these two constants.

Important criteria of ripening are body breakdown and flavor characteristics. Data obtained by four judges on this phase at the end of 2 months are shown in table 4. After two months at 60° F. for this particular lot of cheeses, the flavor score ranged from 38.5 to 39.9, indicating that the cheese flavor was of good quality. The body score ranged from 27.6 to 29.3, a wider variation than that obtained on flavor. The more numerous criticisms referred to flavor at the end of 2 months were slightly bitter and slightly oily, while the body was criticized mostly for being too corky and firm. However, when the same cheeses were

scored again by two judges at the end of 6 months (3 months at 60° and 3 months at 36° F.), the cheeses had deteriorated in flavor but most had improved in body. Most of the criticisms on flavor were bitter and tallowy, while the only criticism on body was slight firmness.

TABLE 3

The soluble protein and volatile fatty acid values of American Cheddar cheese heated to various temperatures with radio-frequency heat and ripened

Heat treatment of cheese	Soluble protein[a]		Volatile fatty acids[b]	
	After 2 mo. at 60° F.	After 6 mo.	After 2 mo. at 60° F.	After 6 mo.
(°F.)	(%)	(%)	(*ml. 0.1 N acid/100 g. cheese*)	
None	5.95	8.15	35.7	43.9
126	4.93	7.50	28.5	41.7
132	5.09	6.30	27.9	36.2
134	4.74	6.88	20.3	24.0
138	4.51	5.57	19.1	27.0
140	4.70	7.16	27.7	31.0
146	4.51	5.90	17.6	31.1

[a] Soluble protein of control cheese 24 hr. after making = 1.29%.
[b] Volatile fatty acids of control cheese 24 hr. after making = 12.5 ml. 0.1 N acid.
[c] Cured 3 months at 60° F. followed by 3 months at 36° F.

TABLE 4

The flavor, body and total scores of American Cheddar cheese heated to various temperatures with radio-frequency heat and ripened for 6 months

Heat treatment of cheese[a]	Cheese ripened for 2 mo. at 60° F.			Cheese ripened for 6 mo. (3 mo. at 60° F. and 3 mo. at 36° F.)		
	Flavor score[b]	Body score[c]	Total	Flavor score[b]	Body score[c]	Total score
(°C.)						
None	39.1	29.1	93.2	38.3	29.3	92.6
126	39.9	29.3	94.1	37.3	29.3	91.6
132	39.6	29.3	94.0	37.5	29.3	91.8
134	39.0	28.8	92.5	37.0	29.0	91.0
138	38.8	28.8	93.0	37.8	29.0	91.8
140	39.5	28.5	93.2	36.5	29.0	90.0
146	38.5	27.6	91.1	36.8	29.0	90.3

[a] Cheese 2 d. old when heated.
[b] Most frequent flavor criticisms: oily, bitter.
[c] Most frequent body criticism: firmness.

Heating of Camembert cheeses. A number of 8-ounce whole Camembert cheeses were heated with radio frequency heat. These cheeses behaved like aged Cheddar cheese, as when the Camembert wheels were heated to between 60 and 90° F. in the center, it was impossible to handle the surface because of the extremely high temperature. For this reason these cheeses were placed in the heater for time intervals ranging from 0.5 to 2 minutes, instead of measuring temperatures. Only a limited number of cheeses were heated in this manner.

Those heated for 1 to 1.5 minutes were found by various judges to be less prone to have their surfaces turn brown or to have their flavors become ammonical after storing at 60° F. than the unheated control sample. No off-flavors developed. However, in time all the cheeses became brown and overripe.

DISCUSSION

It would be advantageous to develop flavor in raw milk cheese before pasteurizing it, but this does not seem possible under the conditions of this study, as rapid melting of the older cheese occurred. Recognition should be made of the off-flavors, such as oily or bitter, which developed in many of the heated cheeses upon prolonged ripening. The source of these off-flavors is not known, though it may be that the oily flavor has something to do with the actual heating, whereas the bitter flavor may be due to bacteria in the cheese. In the control cheese, for example, a bitter flavor was noticed. It might be possible to have *Streptococcus faecalis* as the predominating bacterial flora in the heated cheese by adding large amounts of this thermoduric organism to the cheese milk. This might provide more uniform ripening with good Cheddar flavor.

In the manufacture of Camembert cheese it would be advantageous to delay the ripening process of the cheese after it had reached its optimum point. A very limited amount of work was done on this problem and the preliminary results indicate some delay is brought about, although only rough methods of estimating temperatures were used.

Care should be taken in the heating of ripened cheese that arcing of the electrodes does not result, for brown discolorations appeared on the cheese if this happened. Proper shaping of the electrodes for the cheese is essential.

Many problems exist in this type of heating, aside from economic questions. For example, no good means of determining the temperature during the heating process is available, nor have those factors been studied which are likely to cause irregularities in temperature throughout the cheese. More information is necessary in regard to proper development of flavor and body, and especially the production of off-flavors. After this information is obtained, the careful standardization of time-temperature relationships will be necessary to assure proper pasteurization. It is felt that more information of this nature should be obtained before the practicability of this type of pasteurization can be evaluated fully.

SUMMARY

The radio-frequency pasteurization of young Cheddar cheese in Parakote packages was found possible. Older cheeses did not stand up well under the heat treatment. Phosphatase negative results were obtained on Cheddar cheese heated by radio frequency currents to 132° F. or above, and then cooled in air.

Curing took place in radio frequency heated cheeses, though not as rapidly as in the unheated controls. Off flavors, including oily and bitter, were noticed in a number of heated cheeses. Their cause was not investigated.

Many problems are present in this type of heating which require additional study.

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EFFECT OF TEMPERATURE AND DRYING ON ACTIVATION OF MALE HORMONE OF COW MANURE[1]

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In our studies of the male hormone content of dairy cows manure (2, 3), the fresh product was dried in a Freas electric oven at a temperature of 45° C. for 48 hours. It had been shown that with increasing temperature up to 85° C., the biological activity of the male hormone present was reduced greatly. It seemed of interest to determine if the fresh manure still was more potent and if other methods of drying would maintain the potency. The results were so contrary to what was expected that the study was extended to other factors which might influence these observations.

EXPERIMENTAL[2]

The fresh manure was collected from cows in the University of Missouri dairy herd. Unless otherwise indicated, the manure was dried in a Freas electric drying oven at uniform temperatures. The day-old chicks used in each experiment were White Plymouth Rocks obtained from the same hatchery. The chicks were fed a starter ration in which the 10 per cent alfalfa meal normally fed was replaced by 10 per cent dry cow manure, or its equivalent. At the end of 4 weeks, the chicks were sacrificed, the combs and gonads were removed and weighed. The comb weights per 100 g. body weight of each sex were added together and divided by two. This figure is the equivalent of the average comb weight of the birds if the groups were composed of equal numbers of the two sexes.

When fresh cow manure was fed, it was mixed daily into the chick starter ration at the rate of 10 per cent dry equivalent. To determine the approximate dry matter content of the fresh manure, a number of samples were weighed before and after drying. The average dry matter content was 15.83 per cent. In practice the figure 15 per cent has been used and 133 g. fresh manure (calculated to contain 20 g. of dry matter) has been mixed with 180 g. of feed to give the equivalent of 200 g. of dry feed. Any feed remaining the next day was discarded.

In certain experiments, the cow manure was dried and rewet to compare with the fresh material. In these experiments 20 g. of dry manure were brought up to 133 g. by the addition of water. The rewet manure then was mixed with 180 g. of feed.

The writer has been asked by many persons whether the male hormone content could be maintained if the cow manure were air- or fan-dried instead of being dried at 45° C., as recommended. To seek an answer to this question, samples of fresh cow manure were dried as follows: (a) Sun-dried. The manure was

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placed out in direct sunlight and stirred daily. It required 96 hours to dry. The average noon-day temperature during the period of drying was 34° C. (b) Air-dried. The manure was exposed in an open building without exposure to the sun. It was stirred daily. It required 192 hours to dry. The average noon-day temperature during the drying period was 28° C. (c) Fan-drying. Conditions were the same as in air-drying except that the manure was fanned constantly. It required 72 hours at an average noon temperature of 27° C. (d) Combination of air-drying and fanning. The manure was air-dried for 48 hours, then fanned for 96 hours at an average temperature of 23° C. (e) Quick drying. It was dried quickly by frequent stirring. (f) Fresh manure collected daily was mixed into the feed as described.

Drying in the sun appeared to be the most effective method involving air drying unless fans were employed (table 1). Rapidity of drying apparently was of importance, yet when the sample was dried quickly by stirring at short intervals the result was disappointing. In the light of results to be reported in a later experiment, the importance of the temperature of drying and of heat after drying may explain the relatively poor results in these experiments.

The pH of a sample of fresh manure was observed to be 6.85; it was reduced to a pH of 4.8 by the addition of 142 g. of meta phosphoric acid. It then was dried at 45° C. for 48 hours. Its biological potency was low.

As a comparison with the daily samples of fresh manure, part of each sample was dried and assayed. While this latter group did not have combs as large as the group fed cow manure dried at one time, they were twice as heavy as the group fed the comparable fresh manure. It thus appeared that the male hormone in the fresh manure was inactive biologically.

In the next series of samples, combinations of drying at 45° C. and wetting and redrying at 45 and 80° C. were tried. After the first drying, rewetting and redrying at 80° C. did not reduce the biological activity but the detrimental effects of drying the fresh manure were confirmed.

One sample dried at 45° C., then redried at 80° C. showed the greatest biological potency of any sample assayed. It suggested that increased biological activity was produced by the higher temperature once the manure was dry.

Neither the type of androgen present nor the possible combination is known in the case of the androgen in cow feces. The fact that the androgen is biologically inactive in the fresh manure when assayed by the oral administration to chicks and is activated by drying at 45° C. suggested a number of possible causes. The two most obvious factors are heat and desiccation.

An experiment was set up to compare the feeding of fresh manure with fresh manure heated for varying periods of time at 45° C. The manure was placed in fruit jars and any moisture lost was replaced. The liquid manure was mixed with the feed. The manure heated for varying periods up to 48 hours showed no increase in biological activity (table 2). Drying at 45° C., rather than heating, apparently was the factor activating the male hormone present in cow manure.

TABLE 1

Effect of the method of drying cow manure on its male hormone activity

Method of drying	Av. drying temp.	Drying time	Male chicks			Female chicks			Male and female
			No. of chicks	Av. body wt.	Comb wt./ 100 g. body wt.	No. of chicks	Av. body wt.	Comb wt./ 100 g. body wt.	Comb wt./100 g. body wt.
	(°C.)	(*hr.*)		(*g.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)
Dried at 45° C.	45	48	23	248.7	105.19	37	238.5	89.34	97.26
Sun-dried	34[a]	96	8	250.3	63.92	11	232.7	37.29	50.60
Air-dried	28[a]	192	8	287.3	39.63	12	265.2	22.54	31.08
Fan-dried	27[a]	72	9	239.2	92.08	11	245.9	76.21	84.14
Air- and fan-dried	23[a]	144	10	288.9	44.14	10	266.4	29.69	36.91
Quick-dried	45	17	10	241.1	47.82	12	200.2	37.42	42.62
Fresh manure			7	252.9	44.80	11	214.1	22.83	33.81
Dried in small lots	45	48	15	230.3	89.15	6	215.3	53.96	71.55
Acidified	45	48	9	262.4	55.35	12	257.2	27.82	41.58
Dry-wet and re-dry at 45° C.	45 45	48	12	241.9	58.26	9	232.9	34.75	46.50
Dry at 45° C., wet, dry at 80° C.	45 80	72 24	22	240.3	114.55	16	239.4	84.91	99.73
Dry at 45° C., heat at 80° C.	45 80	72 24	24	257.8	131.08	15	238.3	93.72	112.40
Dry at 80° C.	80	24	20	251.8	38.14	19	257.9	29.27	33.70
Control group			11	264.5	42.12	10	248.0	26.16	34.14

[a] Av. noon temperature at time of drying.

TABLE 2

Effect of various treatments of fresh cow manure

Treatment of manure fed at 10% equiv. level	Male chicks			Female chicks			Male and female
	No. of chicks	Av. body wt.	Comb wt. 100 g. body wt.	No. of chicks	Av. body wt.	Comb wt. 100 g. body wt.	Comb wt. 100 g. body wt.
		(*g.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)
Fresh manure	10	228.9	27.11	10	207.9	15.85	21.48
Heated 3 hr. at 45° C., wet	10	226.3	29.95	11	207.7	23.79	26.87
Heated 14 hr. at 45° C., wet	13	220.7	30.18	8	187.4	17.36	23.77
Heated 24 hr. at 45° C., wet	13	236.8	30.21	8	197.1	25.18	27.70
Heated 48 hr. at 45° C., wet	11	226.3	30.85	10	208.7	17.13	23.99
Control feed, no manure	10	219.4	35.89	10	202.4	21.40	28.65
Heated 15 lb. pressure, dried at 45° C.	8	241.9	84.50	12	219.8	57.90	71.20
Heated 15 lb. pressure, dried at 45° C., wet	8	187.9	60.94	14	186.6	67.68	64.31
Heated 15 lb. pressure, fed wet	5	198.8	53.15	14	215.9	28.81	40.98
Fresh manure	8	183.0	42.64	11	181.4	86.61	92.91
Dried at 45° C.	11	208.7	97.81	9	193.3	33.83	38.24
Dried at 45° C., wet	10	211.2	86.73	9	180.6	91.19	88.96
Dried at 80° C.	7	212.1	48.82	12	209.8	40.05	44.44
Dried at 80° C., wet	7	212.1	50.89	10	194.4	36.68	43.79
10 mg. Testosterone/kg. feed	7	249.9	134.87	12	201.3	96.23	115.55

Since it is somewhat difficult to picture the drying process causing the hydrolysis of combined androgen, the feeding of fresh manure was compared with similar material heated in an autoclave at 15 lb. pressure for 25 minutes, then cooled and fed wet. The inactivation of the enzymes and microorganisms was without beneficial effect in the feeding of wet manure (table 2). Further, the high temperature treatment (121° C.) was ineffective as a hydrolyzing agent when the hormone was present in a combined form. If the cow manure, after heating at 15 lb. pressure for 25 minutes, was dried at 45° C. and then fed, or if rewet after drying, the biological activity was increased considerably, but it showed less potency than the manure dried at 45° C. without autoclaving. The detrimental effect of drying at 80° C. again was shown. Wherever the feeding of the material in the dry and wet states was compared, the dry material was slightly more effective.

For comparison with the results obtained with cow manure dried at 45° C., a group of chicks was fed 10 mg. testosterone per kilogram of feed. The crystalline hormone at this level was slightly more potent than the hormone present in the manure when dried at 45° C.

In the first experiment, reheating at 80° C. for 24 hours one sample of cow manure which had been dried at 45° C. appeared to increase the biological activity of the male hormone present (table 1). To confirm this work and at the same time determine the optimal temperature for reheating, manure was collected from a single cow for a considerable period and dried at 45° C. by the regular method. When sufficient material was on hand, it was divided into five lots. The first lot was not reheated, but the second to fifth lots were heated for 24 hours at 65, 85, 105 and 125° C., respectively.

The assay indicated that the apparent androgen content of this sample of cow manure dried at 45° C. was slightly higher than that observed in the first experiment and closely approached the average comb weight stimulated by 10 mg. testosterone per kg. feed (table 3). However, by redrying the samples at 65° C. for 24 hours, the average comb weight per 100 g. body weight was increased to 170.44 mg. which compares quite favorably with the average comb weight of 183.59 mg. stimulated by 20 mg. testosterone per kg. feed (3). Heating the dried cow manure at progressively higher temperatures then caused a gradual decline in the biological activity of the male hormone. The final temperature, 125° C., greatly reduced the activity.

DISCUSSION

These data indicate that the male hormone in fresh cow manure is present in an inactive form. Heating and drying cow manure at 45° C. increase the biological activity of the hormone present. Heating fresh cow manure at 45° C. for varying time intervals up to 48 hours without drying was without effect upon the biological activity. Autoclaving fresh cow manure at 15 lb. pressure for 25 minutes to inactivate bacteria and enzyme activity was without effect either from the standpoint of retarding unfavorable changes in the hormone or from the possible beneficial effects of the high temperature (121° C.).

TABLE 3

Effect of heat treatment on manure dried at 45° C.

Treatment of manure fed at 10% level	Male chicks			Female chicks			Male and female
	No. of chicks	Av. body wt.	Comb wt./ 100 g. body wt.	No. of chicks	Av. body wt.	Comb wt./ 100 g. body wt.	Comb wt./ 100 g. body wt.
		(*g.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)
Dried at 45° C.	8	214.9	110.91	12	212.9	103.36	107.14
Redried at 65° C. for 24 hr.	11	226.6	208.26	9	221.0	132.61	170.44
Redried at 85° C. for 24 hr.	11	228.3	139.06	8	206.6	118.16	128.61
Redried at 105° C. for 24 hr.	14	239.6	114.67	5	235.8	107.59	111.13
Redried at 125° C. for 24 hr.	10	218.0	75.95	9	205.0	60.55	68.25

Apparently desiccation, rather than temperature, was the paramount factor in the biological activation of the hormone resulting from the drying of cow manure at 45° C. for 48 hours. However, slow drying, either in the sun or shade or by fanning at room temperature resulted in samples of reduced potency.

If temperatures of drying the fresh manure were increased above 45° C., biological activity again was reduced. About 45° C. apparently is the optimum temperature for the desiccation of fresh cow manure. When desiccation is about complete, continued heating at about 65° C. greatly increases the oral biological activity of the hormone.

In the metabolism and excretion of estrogen, the natural hormone, estradiol, is changed in part to the less active estrogens, estrone and estriol, and, further, these compounds are combined as sulphates and glucuronidates before being excreted. Since the estrogens in urine in the combined form are less active biologically than in the free form, the hydrolysis of urine either by acid or as a result of the action of microorganisms has resulted in the production of urine showing increased biological activity.

The androgens in urine also have been shown to occur in a water-soluble, biologically inactive form. On treatment with acid and heat, the water-soluble complex is split, yielding fat-soluble, water-insoluble androgen, which is biologically active (1). It has been demonstrated that the combined androgen is in part a sulfate ester.

The lack of biological activity in the fresh manure might be taken to indicate that the male hormones are present in a combined form just as they are secreted in urine. In the case of urine, the hormones are activated by the hydrolysis of the compounds by heat or acid to liberate the free forms. If the same situation exists in the case of the male hormone in cow manure, it would seem that the appearance of the free form of the hormone is accelerated by desiccation at a temperature of about 45° C. When this process is about complete the conversion to the free form of the hormone is further effected by holding at temperatures of about 65° C. for 24 hours. By this treatment, the cow manure of individual cows may be shown to contain male hormone with oral biological activity approaching the biological activity of 20 mg. of testosterone per kg. of feed. Since the dried cow manure is fed at the 10 per cent level, it means that 100 g. of dried cow manure may contain the oral equivalent of 20 mg. of testosterone.

At this time it is not possible to say that the desiccation and temperature conditions which increase the oral biological activity of the cow manure do so by hydrolyzing the combined male hormone. Possibly other changes in the compounds affecting their biological activity might be produced by these physical conditions. Only by the isolation and characterization of the compounds present in cow manure can this problem be solved.

SUMMARY AND CONCLUSIONS

1. Sun- and air-drying were less effective than fanning for drying manure, but none of these methods was as effective as drying at 45° C. for 48 hours.

2. Feeding of fresh manure was without effect, indicating that the male hormone in fresh cow manure is biologically inactive.

3. The previous observation that heating fresh manure at 80° C. inactivated the hormone was confirmed; however, when the manure is dried at 45° C., it may be wet and redried at 80° C. or heated at 80° C. for 24 hours without loss of activity.

4. Heating cow manure at 45° C. without drying for periods varying from 3 to 48 hours was without beneficial effect.

5. Autoclaving the manure at 15 lb. pressure for 25 minutes neither improved the activity when fed moist nor seriously depressed the activity when dried subsequently at 45° C.

6. When manure dried at 45° C. was reheated for 24 hours at 65° C., the apparent activity was almost doubled. Heating at both 85 and 105° C. resulted in activity equal to the control sample, but heating at 125° C. inactivated the hormone rather severely.

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THE EFFECT OF PREPARTUM MILKING AND OF FEEDING OF VITAMIN A SUPPLEMENTED RATION ON THE LEUCOCYTE COUNT OF POSTPARTUM MILK SAMPLES

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This paper reports the effects of prepartum milking and the feeding of a vitamin A-supplemented ration on the leucocyte counts of postpartum milk samples. An analysis of the effects of these factors on the composition of postpartum milk will be published elsewhere.[1]

METHODS

Films of milk samples of the first postpartum milkings of 32 mastitis-free (3) cows were stained and examined by the methods prescribed by the American Public Health Association (1). The cows were milked on a 12-hour schedule. Sixteen were subjected to prepartum milking, beginning 3 days before the calculated parturition date. The actual period of prepartum milking varied from 36 hours to 9 days. Of these cows, milked prepartum, five were fed the basal ration and 11 the same basal ration supplemented by vitamin A. The other 16 animals were not milked before parturition. Four of these were fed the basal ration and 12 the supplemented diet.

The leucocyte counts, expressed in millions of cells per ml. were converted to logarithms for statistical analysis. The milk samples of the four differently-treated groups of animals were compared in respect to both the mean leucocyte count and the change in the leucocyte count during the first six milkings postpartum.

RESULTS

The average leucocyte counts of the four groups differed less from one another than the error of the difference. The average log-counts of the prepartum basal and vitamin A groups were 5.76 and 5.84, respectively, and those for the two groups milked only postpartum were both 5.77. In actual counts these correspond to the geometric means shown in the last row of table 1, together with the average amount of milk produced, in pounds.

The number of leucocytes decreased strikingly and very significantly during the six postpartum milkings when averaged over all four groups. This downward trend was somewhat less marked in the prepartum-milked cows than in the others, although the difference in the trend could not be considered as statistically significant. The addition of vitamin A to the ration had no effect upon the trend.

Keyes *et al.* (2) found that the number of leucocytes was the highest in the

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[1] This study is being conducted by H. D. Eaton, Department of Animal Industries, University of Connecticut.

TABLE 1

Average milk production and leucocyte counts of postpartum milkings, for the four groups of experimental cows

Postpartum milking	Prepartum milked cows[a]				Non-prepartum milked cows[b]			
	Basal ration		Vitamin A Ration		Basal ration		Vitamin A Ration	
	Leucocytes	Milk	Leucocytes	Milk	Leucocytes	Milk	Leucocytes	Milk
	(*No./ml.*)	(*lb.*)	(*No./ml.*)	(*lb.*)	(*No./ml.*)	(*lb.*)	(*No./ml.*)	(*lb.*)
1st	676,000	12.26	1,000,000	9.99	1,122,000	14.00	1,202,000	14.53
2nd	851,000	16.58	1,175,000	12.35	2,042,000	7.88	1,112,000	8.37
3rd	537,000	16.92	562,000	15.23	490,000	14.55	617,000	10.69
4th	513,000	19.68	525,000	16.67	355,000	17.38	427,000	14.68
5th	479,000	17.62	555,000	16.77	229,000	17.68	407,000	14.19
6th	490,000	20.68	550,000	15.33	263,000	17.08	302,000	17.25
Av. for group	575,000	17.29	692,000	14.39	589,000	14.76	589,000	13.29

[a] 16 cows: 5 on basal ration (1 Ayrshire, 1 Guernsey, 2 Holsteins, 1 Jersey); 11 on vitamin A ration (4 Guernseys, 3 Holsteins, 4 Jerseys).

[b] 16 cows: 4 on basal ration (2 Guernseys, 1 Holstein; 1 Jersey); 12 on vitamin A ration (2 Ayrshires, 3 Guernseys, 4 Holsteins, 3 Jerseys).

first milkings and dropped to normal (the actual number was not given) within 4 days after parturition.

CONCLUSION

The mean leucocyte count of milk samples of postpartum milkings of healthy cows is not affected by prepartum milking or by the feeding of a vitamin A-supplemented ration.

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OCCURRENCE OF PROTOZOA IN THE BOVINE STOMACH[1]

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Digestion of feeds by cattle is accomplished mechanically, by enzyme action, by chemical reactions and through the activity of microorganisms, such as the bacteria and protozoa in the stomach (23). Some attention has been given to these protozoa, yet much remains to be learned concerning their kinds, distribution and activity.

LITERATURE REVIEW

In 1843, Gruby and Delafond (11) observed large numbers of microorganisms, including ciliate infusoria, in the rumen and reticulum but only dead specimens or fragments in the omasum, abomasum and small intestines. Hastings (13) quoted estimates placing the volume of protozoa at 4.4 to 20.0 per cent of the rumen contents.

Becker and Talbott (6) listed 33 species of protozoa as having been identified in the rumen, with from 2 to 16 species present in single animals. Mangold (22) mentioned 19 species of protozoa obtained from the stomachs of cattle out of 33 reported for eight different ruminants. These microorganisms function in digestion and synthesis of nutrients in the rumen and become available to the host when they, in turn, are digested.

Becker and associates (4, 5) suggested that the rumen protozoa enter the stomach through ingestion of freshly contaminated feed or water, and that they were not ingested in cyst form. Becker (3) believed that the numbers of protozoa were related more closely to kind and quantity of feed eaten than to physiological state of the animal. Johnson *et al.* (19) observed the concentration of bacteria to be greatest 1 hour after feeding (6.5 million/ml.), and of protozoa 15 hours later (840,000/ml.). However, six lambs defaunated by fasting coupled with two copper sulfate treatments at 24-hour intervals were kept free of rumen protozoa for 30 days. They utilized urea as efficiently as did normal lambs. Functioning of rumen bacteria was regarded as possible but not investigated. Protein from protozoa was 86 per cent digested and that of bacteria, 55 per cent. Biological values of these proteins with rats were 68 and 66 per cent, respectively.

Schwarz (26) investigated rumen contents of slaughtered cattle and concluded that one-third of the protein in feeds was converted into bacterial and infusorial protein. He cited Scheunert's view that infusoria were harmless commensals aiding in the mechanical action along with the soaking, maceration and intermixing of the paunch contents, agreeing with Bündle on this point.

Microorganisms of the stomach (anaerobic bacteria and protozoa) functioned in the digestion of cellulose (15, 19, 21, 22, 28) and in the conversion of urea, ammonium carbonate and vegetable proteins into animal proteins available ulti-

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mately to the host. Some products of this activity included cellulose disintegration, gases and ether-soluble compounds, as indicated by Baker (1), Cole *et al.* (7), Hale *et al.* (12) and Hungate (17). Rumen microorganisms also contributed to the synthesis of water soluble vitamins (2, 8, 18, 21, 24, 25), including biotin, pantothenic acid, pyridoxine, riboflavin and thiamine.

Use of a rumen fistula has facilitated investigations of biological functions of microorganisms in cattle. Penstate Homestead Jessie 924062 had a rumen fistula by means of which the rumen contents were sampled by Bechdel *et al.* (2). They found that *Flavobacterium vitarumen* synthesized vitamin B complex in the rumen. Huffman (14) reported that an experimental cow went seriously off-feed upon complete removal of the rumen contents through a fistula upon continued feeding of the same ration. She recovered quickly after fresh rumen contents from a normal steer were placed in her rumen. Rumen digestion in cattle was practically completed 12 hours after eating (12).

Ferber (9) and Ferber and Winogradowa-Fedorowa (10) regarded protozoa as symbionts, observing in sheep and a goat that these organisms built up easily-digested protein in their bodies to be broken down in the omasum and digested. A wether, fasted 102.5 hours, was reduced to a state of "no infusoria" in the rumen. Upon resumption of feeding, the total infusoria increased in 30 days to 1,387/ml. Van Der Wath and Myburgh (27) recently investigated the role of infusoria and bacteria in ruminal digestion with Merino sheep under South African conditions.

Knowledge of bacterial and protozoan function in the ruminant stomach suggests a need for more information concerning the kinds of rumen infusoria in cattle and their distribution. Consequently, a survey was made with animals raised in the station dairy herd to determine the kinds of protozoa present in Jersey cattle of different ages.

METHODS

Samples of stomach contents from the rumino-reticular compartment were taken from six cattle slaughtered commercially and used in testing technics of observation, staining and identification. No protozoa were found in a sample obtained from a high grade Brahman bull. This animal was in reasonable condition and appeared healthy. The sample contained a considerable proportion of fresh citrus pulp and little grass or hay. Nothing is known concerning his previous feeding and history.

Twelve male Jersey calves and one Jersey cow raised in the experiment station dairy herd were studied under feeding conditions shown in table 1.

Each animal was stunned, suspended head downward and bled. The hide was removed, animal eviscerated and samples obtained from the ileum near the distal end, abomasum, omasum and rumino-reticular compartment, in this order. These alimentary contents were collected in sterilized Mason jars or test tubes with little contact with the air. In the Biology laboratory, samples were taken rapidly at room temperature, using clean pipettes to avoid extra exposure to air, and examined under cover slips sealed with plain vaseline. Observations began within

TABLE 1

Protozoa in the stomach of Jersey cattle in Florida

Animal no.	Age in days	Stomach compartment	*Callimastix frontalis*	*Dasytricha ruminantium*	*Diplodinium bursa*	*Diplodinium dentatum*	*Diplodinium denticulatum*	*Diplodinium ecaudatum*	*Diplodinium hegneri*	*Diplodinium kelseri*	*Entodinium bicarinatum*	*Entodinium bursa*	*Entodinium caudatum*	*Entodinium furca*	*Entodinium minimum*	*Eutrichomastix ruminantium*	*Isotricha prostoma*	*Isotricha ruminantium*	*Ophryscolex caudatus*	*Polyplastron multivesiculatum*
		Prior to nursing																		
347-F	0.5	Rumen	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Omasum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Abomasum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
352-F	1	Rumen	...	...	...	...	...	...	...	...	...	..	...	...	...	...	...	...	...	...
		Omasum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Abomasum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Fed milk, concentrates and prairie hay																		
338-F	48	Rumen	...	...	...	...	...	...	...	...	...	x[a]	...	...	x	...	...	...	...	...
		Omasum	...	...	...	...	...	...	...	...	...	n[b]	...	...	x	...	...	...	...	...
		Abomasum	...	...	...	...	...	...	...	...	...	...	...	...	n	...	...	...	...	...
340-F	59	Rumen	...	...	...	...	...	...	...	...	...	x	...	...	x	...	...	...	...	...
		Omasum	...	...	...	...	...	...	...	...	...	x	...	...	x	...	...	...	...	...
		Abomasum	...	...	...	...	...	...	...	...	...	...	...	...	n	...	...	...	...	...
341-F	70	Rumen	x	...	...	...	x	x	...	x	x	x	x	x	x	x	...	...	...	...
		Omasum	x	...	...	...	n	n	...	n	n	x	x	x	x	x	...	...	...	...
		Abomasum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Fed concentrates and prairie hay																		
336-F	80	Rumen	...	...	...	...	...	...	x	x	x	x	x	x	x	...	...	...	...	x
		Omasum	...	...	...	...	...	...	x	x	x	x	x	x	x	...	...	...	...	x
		Abomasum	...	...	...	...	...	...	n	n	...	n	n	...	...	...	...	...	...	n
323-F	160	Rumen	...	x	x	...	...	...	...	...	...	x	x	x	x	...	x	x	...	...
		Omasum	...	x	x	...	...	...	...	...	...	x	x	x	x	...	x	x	...	...
		Abomasum	...	...	...	...	...	...	...	...	...	n	n	n	...	...	...	...	...	...
322-F	168	Rumen	x	...	x	x	x	...	x	x	x	...	x	...	x	x	x	x	...	...
		Omasum	...	...	x	x	x	...	x	x	x	...	x	..	x	n	n	x	...	...
		Abomasum	...	...	n	x	n	...	n	n	n	...	n	...	n	n	n	...	...	...
315-F	168	Rumen	...	...	x	x	x	...	...	x	...	x	x	...	x	...	...	...	...	...
		Omasum	...	...	x	n	x	...	...	x	...	x	x	...	x	...	...	...	...	...
		Abomasum	...	...	n	n	x	...	...	x	...	n	x	...	x	...	...	...	...	...
313-F	193	Rumen	...	x	...	...	...	...	x	...	x	...	x	...	x	...	...	...	...	...
		Omasum	...	x	...	...	...	...	x	...	x	...	x	...	x	...	...	...	...	...
		Abomasum	...	n	...	...	...	...	n	...	n	...	n	...	n	...	...	...	...	...
		Fed concentrates and fresh grapefruit pulp																		
319-F	196	Rumen	...	...	x	...	...	x	...	...	...	x	x	x	...	...	x	...	...	...
		Omasum	...	...	x	...	...	x	...	...	...	x	x	x	...	...	n	...	...	...
		Abomasum	...	...	n	...	...	n	...	...	...	n	n	n	...	...	n	...	...	...
316-F	201	Rumen	...	...	...	...	...	...	...	...	x	x	x	x	...	...	...	...	...	...
		Omasum	...	...	...	...	...	...	...	...	x	x	x	x	...	...	...	...	...	...
		Abomasum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
		Fed concentrates, corn silage and hay																		
58-F	*years* 4.5	Rumen	x	x	...	...	x	x	...	...	x	...	x	x	x	x	x	x	x	...
		Omasum	n	n	...	...	x	x	...	...	x	...	n	x	x	n	n	n	n	...
		Abomasum	...	...	...	...	n	...	...	...	...	...	...	...	...	...	n	n	...	...

[a] x = living protozoa.
[b] n = protozoa non-motile (dead?).

10 to 15 minutes of sampling so as to observe the protozoa in the living state insofar as possible. The samples were examined microscopically in the order of collection.

Aliquot amounts were fixed in 10 per cent formalin, in Schaudinn's fluid and in hot Bouin's fluid. The fixed protozoa were stained with precipitated borax-carmine, with standard alum hematoxylin and triosin. Then they were dehydrated in an alcohol series and mounted in balsam for further species identification by means of the keys of Kudo (20) and Becker and Talbott (6).

RESULTS

No protozoa were found in the digestive tract of either newborn calf, 347-F and 352-F. Clear liquid in the abomasum contained squamous epithelial cells. Some cells also were present in the fluid in the rumen and omasum. One calf had licked sand from the floor of his stall. Neither animal had opportunity to nurse prior to slaughter.

No protozoa were observed in the ileum of any of the calves or in the cow.

No calves fed solely on milk were available for this study. Calf 341-F was believed to have obtained some grass shortly before slaughter, as the stomach contents were green in color. Green inclusions were seen in many of these protozoa, including *Entodinium bursa*. The presence of green inclusions in this species is of particular interest, since Hungate concluded (15, 16) that *Entodinium caudatum* did not digest cellulose.

Five calves from 80 to 193 days old were receiving mixed concentrates and upland prairie hay at time of slaughter. Two of these, 315-F and 322-F, were the only animals in which living rumen protozoa were identified in the abomasum. Considerable gas developed in the rumens of these animals during slaughter, sufficient to force some of the rumen liquid through the esophagus. It is highly probable that the living protozoa in the abomasum were an artifact caused by this pressure. As shown in table 1, from 5 to 12 species of protozoa were identified in the rumen contents of these five animals, 313-F, 315-F, 322-F, 323-F and 336-F. Protozoa seen in the abomasal samples from three of them were non-motile (dead?).

In attempting to follow a clue that feeds may affect the fauna of the digestive system, prairie hay was withdrawn from the rations of 316-F for 10 days, and of 319-F for 11 days, and replaced with fresh grapefruit pulp (peel, rag and seed). Only four species of *Entodinium* were found in the rumen of the first animal when slaughtered, while three of these together with the larger *Diplodinium* were identified in the rumen of the second animal. Blades of prairie hay were present in the rumen contents of 319-F even though none had been offered to him for 11 days.

The 4.5-year old Jersey cow 58-F had been removed from a mixed grass pasture 5 days before slaughter and had received the same kind of mixed concentrates and hay as did the calves, in addition to about 20 lbs. of corn silage daily. This cow's rumen contained 12 species of living protozoa, including two of *Diplodinium*, four of *Entodinium*, two of *Isotricha*, and one species each of *Callimastix*, *Dasytricha*, *Eutrichomastix* and *Ophryscolex*.

Two of the younger calves had only two species of *Entodinium* in the stomach.

The cow and a 5.5-month old calf had 12 different species of protozoa. Eighteen species in eight genera of rumen protozoa were distributed variously among the 11 animals past 6 weeks old. The five species of *Entodinium* each were present in six to nine animals, only two animals possessing all five species. From one to four calves had one or more of six species of *Diplodinium*, none having only this genus. Three animals had both species of *Isotricha*, and a single animal had one species of this genus. Single species of *Callimastix*, *Dasytricha* or *Eutrichomastix* were present in three different animals, the mature cow having all three of them. *Ophryscolex* and *Polyplastron* occurred only in single individuals. The distribution of protozoa is listed in table 1.

SUMMARY AND CONCLUSIONS

This survey verified that the stomach and small intestines of calves were devoid of protozoa at birth. No observations were made on calves receiving milk alone. Protozoa were teeming in the rumen, some living ones persisted in the omasum, nearly all in the abomasum were non-motile (dead?) and none were intact in the ileum. This suggests that their main activity took place in the first-named compartment and that, after being rendered non-motile, they were digested by the host. More observations are needed concerning effects of diet on the microfauna both as to species and numbers. Calves appeared to acquire more species of protozoa as they advanced in age. Eighteen species of eight genera of protozoa were observed in 12 experimental Jersey calves and a cow studied in this herd.

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HOMOGENIZED MILK VII. EFFECT OF AGITATION DURING FREEZING ON THE KEEPING QUALITY OF FROZEN HOMOGENIZED MILK

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Previous studies (5, 6) have proved that homogenized milk of good quality can be stored at usual storage temperatures after thawing without deterioration for longer periods of time than usually are necessary before use. Further, it has been found that homogenized milk of good quality may be kept as long as 120 hours at 1.67° C. before freezing without adversely affecting the keeping quality of the frozen product. A more recent study (7) has shown that the addition of sodium citrate with ascorbic acid increases the time that homogenized milk can be stored in the frozen state without protein flocculation or flavor deterioration.

Reports on the use of agitation during the freezing of homogenized milk have not been found. However, experimental work reported by Doan and Baldwin (9) indicates that the destruction of the fat emulsion in milk and cream frozen without agitation is caused by internal pressures developed in the congealing mass. This is a result of initial surface freezing followed by the expansion of water in the body of the product as it is converted to ice. Later, Doan and Leeder (10) reported that the internal pressure was greatly reduced, if not eliminated, and the fat emulsion of the product little affected when concentrated milk was partially frozen in an ice cream freezer with agitation and then filled into small containers for final freezing and storage. Civtl (8) analyzed the outer layer, the part which froze first, as well as the top, middle and bottom portions of the remainder of the sample and found that the central portion was richer in fat, casein, albumin globular sugar and chloride ion than the upper or lower portions. The outer layer was found to be the poorest in these constituents. Babcock *et al.* (3, 4) and Trout (13) have shown that when homogenized milk was frozen, the solid components tended to concentrate in the lower portion of the sample. Other studies (2, 11, 12, 14) have also shown that freezing and storage temperatures affect the physical character of homogenized milk.

The present experiments were undertaken to determine the effect of agitation during freezing on the keeping quality of frozen homogenized milk.

EXPERIMENTAL

Homogenized milk with a fat content of 3.8 per cent pasteurized by holding at 155° F. for 30 minutes and packaged in paper containers by a commercial dairy was used. Quart samples were used to determine the efficiency of the agitation as shown by milk solids distribution. One-half pint samples were used to determine keeping quality as shown by protein flocculation and flavor.

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The samples were frozen and stored at about – 17.8° C.[2] One half of the samples were placed in the freezer and kept in a stationary condition during freezing. The other half of the samples were frozen in the same freezer by placing them in a box attached to a wheel which was revolved by a motor at the rate of 35 r.p.m. (figure 1). This group of samples then was rotated for 72 hours to insure that they would be frozen solidly before they were removed from the rotator. All but three of the quart samples were removed from the freezer shortly after freezing and divided, while in a frozen condition, into top, middle and bottom portions. Each part was thawed and analyzed for fat, protein, total solids

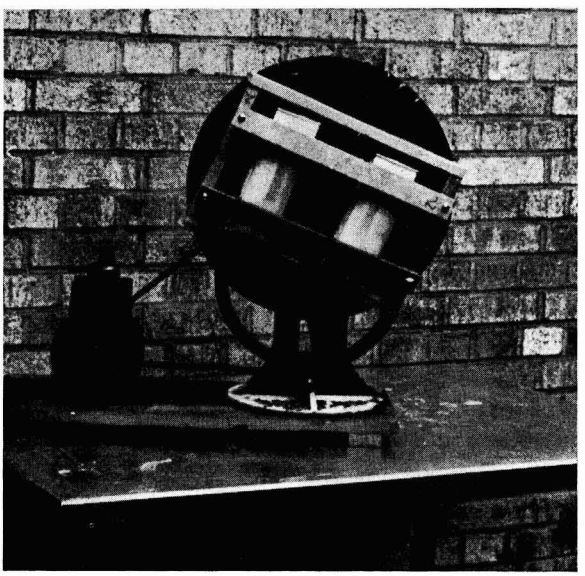

FIG. 1. Rotator used for agitating homogenized milk during freezing.

and ash content to determine the distribution of the milk solids. In addition to the chemical analyses, the milk solids distribution was verified by determining the freezing point of the respective portions of the thawed milk. Chemical analyses were made by methods described in a previous report (3). The freezing point was determined by using a Hortvet cryoscope in accordance with AOAC methods (1). The three quart samples were removed from the rotator after freezing and left in the freezer in a stationary upright position. They were removed after 22, 39 and 82 days and divided while frozen into two equal portions. Each portion was analyzed for total solids, fat, and protein. The degree of separation in each portion also was determined. The half-pint samples were removed and thawed for examination at intervals of about 10 days, beginning with

[2] The thermostat controlling the temperature of the freezer had a sufficient lag to cause a maximum temperature variation of about 8° C.

the 56th day of storage. The degree of separation of the milk was measured by determining the amount of sediment in 50 ml. quantities by centrifuging as was done in previously reported experiments (2). Flavor determinations were made by a panel of three men experienced in milk judging.

The effect of rotating during freezing on the distribution of solids in frozen homogenized milk is shown in table 1. When quart samples of homogenized milk

TABLE 1

Effect of rotating during freezing on the distribution of solids in frozen homogenized milk as shown by chemical analysis and freezing point

Sample number	Method of handling	Section of sample	Freezing point after thawing	Fat	Protein	Total solids	Ash
			(° C.)	(%)	(%)	(%)	(%)
1	Stationary	Top	−.397	2.84	2.54	9.77	0.58
		Middle	−.511	3.09	3.06	11.64	0.71
		Bottom	−.657	3.97	3.85	14.68	0.86
2	Stationary	Top	−.389	2.80	2.52	9.55	0.55
		Middle	−.499	3.18	2.97	11.60	0.67
		Bottom	−.674	3.69	3.95	14.93	0.85
3	Rotated	Top	−.500	3.36	3.11	11.75	0.70
		Middle	−.527	3.36	3.23	12.24	0.71
		Bottom	−.532	3.47	3.23	12.36	0.75
4	Rotated	Top	−.474	3.20	2.91	11.22	0.65
		Middle	−.537	3.52	3.24	12.36	0.73
		Bottom	−.539	3.40	3.25	12.38	0.73
5	Stationary	Top	−.383	2.82	2.44	9.32	0.55
		Middle	−.499	3.36	3.02	11.55	0.69
		Bottom	−.687	4.42	4.02	15.38	0.90
6	Rotated	Top	−.525	3.65	3.22	12.23	0.72
		Middle	−.583	3.99	3.50	13.39	0.79
		Bottom	−.422	2.99	2.64	10.09	0.61
7	Rotated	Top	−.442	3.10	2.68	10.42	0.61
		Middle	−.531	3.67	3.11	12.29	0.73
		Bottom	−.590	3.87	3.49	13.33	0.79
8	Rotated	Top	−.524	3.58	3.21	12.23	0.73
		Middle	−.540	3.65	3.14	12.21	0.73
		Bottom	−.526	3.63	3.15	12.16	0.73
9	Rotated	Top	−.506	3.54	3.12	11.81	0.68
		Middle	−.552	3.78	3.28	12.60	0.76
		Bottom	−.520	3.60	3.15	11.96	0.71

were rotated during freezing, the concentration of milk solids was practically the same in the top, middle and bottom sections. The control samples, which were frozen in a stationary position, gave results similar to those previously reported (3, 4) in that there was a tendency for the solids to settle toward the bottom. There was a greater concentration in the bottom third than in the middle third and a greater concentration in the middle third than in the top third. When the quart samples were rotated during freezing, the freezing point of the top, middle and bottom sections of the milk after thawing was practically the same. In those samples that were not rotated during freezing, the settling of the solids was reflected in the freezing point of the different sections of the milk. In each case the freezing point of the middle section was lower than the freezing point of

the top section and the freezing point of the bottom section was lower than that of the middle section.

The effect of rotating during freezing on the distribution of solids in frozen homogenized milk after storage is shown in table 2. When homogenized milk

TABLE 2

Effect of rotating during freezing on the distribution of solids in frozen homogenized milk after storage

Days in storage	Total solids		Fat		Protein		Sediment in 50 ml.	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
	(%)	(%)	(%)	(%)	(%)	(%)	(*ml.*)	(*ml.*)
22	12.55	12.37	3.73	3.68	3.25	3.22	0.02	0.02
39	12.07	12.16	3.62	3.61	3.15	3.16	0.02	0.03
82	12.28	12.17	3.64	3.61	3.21	3.14	0.02	0.03

was rotated during freezing and then stored in a stationary upright position the milk solids remained evenly distributed throughout the milk for 82 days. This further confirms the results previously reported (4) that when homogenized milk was frozen, the concentration of the milk solids in the lower portion of the sample took place during the freezing process and apparently there was no further movement of these solids after the milk was frozen. The degree of separation was similar in the top and bottom sections of the milk.

Having shown that rotating while freezing prevented a concentration of the milk solids in the bottom of the quart container, the half-pint samples were used to determine the effect of rotation during freezing on the keeping qualities as indicated by flavor and the degree of separation. These results are shown in table 3. Agitation during freezing did not improve the keeping quality of

TABLE 3

Effect of rotation during freezing on the keeping quality of frozen homogenized milk

Days in storage	Method of handling	Sediment	Flavor
		(*ml./50 ml.*)	
56	Stationary	0.03	.....
70	Stationary	0.10	.....
77	Stationary	0.30	.....
85	Stationary	0.5	Oxidized
85	Rotated	0.9	Oxidized
92	Stationary	0.06	Oxidized, stale
92	Rotated	0.03	Oxidized, stale
97	Stationary	0.10	Sl. Oxidized, stale
97	Rotated	0.35	Oxidized, stale
103	Stationary	1.4	Oxidized
103	Rotated	1.2	Oxidized
112	Stationary	1.4	Oxidized, stale
112	Rotated	1.5	Oxidized, stale
118	Stationary	1.6	Oxidized
118	Rotated	1.6	Oxidized

homogenized milk. Furthermore, separation, as shown by the amount of sediment in 50 ml. portions, was not delayed by rotating the milk while freezing.

CONCLUSIONS

When homogenized milk was agitated by rotating at 35 r.p.m. during freezing the chemical analysis and freezing point of various sections of the sample showed that the milk solids remained evenly distributed throughout the sample.

Preventing the concentration of milk solids in the lower portion of homogenized milk by rotating it during freezing does not improve its keeping quality.

Acknowledgement is extended to Elmina Dickson and Edith Giltner for assistance with the analyses.

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THE EFFECT OF THE ADMINISTRATION OF VARIOUS FATTY ACIDS ON THE BLOOD KETONE LEVELS OF RUMINANTS[1]

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The widespread occurrence of ketosis in dairy cattle has made this a disease of practical as well as of theoretical importance. Considerable work has been done *in vitro* on the precursors of the ketone bodies, and some work has been done *in vivo* with laboratory animals, but very little has been done with ruminants. Since ketosis is a much greater practical problem in ruminants than in any other species, and since ruminants very often do not react in the same manner as smaller laboratory animals, it was deemed desirable to study this problem using ruminants as experimental animals. A preliminary report of this work appeared earlier in abstract form (12).

REVIEW OF LITERATURE

Jowett and Quastel (6) incubated normal saturated fatty acids containing two to ten carbon atoms in the presence of liver slices. They concluded that the four, six and eight carbon compounds produced acetoacetic acid most readily, that the ten carbon acid was slightly less active and that acetic acid was considerably less active. Propionic acid did not form acetoacetic acid, and the other odd-numbered acids produced very small amounts. Medes *et al.* (10) showed that ketone bodies arise as intermediates of acetate oxidation in animal tissues *in vitro*. Lehninger (7) has succeeded in obtaining liver suspensions which under the proper conditions readily oxidize all of the normal saturated fatty acids containing four to eighteen carbon atoms to yield acetoacetic acid as end product.

MacKay *et al.* (8) fed acetic acid to a phlorhizinized dog and to fasting rats and demonstrated an increased production of ketone bodies. Swendseid *et al.* (13), using the heavy isotope of carbon, C^{13}, showed that acetic acid takes part in the synthesis of the acetone bodies in the fasting rat. Forbes (5) administered acetic acid to one goat and observed no increase in ketone body excretion. MacKay *et al.* (9) fed normal, saturated fatty acids with four, six, eight, and ten carbon atoms to rats and showed that all were ketogenic, even when adequate amounts of carbohydrate were fed.

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[1] These data were taken from a thesis submitted by L. H. Schultz to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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More recently Phillipson (11) demonstrated that the lower fatty acids were important products of bacterial fermentation in the rumen, and that the acids produced were primarily acetic, propionic and butyric, with acetic predominating. These acids were shown to be absorbed directly into the blood stream from the rumen, reticulum and omasum. Folley and French (4) have shown that ruminant tissues apparently have a greater ability to metabolize acetate than do those from non-ruminants.

EXPERIMENTAL PROCEDURE

Various fatty acids were administered, either orally or intravenously, to goats and the blood ketone levels were followed. The animals used were four mature female goats which averaged 98 lb. in weight and had been in lactation about 5 months. Except for one test where they were fasted, the goats were fed and handled normally. The goats were used at random in the course of the experiments. However, the fatty acids which gave positive results all were tested at one dosage level on the same goat to check the differences obtained in blood ketone levels. One study on acetic acid administration was made with a phlorhizinized goat. The goat used for this experiment was a young male castrate goat weighing 60 lb. The animal was fed normally. One g. of phlorhizin dissolved in 3 ml. of propylene glycol was administered for 3 days prior to the study. The animal was placed in a metabolism cage and complete daily urine collections were made.

The fatty acids used were acetic, propionic, butyric, caproic, caprylic, capric and oleic. Other organic acids tested were lactic and succinic. Corn oil also was used. For oral administration, the liquid fatty acids and the organic acids were diluted with 400 ml. of water and introduced into the rumen by means of a stomach tube. Solid fatty acids and corn oil were administered by capsule. Fifteen g. dosages were used first and then all of the experiments were repeated at least once using 30 g. dosages. For intravenous injections only acetic and butyric acids were used, and they were diluted with 250 ml. of physiological saline and injected into the jugular vein. A single 10 g. dosage was used for acetic and two dosages, one of 5 g. and one of 10 g., were used for butyric acid in the injection studies.

Blood samples were taken immediately prior to the administration of substances to be tested, and usually at 0.25, 0.5, 1, 1.5, 2, 3 and 4 hr. intervals afterward. Normal values were determined for a 3-day period prior to the start of this study. They ranged from 1.1 to 4.0 mg. per cent total ketone bodies.

Blood ketone determinations were carried out on a Folin-Wu (3) blood filtrate according to the method of Behre and Benedict (1). The colorimetric determination of acetone in the distillate was carried out according to the method of Block and Bolling (2), using a Coleman spectrophotometer. All figures given represent total ketone bodies expressed as acetone.

RESULTS AND DISCUSSION

Contrary to the results obtained by other workers with tissue slices and with intact rats and dogs, acetic acid showed no definite ketogenic activity when administered orally to goats, as determined by following blood ketone levels. It is recognized, however, that our experiments were set up somewhat differently

TABLE 1

Total blood ketone levels following oral administration of acetic acid to normal, fasted and phlorhizinized goats

Time after administration	Normal		Fasted		Phlorhizinized
	Amt. administered		Amt. administered		Amt. administered
	15 g.	30 g.	15 g.	30 g.	30 g.
	Total blood ketones		Total blood ketones		Total blood ketones
(*Hr.*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)
0	1.5	1.3	1.9	1.2	2.7
0.25	1.9	1.5	2.9	0.9	3.0
0.5	1.5	3.4	1.1	0.8	2.8
1	2.2	3.9	1.2	1.0	2.1
1.5	2.5	3.8	.....	.....	.....
2	4.0	3.3	1.8	1.1	2.9
3	1.6	1.4	1.8	1.7	3.9
4	1.9	1.2	2.3	1.8	4.1

than those in which rats and dogs were used. As shown in table 1, acetic acid was given in 15 and 30 g. doses to goats fed normally and to goats which had been fasted for 36 hr. No significant changes were noted in blood ketone levels in any

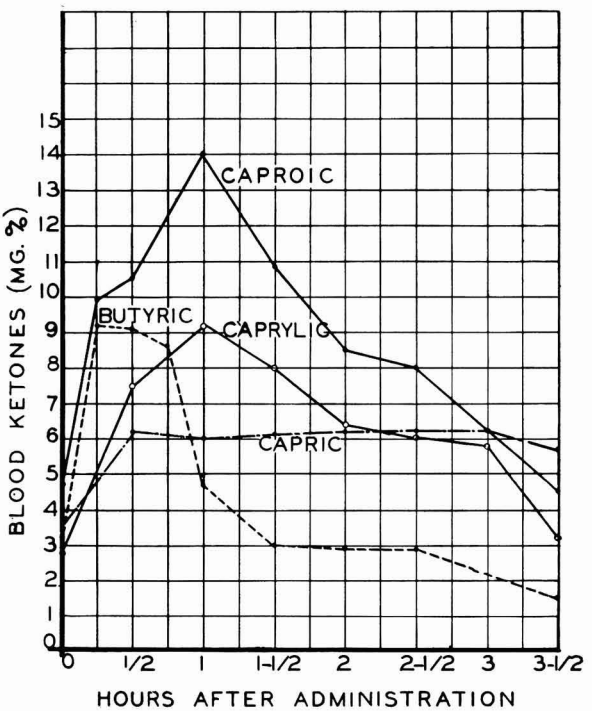

FIG. 1. *The effect of oral administration of 30 g. of various fatty acids on the blood ketone level.*

of the trials. The goat which had been treated with phlorhizin was excreting 35 to 40 g. of sugar daily, but the blood sugar values were within the normal limits. Again no significant changes were noted in blood ketones following acetic acid administration, as shown in table 1.

The fatty acids containing four, six, eight and ten carbon atoms caused definite increases in blood ketones following administration. Four trials were made on butyric acid and at least two on each of the other acids. Figure 1

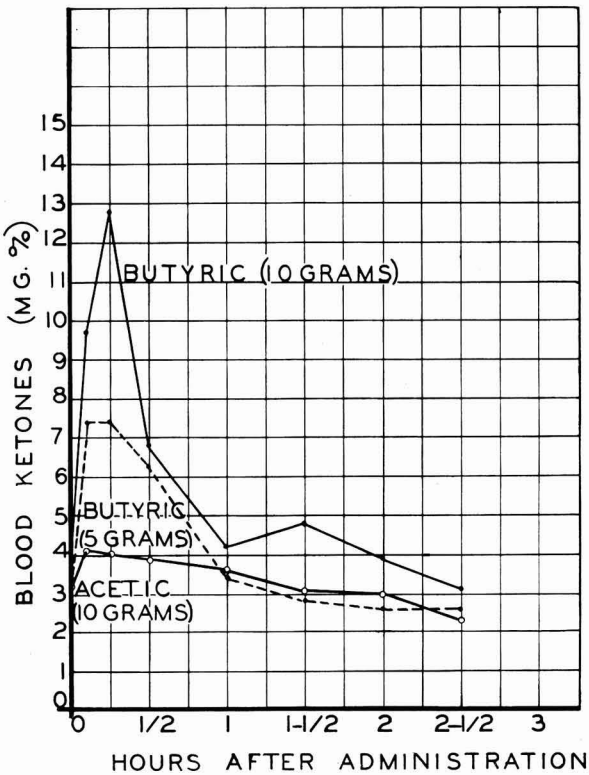

FIG. 2. *The effect of intravenous injection of acetic and butyric acids on the blood ketone level.*

shows the results of the 30 g. dosage level when each of the four acids was administered to goat no. 45. Butyric caused the most rapid increase in blood ketones, the peak being reached in 0.5 hr., and the values returned to normal more rapidly than in the case of the other acids. Peaks of blood ketones were reached in about 1 hr. for the other acids. Caproic acid caused the greatest rise in blood ketones and capric the least, with butyric intermediate. This was the general observation in all of the tests, although there were some trials where butyric caused a greater increase than caproic. In general, the longer the acid chain

the longer it took for the blood values to return to normal. In most cases the values were back to normal in 3 to 4 hr.

Essentially the same results were obtained by intravenous injection of acetic and butyric acids as with oral administration, indicating that the rumen microorganisms were not involved in the results obtained from oral administration. As shown in figure 2, 10 g. of acetic acid injected intravenously caused no change in blood ketone levels beyond the normal variations. Butyric acid, on the other hand, in both 5 and 10 g. dosage levels, caused definite and rapid increases in blood ketones, maximum values being reached in 15 min. Smaller amounts were needed intravenously than orally to give comparable increases in blood ketones.

Several other fatty or organic acids, as well as corn oil, were tested for ketogenic activity with essentially negative results. Table 2 shows the total blood

TABLE 2

Total blood ketone levels following oral administration of miscellaneous acids and corn oil

Time after administration	Substances administered (30 g.)					
	Propionic acid	Lauric acid	Oleic acid	Lactic acid	Succinic acid	Corn oil
	Total blood ketones					
(*Hr.*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)
0	3.3	2.4	4.4	2.2	1.1	2.8
0.5	1.0	2.4	4.0	3.3	1.1	2.7
1	1.8	2.8	3.2	2.1	1.0	3.0
2	1.4	3.4	3.2	1.0	0.8	2.5
3	1.7	3.3	2.0	1.3	0.8	2.4
4	1.8	4.4	1.2	1.6	0.8	2.7

ketone levels following oral administration of 30 g. dosages of six different substances. Propionic acid caused no perceptible increase, thus confirming work with tissue slices and laboratory animals. Lauric acid gave negative results under the conditions of this study. Oleic acid failed to cause any increases in blood ketones. Lactic and succinic acids, as well as corn oil, also showed negative results.

SUMMARY

The oral administration of acetic acid is fasted, non-fasted or phlorhizinized goats did not result in an increase in blood ketones. Butyric, caproic, caprylic and capric acids administered orally caused increases in blood ketones of 5 to 10 mg. per cent. Maximum levels of ketones were usually reached in 15 min. for butyric acid and in 1 hr. for the other three acids. The greatest increases were observed with butyric and caproic acids. Values returned to normal in about 3 hr. Propionic, lauric, oleic, lactic or succinic acids, administered in equivalent amounts, caused no significant rise in blood ketones. Corn oil also gave negative results.

Intravenous injection of acetic or butyric acids gave results similar to oral administration. Acetic acid caused no significant changes, while butyric acid

caused rapid increases in blood ketones. Less acid was needed intravenously than orally to give comparable increases in blood ketones.

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ABSTRACTS OF LITERATURE

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BOOK REVIEWS

632. Elementary experiments in dairy bacteriology. R. N. Doetsch and M. L. Speck. Burgess Publishing Co., Minneapolis, Minn. 62 pp. $1.75. 1949.

This manual presents in logical sequence a series of experiments designed for a first course in Dairy Bacteriology for students with a background in general bacteriology and in general chemistry. Twenty-three experiments are outlined and lists of materials required for each experiment are given in an appendix. A list of journals and books for possible collateral reading also is presented, but, with a few exceptions, these publications are not cited specifically in the different experiments. Although *Bergey's Manual* is mentioned once in the text and quite a few statements concerning classification are made, this book is not included in the list of references. Blanks are provided for recording the pertinent data from each experiment and introductory remarks are made for each experiment. In the opinion of the reviewer, the relation of utensil contamination and later opportunities for growth in the case of coliform bacteria are not presented with quite sufficient emphasis, the impression being left that these organisms are predominantly of direct grain and fecal origin. The spiral binding is a convenience. This manual should prove very valuable to those who wish to teach from a fixed laboratory manual, as it seems to the reviewer to be the best publication of this type available. F. E. Nelson

633. Advances in enzymology. Vol. IX. F. F. Nord, editor. Interscience Publishers, Inc., New York, N. Y. 760 pp. $9.00. 1949.

This volume maintains the high standard set by previous volumes in this series. The twelve review papers cover a wide variety of topics in an authoritative and well-documented manner. The authors represent a cross-section of European and American leaders in the various fields surveyed. The author and subject indices to the current volume assist greatly in the finding of specific material. A cumulative index of all 9 volumes which have appeared to date is included.

The chapter on "Metabolism of Semen", by T. Mann, contains considerable material on bovine semen and 336 references are cited. "The Mechanism of Fertilization in Metazoa", by John Runnström, contains much material of a fundamental nature which would be of interest to those in breeding work. Other chapters of interest to those in one or more dairy fields include: "Some Aspects of Reversible Step Reactions", by L. Michaelis; "Kinetics of Biological Reactions with Special Reference to Enzymic Processes", by A. E. Stearn; "Photochemistry of Enzymes, Proteins and Viruses", by A. Douglas McLaren; "The Nature of Viruses", by M. A. Lauffer *et al.*; "Nitrogen Metabolism of Higher Plants", by H. E. Street; "Chemistry and Enzymology of Nucleic Acids", by F. Schlenk; "Principles of Enzymic Histo- and Cytochemistry", by D. Glick; and "Industrial Biosynthesis. Part I. Fats", by A. Hesse.
F. E. Nelson

634. Principles of Veterinary Science. F. B. HADLEY. 521 pp., 4th Ed., Ill. W. B. Saunders Co., Philadelphia and London. 1949.

This text is of special interest to pre-veterinary students and persons interested in pursuing careers in fields related to animal husbandry. The first part of the book includes a rather comprehensive picture of the anatomy and physiology of animals, and the second part deals with animal diseases. Physiology and general function are especially emphasized.

Quite a few changes have been made in this new edition. Several chapters have been revised, and one chapter, "The Animal World", is entirely new. According to the author, this chapter was added "to give an idea of the great range of animal life". Due to the decrease of importance of horses on farms today, a great deal of space formerly alloted to horses has been reduced. With the increasing interest and importance of dairy cattle, considerably more attention has been given to them.

This book is written in a manner for rapid and thorough comprehension. Anyone interested in

any phase of livestock production would find this edition of considerable benefit. All chapters are sub-headed and divided into topics of chief concern. At the end of each chapter are a number of questions, the answers to which thoroughly summarize the contents of the chapter.

T. M. Ludwick

635. Some effects of feeding iodinated casein for a long time to cattle, swine and white rats. SVEN DYRENDAHL (English translation by Ebba Ericksson). Royal Vet. College, Stockholm. Pp. 3–116. 1949. (146 ref.)

Thirty-eight calves and 82 Yorkshire pigs received 1 g. /30 kg. live weight of iodinated casein or (Exp. 2-pigs) 1 g. /100 kg. live weight daily. Three hundred rats received varying levels, and radioactive phosphorus isotope P^{32} 40 min. before being killed. Poor flesh, osteoporosis with stiffness of gait, accelerated heart and respiratory rate after exercise, increased skin and rectal temperatures and loose feces characterized treated cattle. Exophthalmus of the eyeball in cattle, muscular tremor, nervousness and irritation also were noted. Reduced blood sugar was accompanied by lower liver glycogen content, based on blood analyses and liver biopsy samples. Periodical serous nasal discharge and coughing affected treated animals. Also, they had increased appetites, were slightly heavier but stored less body fat than did the normal animals. Cattle have been on test 3.5 yr., while pigs were slaughtered at 100 to 120 kg. live weight. Physical reactions of all animals indicated desire to lose excess body heat.

Phosphorylation processes in the liver and heart in rats which had been fed 0.01 g. of iodinated casein daily for 30 d. were observed with aid of radioactive phosphoric isotope P^{32}. They were injected with 5 c. of P^{32} in glucose solution intraperitoneally 40 min. before being killed. Liver and heart were removed, placed in 15% trichloracetic acid solution, then ground and washed in trichlor-acetic acid, so that the filtrate contained 100 ml. concerning the liver and 50 ml. regarding the heart. Analyses, and determinations of impulses/min. by the Geiger-Müller counting chamber were made on parts of this solution. Phosphorylation rate of liver and heart was higher in iodinated casein-treated animals than in controls. Nipples of treated rats were enlarged.

R. B. Becker

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

636. In vitro effect of certain antibacterial agents on organisms encountered in bovine mastitis. M. E. KRAFT AND G. R. SPENCER. Univ. of Wisconsin, Madison. Proc. Soc. Exptl. Biol. Med., **70,** 1: 176–179. Jan., 1949.

Various antibacterial agents were diluted to the approximate level which could be attained for a period of 10 to 12 hr. after intramammary infusion in cows. Subtilin was effective in high dilution against gram positive organisms. Streptomycin was more effective against gram positive cultures than against gram negative organisms. *S. aureus* and *S. typhimurium* were resistant to the sulfonamides. *Str. agalactiae* and *C. pyogenes* were susceptible to sulfapyridine, sulfone, sulfadiazine and sulfamerazine. *E. coli* was susceptible to sulfapyridine, sulfadiazine, sulfamethazine and sulfamerazine, among which the last two showed great activity. Sulfamethazine, sulfadiazine and sulfamerazine were effective against *Br. abortus*. *Ps. aeruginosa* was inhibited by sulfamerazine and sulfapyridine and sulfadiazine was the most effective antibacterial agent. R. P. Reece

637. Penicillin in Str. agalactiae infection: Trials made in Great Britain in 1945 and 1946. Anonymous. Vet. Record, **61,** 19: 235–237. May 7, 1949.

These data on field trials on the use of penicillin for *S. agalactiae* were accumulated by members of the Mastitis Conference of the Agricultural Research Council of Great Britain working at 6 different laboratories.

Penicillin Field trials were made on 1363 *S. agalactiae* infected quarters over a 2-yr. period. The examination consisted of plating 0.1 ml. of milk on blood agar plates and reading after 48 hr. at 37° C. for identification of *S. agalactiae*. A quarter was considered infected if it showed 3 positive tests, 2 of which were 1 wk. previous to treatment. Cure was considered to be effective if 7 negative tests were obtained, 3 of which were in the week after treatment and the additional 4 at weekly intervals.

Following pilot trials, it was decided to compare the efficacy of the following dosage levels and intervals in the 1st trial: 2 doses of 20,000 units with a 24-hr. interval, 4 doses of 20,000 units with a 24-hr. interval, 4 doses of 10,000 units after successive milkings, and a single dose of 100,000 units. All penicillin injections were made in 50 ml. of water and all quarters were treated. In the 2nd trial, dosage levels and intervals were modified as follows: 2 dosages of 20,000 units with a 24-hr. interval, 2 doses of 40,000 units with a 24-hr. interval, and 2 doses of 100,000 units with a 24-hr. interval. In addition, the penicillin was injected in 10 ml. of distilled water instead of 50 ml. by one of the laboratories.

Because of the larger numbers, results were expressed in terms of quarters cured, which makes

them about 10% higher than on a cows cured basis. The total results showed that one dose of 100,000 units is inadequate and gave only 58% cure; 2 doses of 20,000 units at 24-hr. intervals resulted in 80% cure and increasing the dosage level at this interval to 40,000 or 100,000 showed no advantage; 4 doses of 10,000 units at successive milkings or 4 doses of 20,000 units in a 24-hr. period were no better than 2 doses at the 24-hr. interval, all being approximately 80% effective.

R. P. Niedermeier

638. A comparison of the immunizing value in cattle of dead antigens and S. 19 Br. abortus vaccine. A. McDiarmid, Agr. Research Council, Field Station, Compton, Berks. Vet. Record, **61,** 22: 305–308. May 28, 1949.

Sixty Ayrshire heifers ranging in age from 22 to 30 mo., none of which had previously been bred, were used in this study to compare the immunizing value of dead vaccines with that of the avirulent vaccine made from strain 19. These heifers were divided into 5 groups of 12 each, with group 1 serving as a control, group 2 was vaccinated with S. 19 vaccine, group 3 was vaccinated with an antigenic fraction of *B. abortus* strain 544 and the remaining 2 groups were vaccinated with dead *B. abortus* (strain 544) suspended in lanolin and liquid paraffin. In all groups except no. 4, the antigens used were prepared from an estimated 60 billion bacillary bodies, and in group 4 the dead vaccine contained 10 times this number. When the majority of the heifers were 5 months pregnant, an infective dose (130 million organisms) of virulent *B. abortus* (strain 544) organisms was given via the conjunctival sac.

At parturition, cultural and biological examinations were made of blood, cotyledon, colostrum and fetal stomach contents. Blood samples were also taken at regular intervals during the experimental period and the agglutination test run. Results of these tests are given in detail. In general, these data demonstrated that the living avirulent S. 19 vaccine is better than dead antigens in all cases, including group 4 where the organisms used to prepare the vaccine were increased 10 times. The authors suggest that one might expect a better degree of immunity by large, repeated doses of one of the dead antigens. The lanolin vaccine seemed to be the most effective of the dead antigens. R. P. Niedermeier

639. The stability of the avirulent characters of Brucela abortus strain 19 and strain 45/20 in lactating and pregnant cows. A. W. Taylor and A. McDiarmid. Vet. Record, **61,** 23: 317–318. June 4, 1949

This study consists of 2 parts. In part I, 28 non-pregnant Ayrshire and Holstein-Friesian cows that had calved once and were negative to the aggultination test were used. All but 3 of these cows were lactating at the time they were vaccinated. The subcutaneous method of vaccination was used and 8 cows received 5.0 ml. of S. 19 vaccine; 8 were given 2 inoculations at 3-wk. intervals of 45/20 vaccine, and 12 cows served as non-vaccinated controls. These cows were housed and grazed together. The cows were bred beginning 2 mo. after vaccination. Milk samples were taken at 2-wk. intervals and examined culturally and in addition biologically for the vaccinated cows. Aggultination test was run monthly. At parturition, blood samples for the agglutination test, colostrum and cervical swabs were taken for cultural and biological examination. The prepartum sampling procedure was continued for approximately 2 mo. postpartum. Results showed that no *Br. abortus* was excreted in the milk of the vaccinated cattle, and no infection appeared in the control animals from either strain 19 or 45/20.

In part II, each strain was introduced intravenously in a pregnant negative cow at sufficient dosage level to cause abortion. At abortion it was recovered by culture from the cotyledon and in as short a time as possible another pregnant negative animal was inoculated. Each strain was passaged 7 times in this manner through pregnant cattle. In this process S. 19 remained unchanged in regard to its ability to grow in air and its accepted virulence for guinea pigs. Strain 45/20 became highly virulent by the 7th passage and became CO_2 sensitive. Its inability to produce agglutinin was lost between the second and third passage. R. P. Niedermeier

640. The mucus agglutination test for the diagnosis of bovine trichomoniasis. A. E. Pierce. Vet. Record, **61,** 25: 347–348. June 18, 1949.

Results are given for mucus agglutination tests on 465 mucus samples from clean, suspect and infected herds in the British Isles. Only the 1:10 dilution reading was used. The physical properties of the mucus were observed and the sample was then classified as oestral, post oestral, vaginal, pregnant, purulent or aqueous, prior to running the agglutination test. The successful application of the mucus agglutination test, as well as the ability to detect microscopically *T. foetus* in the various types of mucus samples is discussed. The author concluded that the mucus agglutination test is more accurate than the blood agglutination test. Inasmuch as several known infected animals failed to react to the mucus agglutination test and agglutinin may persist after recovery, the direct microscopic examination still is necessary,

but based upon this data the suggestion is made that microscopic examination be applied to only those types of mucus most likely to contain the organism. R. P. Niedermeier

641. Some observations on milk fever. A. ROBERTSON. Vet. Record, **61,** 24: 333–339. June 11, 1949.

Data given include observations on 19 cases of milk fever. Blood analyses for serum Ca, Mg and inorganic P before treatment, soon after treatment and after recovery periods are given. In several of the cases when the blood Ca was brought back to the normal level by Ca borogluconate treatment, a cure was not effected and the author points out that this supports the contention that blood Ca level is not alone responsible for milk fever. Upon statistical analysis of the data, no correlation was found between blood Mg levels and the symptomatology classification in regard to excitement, coma, narcosis or alertness. An interesting review of the historical background of research on milk fever is included, as well as a lengthy discussion by research men who have worked on this disease. R. P. Niedermeier

Also see abs. no. 634, 655, 657.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

642. Stabilization of butter. W. S. MUELLER (assignor to the U. S. of America as represented by Secretary of War). U. S. Patent 2,472,119. 16 claims. June 7, 1949. Official Gaz. U. S. Pat. Office, **623,** 1: 128. 1949.

Butter is protected against rancidity and decolorization by tetrachloroparabenzoquinone.

R. Whitaker

Also see abs. no. 726.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

643. Consumer preference as related to acidity, curd size and creaming of cottage cheese. W. H. E. REID, Univ. of Missouri, Columbia. Milk Dealer, **38,** 9: 43–44, 122–126. June, 1949.

Many manufacturers of cottage cheese use the acid test of the whey or of the curd as an index to when the curd has sufficiently firmed or is ready for cutting. A survey indicated that the acidity of the whey should be between 0.45 and 0.50%. Acidity of the curd varied from 0.57 to 0.65%.

The curd size varies in different regions and in some markets it is necessary to furnish both the "Popcorn Kernel" and the "Smearkase" types to satisfy cottage cheese consumers. When creaming the cottage cheese, care should be applied to avoid damaging the curd particles. The 3 defects commonly found in body and texture are mealiness, lumpiness and a soft, pasty texture.

The physical properties of cottage cheese manufactured from skimmilk reinforced with about 50% of nonfat dry milk solids are very comparable with cottage cheese manufactured from normal skimmilk. This was evidenced by the fact that several thousand lb. of this cottage cheese received favorable reception from the consuming public.

Nonfat dry milk solids are satisfactory for making cottage cheese. A concentration of 20%, i.e., 20 lb. of nonfat dry milk solids to 80 lb. of water is recommended. C. J. Babcock

644. It's all cottage cheese. CAROLINE B. MENUEZ, Paper Cup and Container Institute, Inc. Am. Milk Rev., **11,** 5: 10, 12. May, 1949.

The results of a survey revealed wide differences in types of cottage cheese offered for sale. Cottage cheese was classified according to six different types, based upon size of curd and relative amount of creaming. Variation in preference in different localities was pointed out. Potential marketing possibilities are emphasized by the fact that the per capita annual consumption in California is 6 lb. while the national average is 2 lb.

D. J. Hankinson

645. A comparison of starters for use in Roquefort-type cheese. J. CLARKE AND N. S. GOLDING. State College of Washington. Natl. Butter Cheese J., **40,** 7: 27–29, 54. July, 1949.

Carbon dioxide is a limiting factor in mold growth. Therefore, a lactic starter producing little or no carbon dioxide would appear to be desirable for use in making Roquefort-type cheese. The purpose of this study was to determine whether pure lactic cultures or those containing associate types of organisms were most desirable for this use. A comparison was made of the following starters: a commercial starter containing associate types of organisms (*Leuconostoc citrovorum* and *L. dextranicum*) and organisms of the *Streptococcus lactis* group; a pure culture of *S. lactis;* a pure culture of *S. cremoris.* Three lots of cheese were made with each of these starter cultures. Two different strains of *Penicillium roqueforti* were used.

Cheeses made with commercial starter had more abundant mold growth than that made with either of the pure cultures. Cheeses made with the pure cultures had dry textures; those made with the commercial culture had wet or leaky texture. Characteristic Roquefort flavor could not be correlated with high total volatile acidity. Cheeses made with pure cultures were criticized

for bitter taste and foreign mold flavors. The use of commercial type starter made equal or better cheese than either of the pure cultures.

H. E. Calbert

646. Report on sampling, fat and moisture in cheese. W. Horwitz and Lila Knudsen, Food and Drug Admin., Federal Security Agency, Minneapolis 1, Minn. and Washington 25, D. C. J. Assoc. Offic. Agr. Chemists, **32,** 2: 303–309. 1949.

A more extensive study of the official and modified methods for fat and moisture in cheese was conducted and analyzed statistically. The experiments were designed to determine (a) the variation between laboratories, (b) the variation between collaborators within a laboratory, and (c) the variation between duplicate samples run by the same collaborator. Results obtained in over 700 determinations are plotted graphically. Analyses of variance were performed on the data for each method. In most cases the variation between laboratories contributed a significant amount of variation. The difference between collaborators within a laboratory was very significant also. Variations are shown in terms of the standard deviation and in terms of variation to be exceeded 1 time in 20 ($P = 0.05$ limits).

When the A.O.A.C. method was used for determining moisture, the results obtained between laboratories had a standard deviation of ± 0.29 and $P = 0.05$ limit of 0.57; the draft oven method values of ± 0.31 and ± 0.61, respectively. Results between collaborators within a laboratory using the A.O.A.C. method had a standard deviation of ± 0.25 and a $P = 0.05$ limit of ± 0.49; the draft oven method gave values of ± 0.27 and ± 0.53, respectively. Results by one collaborator using the A.O.A.C. method had a standard deviation of ± 0.16 and a $P = 0.05$ limit of ± 0.31; the draft oven method gave results of ± 0.20 and ± 0.39, respectively.

When fat determinations were made by the A.O.A.C. method, the results obtained between laboratories had a standard deviation of ± 0.24 and a $P = 0.05$ limit of ± 0.47; a method employing direct weighing into a Mojonnier tube gave values of ± 0.32 and ± 0.63, respectively. Results obtained between collaborators within one laboratory, using the A.O.A.C. method, had a standard deviation of ± 0.19 and a $P = 0.05$ limit of ± 0.37; the method employing direct weighing into a Mojonnier tube gave values of ± 0.28 and ± 0.55, respectively. Results obtained by one collaborator, using the A.O.A.C. method, had a standard deviation of ± 0.15 and a $P = 0.05$ limit of ± 0.29; the method employing direct weighing into a Mojonnier tube gave values of ± 0.19 and ± 0.37, respectively.

F. J. Babel

647. Method of processing cheese and package therefor. F. M. Fisher and H. C. Hopp (assignors to Standard Cap and Seal Corp.). U. S. Patent 2,471,867. 1 claim. May 31, 1949. Official Gaz. U. S. Pat. Office, **622,** 5: 1496. 1949.

Instead of placing freshly milled curd in conventional hoops, it is placed in molds which are lined with a non-hydroscopic envelope, such as cellophane. The product in the lined mold is placed under vacuum, and the envelope sealed. Release of the vacuum causes the envelope to shrink around the curd causing it to knit together and acting as a protective coating during curing.

R. Whitaker

Also see abs. no. 655, 671.

CONDENSED AND DRIED MILK; BY PRODUCTS

F. J. DOAN, SECTION EDITOR

648. Stabilizing evaporated milk. H. E. Otting, L. H. Chrysler and E. F. Almy (assignors to M and R Dietetic Laboratories, Inc.). U. S. Patent 2,473,493. 11 claims. June 14, 1949. Official Gaz. U. S. Pat. Office **623,** 2: 607. 1949.

Evaporated milk having a total solids content greater than the usual 26% is easily sterilized without coagulation by incorporating a small portion of mineral modified milk having a Ca/P ratio of 0.15/0.75.

R. Whitaker

649. Filtrations-probleme bei der Molkeaufbereitung. (Filtration problems in preparing whey.) English summary. F. A. Friedel. Die Milchwissenschaft, **3,** 10: 292–296. Oct., 1948.

In order to obtain clear whey for human consumption the whey is heated to 100° C. and held for 10 to 15 min. The heat-coagulated proteins are permitted to settle, preferably at pH 4.5, during a period of 12 hr. The supernatant is filtered through a Berkfeld wash filter consisting of a layer of infusorial earth, which treatment renders the liquid clear. An illustration of a schematic procedure is given.

I. Peters

650. Korrektur der spindelanzeige bei molke. (The correction of lactodensimeter readings in whey.) English summary. G. Roeder. Die Milchwissenschaft, **3,** 11: 335–340. Nov., 1948.

The expansion coefficient was found to be the same for rennet- and acid-type whey at temperatures of 10 to 30° C. Based on the expansion coefficient of whey, the specific gravity for each temperature was calculated, taking into account the glass correction factor of the lactodensimeter.

Thus a table was formulated for the determination of the specific gravity of whey with readings taken at 10 to 30° C. corrected to 15° C. The calculated and actual values obtained were in sufficient agreement for the degree of accuracy required in this test. I. Peters

Also see abs. no. 659, 661, 668, 669.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

651. A modified frost little plate method for routine bacteriological control of milk. E. L. E. Humphries, S. Hues & Sons, Ltd., Liverpool. Dairy Ind., **14,** 4: 389–391. Apr., 1949.

A description of a modification of the original Frost technique is given in some detail. The technique consists essentially of preparing a 1 to 10 dilution of the milk to be tested in quarter-strength Ringer solution in the ordinary way. A sterile platinum loop calibrated to deliver 0.01 ml. is used to measure 0.01 ml. of this dilution, and to mix thoroughly with 3 to 4 drops of molten agar previously deposited on a sterile 3 × 1 in. micro slide, and finally to spread the inoculated agar over an area of 4 cm.^{2} After hardening, the slide is at once placed in a moist chamber and incubated overnight at 37° C. Next morning the slides are placed in a water-oven at 100° C., dried, washed with acetone, then water, stained with dilute aqueous methylene blue, again washed with water and returned to the oven to dry.

A description of a microscopic counting aid is given which aids in reducing fatigue as well as errors. G. H. Watrous, Jr.

652. Doppelkombinierte methode zur indirekten keimzahlbestimmung der milch. (The combination of two methods for the indirect count of bacteria in milk.) English summary. A. Todoroff and K. Assenowa. Die Milchwissenschaft, **3,** 10: 300–302. Oct., 1948.

A growth medium consisting of equal parts of peptone whey agar and nutrient broth agar when used in petri plates and in deep cultures permits the development of a large variety of bacteria differing nutritionally as well as in their demand for oxygen. Incubation at 32 to 35° C. for 48 hr. is recommended. For yeasts and molds a medium consisting of equal parts of peptone whey agar and wort agar is recommended for plating, using an incubation temperature of 30° C.

I. Peters

653. Method of deflocculating bacteria. G. Green (assignor to Syntron Co.). U. S. Patent 2,472,419. 5 claims. June 7, 1949. Official Gaz. U. S. Pat. Office, **623,** 1: 202. 1949.

To provide a means of breaking up clumps of bacteria, prior to counting, the medium is subjected to mechanical vibration of frequencies of 3,600 to 7,200 vibrations/min. R. Whitaker

654. A note on morphological differences between strains of Streptococcus cremoris. H. R. Whitehead and G. J. E. Hunter, Dairy Research Inst., Palmerston North, New Zealand. J. Gen. Microbiol., **3;** 43–45. 1949.

Cultures of *Streptococcus cremoris* were grown in milk for 5 hr. at 37° C., rather than at the optimum of 30° C., and then examined under the microscope. The appearance of involution forms served as a characteristic which was considered to be significant in the identification of various strains of this organism.

J. J. Jezeski

655. Penicillin in relation to acid production in milk by starter cultures used in cheddar cheese making. H. Katznelson and E. G. Hood, Dept. of Agr., Ottawa, Can. Science, **109,** 2837: 490. May 13, 1949.

The carry over of penicillin used as a treatment for mastitis into milk used for cheese may be great enough to inhibit the activity of acid producing bacteria. Pasteurization of milk failed to inactivate the antibiotic. M. Loewenstein

656. Zur taxonomie der mikroorganismen. (On the taxonomy of microorganisms.) English summary. A Meyn. Die Milchwissenschaft, **3,** 10: 297–300. Oct., 1948.

A discussion dealing with the importance and method of systematic classification of microorganisms is presented. I. Peters

657. The identification of Brucella abortus strain 19 by dye bacteriostasis. H. B. Levine and J. B. Wilson, Univ. of Wisconsin, Madison. J. Infectious Diseases, **84,** 1: 10–14. Jan.-Feb., 1949.

The work was undertaken to find an *in vitro* test to replace the quinea pig virulence test for the identification of strains of *Brucella abortus* which do not require added CO_2 on primary isolation. The tolerance to 6 basic thiazin dyes was studied with 97 cultures of *Br. abortus*. The cultures included 31 cultures of strain 19 from various sources, as well as virulent, relatively avirulent and aberrant cultures of human and animal origin. The dyes used were thionin, thionine blue, methylene blue, azure A, azure B and azure C. The organisms were streaked onto plates of Bacto-Tryptose agar to which had been added after sterilization the desired amount of sterile dye solution. The criterion of differentiation was the fail-

ure of strain 19 to grow at a dye level that did not inhibit other strains.

All of the strain 19 cultures were completely inhibited by 0.4 mg. thionine blue/l. of medium. All of the virulent strains tolerated at least 50% more of the dye. For routine testing the authors recommended a dye level of 0.4 to 0.5 mg./l. and an incubation period of 5 d. J. F. Cone

658. Today's milk is safe milk. A National Dairy Council Digest. Am. Milk Rev., **11,** 5: 44–45. May, 1949.

Largely because of pasteurization, disease outbreaks due to milk have declined in recent years. At the same time outbreaks due to other foods increased. Milk borne outbreaks are largely confined to small cities and rural areas where pasteurization is less common. Eradication plans for animal diseases communicable to man, such as tuberculosis and brucellosis, are important health measures. The effect of pasteurization on the food value of milk is briefly discussed.

D. J. Hankinson

Also see abs. no. 636, 637, 638, 639, 640, 645.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

659. Vergleich verschiedener fettbestimmung's methoden bei der akwendung auf milchpulver under kindernährmittel. (A comparison of various methods for the determination of fat in milk powder and baby foods.) English summary. W. Mohr and J. Häsing. Die Milchwissenschaft, **3,** 11: 321–327. Nov., 1948.

Dried powdered whole milk, skimmilk, buttermilk, whey and cream, as well as baby foods and butter, were analyzed for fat content by five different methods. The Schmidt-Bondzynaki method gave variable results, depending on the heat treatment of the respective milk powder. The Roese-Gottlieb method showed low values with rancid fat powder but not with normal fat powder. Schlowmer carbon tetrachloride method gave variable low values, whereas the Grossfeldt pure extraction method gave accurate values, provided the extraction time was of sufficient duration. This method measures also the phosphatides and therefore gave higher values than did the Weibull-Stoldt method. The latter gave accurate reproducible values in all instances and is recommended for the determination of fat in dried milk and whey. The method is as follows: A 20 g. sample is suspended in 100 ml. cold water and 60 ml. fuming HCl (spec. gr. 1.19). This mixture, with pumice added, is heated on a water bath with intermittent stirring until foaming ceases. The container then is covered with a watch glass and heating continued on an asbestos gauze over a direct small flame and boiled for 20 min. with intermittent stirring. Boiling water is added and the hot mixture filtered through a moistened folded filter paper of 27 cm. diam. After 3 washings with hot water and careful draining, the filter is folded, placed on a watch glass and dried for 3 to 4 hr. in a drying oven at 105° C.

The filter without thimble is placed directly into an extraction apparatus containing a pad of fat-free cotton at the bottom. Extraction in the Soxhlet apparatus is continued for 3 hr. using 175 ml. ether. The ether is evaporated and the fat is dried to constant weight at 105° C. The limits of error by this method for whole milk powder is ± 0.1 per cent. I. Peters

660. Report on the detection of added water by the serum tests. H. J. Hoffman, Dept. of Agr., Dairy and Food, St. Paul, Minn. J. Assoc. Offic. Agr. Chemists, **32,** 2: 309–317. 1949.

Results obtained in a collaborative study indicate that the official serum methods only serve to indicate to the analyst that added water may be present in a milk sample. Milk samples falling below the present standards (38.3 sour serum, 36 copper serum and 39 acetic serum) contain added water. The methods gave no indication of the amount of added water. A suggestion was made not to prosecute for added water unless cryoscopic results were available. Data obtained by the serum methods were not uniform. It was recommended that the serum methods be confined to their present limitations, *i.e.,* indicating the presence of suspected samples. F. J. Babel

661. Methanol extraction of lactose and soluble proteins from skim milk powder. A. Leviton, Bur. Dairy Ind., USDA, Washington, D. C. Indus. Eng. Chem., **41,** 7: 1351–1357. July, 1949.

Earlier work on the separation of soluble proteins and lactose from whey powder has been extended to fluid and dried skim milk. The proteins of spray-dried skim milk were almost completely precipitated by 62% methanol at – 15° C. The dried milk-methanol mixture was centrifuged within a few minutes after mixing to remove the protein precipitate. Lactose of good quality crystallized from the filtrate during 15 hr. and was recovered by centrifuging. The protein product was soluble in water and showed no significant change in particle size distribution from that in the original milk. The protein product recovered comprised 42.2% of the solids of skim milk and it contained 74.1% protein of which 81% was casein. The influence of solvent-powder ratio, the effect of methanol concentration, of higher extraction temperatures and the properties

of the lactose and the protein complexes were determined. Best results were obtained when 20 g. of powder were extracted with 100 ml. of 62% methanol. The results indicate that, for many industrial purposes, extraction at room temperature would produce a satisfactory soluble protein product. For best results the skim milk used should be a soluble spray-dried product in which the lactose has not crystallized. The process can be applied to fluid and concentrated skim milk, but with these products there is an alcohol rectification and a serious filtration problem. A study of the extraction of the fluid skim milks yielded interesting data on the constitution of the proteins in milk. B. H. Webb

662. Elektronen-mikroskopische Grössenbestimmung der Calciumcaseinatteilchen in Kuhmilch. (Electron microscope determination of size of calcium caseinate particles in cow milk.) NITSCHMANN, Hs., Univ. of Bern, Switzerland. Helvetica Chimica Acta, **32:** 1258–1264. 1949.

Preparations were made from skim milk diluted with 0.01 *M* $CaCl_2$ or treated with formalin and then diluted with distilled water. Simple dilution with distilled water allowed dispersion of some particle aggregates. The preparations were made on glass, gold shadowed and removed on a formvar lacquer film. The most common particle size is 80–120 mμ, with considerable numbers of particles in the 40–80 mμ and 120–160 mμ classes. Some particles are as large as 280 mμ. These values agree reasonably well with certain other values which have been reported.

F. E. Nelson

663. The binding of organic ions by proteins. Comparison of native and modified proteins. I. M. KLOTZ AND J. M. URQUHART. Northwestern Univ., Evanston, Ill. J. Am. Chem. Soc., **71,** 5: 1597–1603. May, 1949.

A comparative quantity study was made of the binding of a common anion, methyl orange, by a group of proteins, mostly of crystalline nature, under very identical environmental conditions. The extent of binding of methyl orange was measured by a differential dialysis technic. Bovine plasma proteins, fractions II (γ-globulin) and III-1 (β2-globulin), did not bind methyl orange. The extent of binding by friction IV-1 (α2-globulin) is quite small, while the crystallized albumin fraction showed significant binding properties. Among the non-plasma proteins examined only β-lactoglobulin showed appreciable uptake of methyl orange. Modification of proteins, such as acetylation, decreases the affinity of albumin for anions. Where the number of cationic loci are not decreased, such as the conversion of the ϵ-ammonium groups of lysine to guanidinium groups, the binding ability of serum albumin remains unaltered. H. J. Peppler

664. Compounds with "folic acid" activity. A. Z. HODSON, Pet Milk Co., Greenville, Ill. Arch. Biochem., **21,** 2: 330–334. Apr., 1949.

The activity of possible interfering compounds in the folic acid assay of milk were compared with that of pteroylglutamic acid for *Lactobacillus casei* and *Streptococcus faecalis*. Under the conditions tested, ribonucleic acid, uric acid, glutamine and orotic acid did not interfere in assays for folic acid while desoxyribonucleic acid and 5-methylthiouracil are active for *L. casei* and *S. faecalis*. The results do not explain the discrepancies observed in the folic acid assay of milk nor do they necessarily invalidate the values already reported for milk and other foods.

H. J. Peppler

665. The digestion of acetyl proteins by pancreatin. B. M. HENDRIX AND W. J. WINGO, Univ. of Texas. Arch. Biochem., **21,** 2: 431–36. Apr., 1949.

The digestive action of pancreatic extract upon acetylated casein, edestan and egg albumin, and their alkali-treated derivatives, was determined, and the nature of the binding of the acetyl group to the protein was studied. As much as 69% of the acetyl casein was digested, based on the amino nitrogen set free by the native protein. The digestibility of acetyl casein differed only slightly from that of its alkali-treated derivative. Less acetyl was liberated from acetyl casein by pancreatic digestion than was removed by the solution of the protein in dilute NaOH. The acetyl removed from alkali-treated acetyl casein by pancreatin amounts to less than 5.5% of the acetyl bound to the casein prior to enzyme digestion or alkali treatment. The liberation of acetic acid from acetyl proteins is believed due to esterases of the pancreatic extract. These esterases split the acetyl group bound by oxygen linkage to various groups in the protein molecule, such as the phenolic hydroxyl of tyrosine, other hydroxy-amino acids and carbohydrate groups.

H. J. Peppler

666. Process of preparing modified protein. I. A. PARFENTJER (assignor to American Cyanamid Co.). U. S. Patent 2,473,255. 3 claims. June 14, 1949. Official Gaz. U. S. Pat. Office, **623,** 2: 547. 1949.

A protein of improved nutritional value is made by digesting casein with pepsin at pH range 2 to 8, until most of it is water-soluble at pH 4.6. The desired fraction is salted out with 25 to 35% by

weight of $(NH_4)_2SO_4$, then the salt is removed by dialysis. R. Whitaker

667. A capillary-ascent test tube method for separating amino acids by filter paper chromatography. L. B. ROCKLAND AND M. S. DUNN, Univ. of Calif., Los Angeles. Science, **109,** 2839: 539. May 27, 1949.

A rapid, convenient, capillary-ascent test tube method for separation of less than γ quantities of amino acids by filter paper chromatography is described. M. Loewenstein

Also see abs. no. 642, 646, 648.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

668. Apparatus for the desiccation of organic substances. F. E. HURD. U. S. Patent 2,471,035. 4 claims. May 24, 1949. Official Gaz. U. S. Pat. Office, **622,** 4: 1133. 1949.

Milk, fruit juices and the like are first supercooled in a pressure tank and then sprayed into a vacuum chamber at 29 in. Hg or lower, where the product freezes in the form of extremely small droplets. The moisture sublimes as the particles fall to the bottom of the cone shaped chamber and the dry powder is removed and packaged. Heat may be applied to the nozzle to prevent freezing of the liquid on the nozzle. The chamber also is jacketed and insulated to permit control of the temperature within the chamber. R. Whitaker

669. Spray device. R. E. MEADE, N. E. TAYLOR AND J. F. CREWS (assignors to Western Condensing Co.). U. S. Patent 2,473,035. 6 claims. June 14, 1949. Official Gaz. U. S. Pat. Office, **623,** 2: 490. 1949.

The chief novel feature of this centrifugal spray wheel for atomizing fluids like milk, whey, etc. is a series of teeth on the rotating wheel upon which the material impinges and is thrown outwardly from the device in a fine mist. R. Whitaker

670. Heat exchange system. R. E. OLSON (assignor to Taylor Instrument Co.). U. S. Patent 2,472,984. 2 claims. June 14, 1949. Official Gaz. U. S. Pat. Office, **623,** 2: 477. 1949.

Details are given of a flow diversion valve and an automatic system of operation for the valve for ensuring the proper temperature of milk entering the holding tube of a high temperature-short time pasteurizer for milk. R. Whitaker

671. Protecting cheesemakers' profits with instruments. J. MEYER, Minneapolis-Honeywell Regulator Co., Brown Instrument Div., Philadelphia, Pa. Natl. Butter Cheese J., **40,** 6: 32–33, 50–51. June, 1949.

A "continuous balance" electronic potentiometer is a new device that can be used as a control instrument to prevent small fluctuations of temperature when high temperature-short time pasteurization of milk for cheesemaking is used. For smaller operations using the holding method of pasteurization, a standard dairy thermometer with built in timer, controller and signal lights now is available, serving as a means of automatic control in vat pasteurization. H. E. Calbert

672. A guide to checking high temperature short time pasteurizer. P. J. DOLAN, JR., Calif. Dept. Agr. Am. Milk Rev., **11,** 5: 40–41. May, 1949.

Cardinal points in checking performance and compliance of high temperature short time units with ordinance requirements are divided into 4 phases. The 1st phase, construction and arrangement of equipment, includes suitable clearance around equipment, sanitary construction, condition of plate gaskets, raw milk surge tank location, raw milk pressure relationship to pasteurized side, characteristics of the timing pump, slope of the holding tube, recording and indicating thermometer construction, and flow diversion valve construction. The 2nd phase is thermometer and controller accuracy. The 3rd phase is thermometer temperature controller and flow diversion valve response. The 4th phase, holding time, is determined by salt solution injection into water flow, using an electrode and meter as an indicating device. Filling time for a 10 gal. can may be used as a quick check if established when the unit is operating properly. Timing should be carried out as in operation—in diverted flow and with and without homogenizer in operation. The timing pump should be sealed at its maximum speed. D. J. Hankinson

673. Receptacle handling apparatus. I. H. KENDALL (assignor to Cherry Burrell Corp.). U. S. Patent 2,473,955. 7 claims. June 21, 1949. Official Gaz. U. S. Pat. Office, **623,** 3: 870. 1949.

This device takes inverted milk cans from a can washer, turns them right end up and delivers them on a conveyor with the lids in place. R. Whitaker

674. Ice cream machine with perforated screw agitator. G. H. G. ESPINASSE AND J. P. C. ESPINASSE. U. S. Patent 2,474,730. 3 claims. June 28, 1949. Official Gaz. U. S. Pat. Office, **623,** 4: 1176. 1949.

The chief feature of this ice cream freezer is a screw conveyor type of agitator which operates within a refrigerated cylinder. R. Whitaker

675. Refrigerating-plant efficiency. J. F. HYAM, Sarnia, Ont. Power, **93,** 6-A: 142. Mid-June, 1949.

An equation and an alignment chart are presented for determining the efficiency of refrigerating plants under various temperature conditions. H. L. Mitten, Jr.

676. Good purge means more tonnage. T. G. HICKS. Operating Engineer, **2,** 6: 36–7. June, 1949.

High condenser pressure, poor condenser heat transfer, increased power input and formation of explosive mixtures may indicate an air-bound system. Air and other noncondensable gases collect on the high side at the point of lowest pressure. This point may shift from the receiver to the condenser and back.

A purger is connected to the horizontal receiver on the top. It may be connected just above the liquid level in the case of vertical receivers. Location of purge line connection to condensers depends upon the type of condenser. With double-pipe condensers, correct location is near liquid-refrigerant outlet. Atmospheric condensers should have the purge line connected at the refrigerant outlet. Bleeder-type condensers should have the purge connection on the top pipe. With the horizontal shell-end-type condenser, purge lines are connected on the top at each end, while with the vertical, they are connected a few inches above the liquid refrigerant outlet. The purge connection is made at the liquid outlet in the case of evaporative condensers. Purge the receiver, then the condenser. Do not purge the condenser while it is operating. H. L. Mitten, Jr.

677. How much does your steam cost? W. SHINN AND W. ARROTT, Operating Engineer, Albany, N. Y. Operating Engineer, **2,** 7: 28–9. July, 1949.

The cost of steam, electricity, heat and other services from the power plant is important to the engineer, manager and the company's customers. Cost records show the cost of operation. Figuring costs is easy when dollars are substituted for make-up water, gal. of fuel oil or lb. of steam. An example of cost records and calculations is presented. H. L. Mitten, Jr.

678. Die wärmepumpe in der milchindustrie. (The heating pump in the dairy industry.) English summary. K. H. SUTTOR. Die Milchwissenschaft, **3,** 11: 340–344. Nov., 1948.

The discussion is concerned with the usefulness of installing a heating pump in dairy plants in order to utilize the waste heat resulting from such operations as cooling of heated milk, etc. A heating pump may prove to be economical only in plants where the cost of fuel is high as compared with electricity. Other items to consider are: (a) the discontinuous supply of waste heat throughout the day, (b) the immediate utilization of waste heat or the need for storing it for later use, and (c) the seasonal use of waste heat. I. Peters

679. Der trennvorgang in der schleudertrommel. (The process of separation in a separator bowl.) English summary. W. WILSMANN. Die Milchwissenschaft, **3,** 10: 302–309; **3,** 11: 327–334; **3,** 12: 366–371. Oct., Nov., Dec., 1948.

This is a theoretical discussion of the separator bowl. Calculations and diagrams dealing with the principles of efficient separation and clarification of milk are presented. I. Peters

680. Proper air filter maintenance pays industry in good results. B. G. EVANS, Eli Lilly and Co., Indianapolis, Ind. Heating, Piping Air Conditioning, **21,** 7: 86–8. July, 1949.

If filters are not cleaned or replaced when dirty, they may impede air flow and may allow dirt to be carried through because of high velocities in sections of the filter.

Some filters work better for a given application than others. A wire mesh cleanable filter is used for filtering the air supplied a building. This type filter removes enough impurities to make the air tolerable for ventilation. It has a large dust holding capacity. It may be cleaned with a solution such as kerosene by dipping and setting up to dry.

Where high purity air supply is needed, but the installation of an electrostatic filter is not justified, the author uses a filter consisting of multiple layers of paper lapped over a serrated frame. Sprayed with oil, this filter has a high efficiency of dust collection. Because of low dust holding capacity, it must be changed frequently. The electrical precipitation filter is used in applications with rigid air filtration requirements. Stationary cell-type filters are cleaned by directing a hot jet of water at 200 to 210° F. against the ionizing wires and plates.

Traveling plate electrostatic filters are used where high efficiency and large dust holding capacity are required. The plates are built into a continuous screen which rotates and dips into an oil bath for continuous cleaning.

Maintenance of filters is of prime importance for efficient removal of impurities and proper ventilation. H. L. Mitten, Jr.

681. Design standards for grade A plant producer farm dairies. G. L. NELSON, Okla. A. and M. College, Stillwater. Agr. Eng., **30,** 6: 271–273. June, 1949.

Considerations leading to the design standards presented are those which have been developed cooperatively by the Oklahoma A. and M. College, Oklahoma State Health Department, and 19 county and city health departments.

The building described is congruent with the U. S. Milk Ordinance and Code; it contains a milking room, a feed room and a milk room. Herd shelter is to be provided elsewhere. There should be a feed alley and litter alley in the milking room; these will increase wall and floor requirements but decrease the work travel tremendously. The milking room should be of such size as to permit controlled grain feeding. Fifteen min. feeding time is a reasonable time. Where a two-unit milker is used, and 5 min. is allotted for preparing and milking each pair, a minimum of 6 stanchions is needed.

Proper drainage of the milking room wastes depends upon the natural drainage of the site. Milk room wastes should not be permitted to flow through the milking room.

Mangers should be 12 in. higher than stall platforms. Milk rooms may be ventilated by vertical flue ventilators. Design data which specify the ventilation rate are not known. In the milking room a 24-in. diameter flue will remove moisture at the rate produced by 6 cows if the outside temperature is 25° F. with relative humidity of 50% and air velocity is 5 mph. and the inside temperature is 35° F. with 75% relative humidity. Several designs and some comparisons are illustrated.

H. L. Mitten, Jr.

682. Calculations for milkhouse heating purposes. C. P. WAGNER AND M. NABBEN, Northern States Power Co., Minneapolis, Minn. Agr. Eng., **30,** 6: 294–296. June, 1949.

Milkhouse heating is desirable to protect the water system, to remove ice from the floor, to prevent condensation on walls and to provide for personal comfort of workers. A controlled temperature of 40° F. gives milkhouse protection. Milkhouses in Minnesota of the same size, heated to 40° F., require from 2000 to 8000 kw-hr./season, depending upon the insulation values and degrees of infiltration. A Minnesota milkhouse that can be heated to 40° F. by 2000 kw-hr. is calculated to require 3200 kw-hr. for heating to 50° F.

Heat requirements of a building may be reduced by locating one or more sides against another building, serving through connecting vestibule, insulating and reducing infiltration. New milkhouse location plans should be made considering heating. In the Minneapolis area the annual value of good insulation and close fitting storm door and windows is $40 to $80 with electricity at 2¢/kw-hr. and heating to 40° F.

Heat removed from milk may be used to heat the milkhouse, for the ordinary mechanical refrigerator acts as a heat pump. The radiator (condenser) has an output of approximately 2.3 kw-hr. (7850 BTU) for each 10 gal. can of milk handled (milk temperature reduced from 95° F. to 40° F.). The milk cooler may be supplied with well water to supplement the milk as a heat supply. A milk cooler compressor of 0.75 hp. or larger would be required for this application. The use of the milk cooler as a heat pump is still in the experimental stage.

Additional methods of applying heat are direct radiation, storage water heater, radiant heat (wall or floor), heat pads and heat lamps. Fan-type, direct radiation heaters seem to be the best units for supplementary heating.

The well-insulated milkhouse of 1000–12000 ft.^{3} can be expected to require 0.75 kw-hr. of direct heat/degree-day. The same milkhouse may be heated with the milk cooler operating as a heat pump for 0.25 kw-hr. or less/degree-day. This includes energy used to cool the milk.

H. L. Mitten, Jr.

683. Are your trap discharge lines right size? H. G. EBERT, Yarnall-Waring Co., Philadelphia, Pa. Operating Engineer, **2,** 6: 26–7. June, 1949.

Trap discharge-line size is often as important as a properly sized trap. Charts are presented which give % of flash when condensate enters a trap, equivalent length of pipe to allow for the fittings in the line, and discharge-pipe diameter. Discharge lines sized by these charts will help traps drain equipment rapidly. H. L. Mitten, Jr.

684. Safety is big business here—$640.00 saved in two years. W. ARROTT, Operating Engineer, Albany, N. Y. Operating Engineer, **2,** 7: 20–22. July, 1949.

The safety program of the 74th St., New York power plant is described. Step one in any program is education. The men who run the plants should be responsible for safety and for teaching safety to the workers. Classes were built around films of the National Safety Council. The second step is a safety program. Competition builds enthusiasm among workers. The third step is safety follow-up. After each accident, the follow-up determines the cause, and steps are taken to prevent recurrences. Safety saves working time and boosts morale. H. L. Mitten, Jr.

685. Low down on wearing rings. I. J. KARASSIK AND R. CARTER, Worthington Pump and Machinery Corp., Harrison, N. J. Operating Engineer, **2,** 7: 38–39. July, 1949.

Centrifugal pumps have running joints between their impeller and casing. Clearance in these joints is usually between removable wearing rings. The different types of rings are presented by diagram and discussed as to construction, location, mounting and clearances. H. L. Mitten, Jr.

Also see abs. no. 713.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

686. The ice cream market in the home. Anonymous. Ice Cream Trade J., **45,** 7: 34, 36, 77–79. July, 1949.

According to a survey just made by the U.S.D.A., the average family of 3.42 persons in 68 cities spends 42.5¢ for ice cream for home consumption. The amount spent ranged from 17¢/wk. for families whose income was under $1,000/yr. to 73.2¢ for families with incomes of $7,500 or over. The average family spent $25.57 weekly for food, 1.66% of which was spent for ice cream.

Assuming that the average per capita consumption in 1948 was 16.23 qt./person, the average family would have consumed 55.5 qt./yr., or $38.50 worth at 70¢/qt. The government study indicated that about half of the ice cream produced is going into the home, the other half being consumed at the point-of-sale. The amount of money spent for ice cream ranked high when compared with the amount spent for other dairy products. The average family spent $2.21/wk. for fluid milk, 65.6¢ for butter, 52.6¢ for all types of cheese and 42.5¢ for ice cream. W. H. Martin

687. What the industry should do about costly practices that are unsound and unethical. J. H. MEEHAN, Phila. Dairy Products Co. Ice Cream Trade J., **45,** 6: 46, 48, 99. June, 1949.

The unsound and unethical pratices which have beset the ice cream industry are unfair to the producers as well as the dealers. Management, which is at fault, should stop this cutthroat competition and train their salesmen to sell ice cream on the basis of intelligent constructive sales instead of underselling competitors and bribing dealers with cabinets and neon signs. Salesmen should stress selling more gallonage to their customers instead of fighting for other companies' customers; thus the gallonage sales would increase, the cost of ice cream decrease, and better feeling would result among members of the association. W. H. Martin

688. Snowballs in July. Anonymous. Ice Cream Trade J., **45,** 6: 44. June, 1949.

An unexpected but very welcome advertisement of ice cream will reach 43 million readers through the efforts of Durkee Famous Foods campaign to acquaint the public with Coconut Snowballs. These "snowballs" are ice cream covered with chocolate and topped with coconut. They are sold in the Stork Club and other eating places for as high as $1.50, but are really very inexpensive so they should appeal to the fountain and home consumers. This extensive advertisement will start just as the June Dairy month campaign ends, thus keeping public attention focused on ice cream. W. H. Martin

689. While the show goes on, there's gallonage in theatres. T. E. HEIDENREICH, JR. Ice Cream Trade J., **45,** 5: 46, 48, 113–114. May, 1949.

Theatre operators, hunting a new source of revenue to support their dropping ticket sales, have provided a comparatively new "dry stop" for ice cream manufacturers. In a recent *Motion Picture Herald* survey of 16,880 theatres, 20% or 3,201 were selling ice cream, while 89% sold candy. The gallonage demand is as high, if not higher, in the winter months as in summer, making a year-round market with only a slight slump in Apr.–May and Sept.–Oct. In 1948, theatre gallonage sales increased over the previous year's sale and so far in 1949 the increase has been substantial.

The few retail difficulties such as proper storage cabinets, an ice cream product which will leave a minimum of litter in the theatre, and the method of selling either by peddling in the aisles or selling from the lobby stands are being considered and overcome. This "dry stop" adds up to some important gallonage for the industry in years to come. W. H. Martin

690. High's converts to self-service. H. HAUG. Ice Cream Trade J., **45,** 5: 52–53, 108. May, 1949.

High's Dairy Products Co. of Washington, D. C., are converting most of their 67 stores from counter-type to self-service after experimenting with a store which moved from 12th to 3rd place in sales volume after self-service had been installed.

Though the equipment for self-service costs about $6,000 as compared to $1,800 for counter sales equipment and the electricity bills run 3 and 4 times higher, the greater volume of business more than pays for the change. There was a 50% increase in milk sales when the open sales cases were substituted for the glass door reach-in type cases, and the ratio of hand-dipped and packaged

ice cream sales changed from 3 hand dipped quarts to 1 packaged quart previously and is now 4 packaged quarts to 1 hand dipped.

The same number of attendants is maintained but they handle a much larger amount of business. Long periods of waiting have been eliminated, inventories are easier and there is no additional administrative problem. W. H. Martin

691. A legislative commission looks at the home delivery of milk. Anonymous. Am. Milk Rev., **11,** 6: 36–39, 41. June, 1949.

This article is a summary of a study in New York City conducted under the direction of Dr. E. C. Young, Dean of the Graduate School at Purdue Univ., at the request of a temporary commission on agriculture created by the legislature of the State of New York in 1945. The report was submitted in March, 1949.

The duties of a home delivery routeman were stated as follows: (a) get vehicle and return it to garage, (b) load and unload, (c) drive to route and return, (d) deliver, (e) collect, (f) record sales and collections and (g) settle accounts and make out order. The average routeman spent 60% of his time for actual service to the customer and 40% for bookkeeping, loading, and unloading, credits, and collections. Wide variations in delivery costs were noted and attributed to differences in consumer dwelling and buying habits, standards of customer service, number of units delivered/customer and responsibilities of the routeman. The cost of the average customer call was 18.5¢ with an average of 2.86 qts./customer, resulting in a cost/qt. of 6.5¢. When the customers lived primarily in row houses the cost/call was 8.6¢. When customers lived in separated homes the cost was 12.4¢/call. Since no price reductions were offered for quantity purchases by multiple route companies, no incentive existed for revising buying habits of customers. Home delivery of milk offers more service to consumers than any other food product. D. J. Hankinson

692. Prevailing wages paid to milk routemen. C. Lewis, Univ. of Missouri, Columbia. Milk Dealer, **38,** 8: 46–47, 138. May, 1949.

A survey of prevailing wages paid to milk routemen gave enough information to conclude that the wages, including commissions, paid these workers range from $59.37 to $87.37, with an average of $66.66/wk. Commissions averaged between 5% and 8% on wholesale routes and a little more on retail routes. The wholesale price of pasteurized milk ranged from 15 to 24¢/qt. with an average price of 17.99¢/qt. The retail price ranged from 17 to 24¢ with an average price of 19.85¢. Homogenized milk had the same price range as retail pasteurized milk but averaged 20.69¢/qt. Wage cost/qt. varied between 1.25¢ to 11¢ with an average of 3.66¢/qt. on wholesale and 3.869¢/qt. on retail. Tables are presented showing distribution of milk prices for Feb., 1949, wage groups disposition, and the weekly wages paid routemen, and prices for retail and wholesale bottle milk for Feb., 1949. C. J. Babcock

693. Selling salesmen to sell. R. G. Peat, Silverwood Dairies, Ltd., London, Ontario. Am. Milk Rev., **11,** 7: 2–4. July, 1949.

This article points out 6 factors which make for successful selling: (a) selection of personnel, (b) training, (c) planned selling, (d) incentive, (e) recognition, and (f) supervision. The use of all factors is summed up under one term—leadership. D. J. Hankinson

694. Where do you break even? M. J. Kluger. Am. Milk Rev., **11,** 6: 2–4, 6. June, 1949.

"Break-even" point is a term used by accountants to indicate the sales volume just necessary to pay for expenses which do not vary with sales volume in addition to the expenses which vary with sales. It is useful as a management tool to maintain sufficient volume to insure profit. This is especially important in the milk industry where the margin of profit is narrow. The break-even point varies between plants and from month to month. It is pointed out that a reduction in volume of sales may result in a net loss to the plant because certain fixed expenses do not change with volume of business. The break-even point can be represented in chart form to give a visual guide for conduct of business. D. J. Hankinson

695. Allocation of costs in multiple products plants. L. C. Thomsen, University of Wis. Natl. Butter Cheese J., **40,** 6: 28–29, 56–58. June, 1949.

In multiple products plants it is difficult to allocate costs of such secondary operations as quality control, and receiving, separating and administrative costs. This paper discusses various methods of allocating these secondary costs to the cost of the major product. A dairy plant must have a sound accounting system before any method of cost allocation is practical. Adoption of standard accounting procedures will result in greater permanency for the accounting system. This will make possible long term comparisons of costs, sales, etc. H. E. Calbert

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

696. The value of urea in protein supplements for cattle and sheep. J. S. Dinning, H. M.

BRIGGS AND W. D. GALLUP, Oklahoma Agr. Expt. Station, Stillwater. J. Animal Sci., **8,** 1: 24–34. Feb., 1949.

Nitrogen balance studies were conducted on 2 yr. old steers and growing wether lambs on maintenance wintering, and fattening rations in which urea was included in varying amounts from 9 to 50% of the total N of the ration. The steers refused to consume all of the rations in which urea furnished 50% of the N, but no difficulty was experienced in getting the lambs to eat rations containing that level of N.

The amount of N retention for both steers and lambs was increased when additional N was furnished by urea. The addition of urea did not increase fecal N but caused an increase in the total N of the urine, largely in NH_3 and urea. Both steers and lambs reacted alike in this respect. Lambs apparently were more efficient in the utilization of urea. Total N retained was about the same in rations in which urea furnished 50% of the N, as in those in which it supplied 25%. Urea increased the apparent digestibility of protein but had no effect on the digestibility of other nutrients. F. C. Fountaine

697. The influence of soybean oil meal upon roughage digestion in cattle. W. BURROUGHS AND P. GERLAUGH, Ohio Agr. Expt. Station, Columbus. J. Animal Sci., **8,** 1: 3–8. Feb., 1949.

The digestion coefficients of dry matter in corncobs and timothy hay were determined by feeding fattening cattle rations to steers. Two rations, 1 with and 1 without corncobs, were fed both at an 8% and at a 15% protein level. Soybean oil meal was substituted for corn to change the protein content of the rations. The digestion of dry matter in corncobs was increased by 14% and that of timothy hay by 17% by the addition of soybean meal to the ration. F. C. Fountaine

698. Further observations on the effect of protein upon roughage digestion in cattle. W. BURROUGHS, P. GERLAUGH, B. H. EDGINGTON AND R. M. BETHKE, Ohio Agr. Exp. Station, Columbus. J. Animal Sci., **8,** 1: 9–18. Feb., 1949.

Five series of digestion trials with beef steers were conducted to determine the effect of protein on dry matter digestion of corncobs or clover hay fed as sole roughages. In 2 series with corncobs and 1 with clover hay, the protein content of the ration was varied by substituting dried skimmilk for mineralized starch. In one series dried skimmilk was added in varying amounts directly to the corncobs and in the final series dried skimmilk was added in different amounts to a constant mixture of starch and corncobs.

In every instance when starch was fed as part of the ration, the addition of dried skimmilk improved the apparent digestibility of the roughage. Adding dried skimmilk to a ration in which no starch was present had little effect on the digestion of the dry matter in corncobs, even though the total ration contained as little as 4% total protein. When starch was present the minimum level of protein for efficient digestion seemed to be between 8 and 12%. F. C. Fountaine

699. The growth of dairy heifers raised chiefly on roughages. O. T. STALLCUP, H. A. HERMAN AND A. C. RAGSDALE. Mo. Agr. Expt. Sta. Bull. 523. 1949.

Twenty-eight Holstein heifers nursed their dams 3–4 d., received whole milk to 6–7 mo., with lespedeza hay, alfalfa hay, sorgo silage, limited concentrates at 3 levels, and pasture in season to 24 mo. Others received limited milk to 8 wk., an 18% protein calf starter, lespedeza hay and pasture in season. Calves were encouraged to take roughage and concentrates early. Normal growth was obtained where about 55% of the crude protein and digestible nutrients were obtained from pasture. Dairy heifers of normal weight and height may be reared from 6 to 24 mo. of age on not more than 900 lb. concentrates, if quality roughage and pasture are provided in abundance. Feed consumptions and body measurements are given. R. B. Becker

700. The metabolism of niacin in ruminants (sheep, goats and calves). P. B. PEARSON, W. A. PERLZWEIG AND F. ROSEN, A. & M. College of Texas, and Duke Univ. School of Medicine. Arch. Biochem., **22,** 2: 191–94. June, 1949.

The urinary excretion of nicotinic acid, N-methylnicotinamide (NMN) and its pyridone, N-methyl-6-pyridone-3-carboxylamide was determined for calves (about 6 wk. old) and mature goats and sheep on a normal diet and following the ingestion or parenteral administration of 2 g. nicotinamide daily for 3 consecutive d. Between 14 and 19% of the niacin ingested by calves and sheep is excreted by the renal pathway as non-methylated nicotinic acid derivatives. Goats excreted only 6.4% when nicotinamide was ingested, but 23% when administered subcutaneously. A small but insignificant increase in the amount of NMN excreted was observed for all three species, thus exhibiting a behavior similar to that reported for the rabbit, guinea pig and horse. On a normal diet neither the calf, goat nor sheep excreted measurable amounts of pyridone. The results obtained with nicotinamide administration further reveal that pyridone is not an end product of niacin metabolism in the calf, and that it is of minor quantitative importance in the goat and

sheep. The observations do not preclude the possibility of both methylated derivatives being intermediates in the metabolism of niacin by herbivora, since the horse and guinea pig have been shown to destroy NMN, while the rabbit oxidizes a considerable quantity of it to the pyridone. The enzyme capable of oxidizing NMN has been found in rabbit liver but not in sheep liver.

H. J. Peppler

701. Studies of the effect of phosphate fertilization on the composition and nutritive value of certain forages for sheep. G. Matrone, R. L. Lovvorn, W. J. Peterson, F. H. Smith and J. A. Weybrew. J. Animal Sci., **8,** 1: 41–51. Feb., 1949.

Phosphate fertilization of Bladen silt loam of the North Carolina Coastal Plain had no effect on the chemical composition of soybean hay grown on it. When measured by gain in wt. and apparent digestibility there was no significant difference in the feeding value for lambs of a ration of soybean hay and raw soybeans grown on phosphate fertilized plots and one grown on check plots.

In the 2nd yr., with cerelose as a concentrate, soybean hay grown on phosphated soil gave significantly greater gains in lamb and had a higher apparent digestibility than soybean hay grown on unphosphated plots. The authors suggest that general conclusions should await results of further investigations. F. C. Fountaine

702. Phosphatic animal-feed supplement. Laboratory and pilot plant production. G. L. Bridger, J. W. Moore and H. M. McLeod, Jr. Tenn. Valley Authority, Wilson Dam, Ala. Animal feeding tests. D. E. Williams, F. L. McLeod, E. Morrell and H. Patrick, Univ. of Tenn. Agr. Expt. Sta., Knoxville, in coop. with Tenn. Valley Authority. Indus. Eng. Chem., **41,** 7: 1391–1400. July, 1949.

A waste material composed chiefly of iron and phosphorus in a form unavailable to plants and animals was converted to a limestone-ferrophosphorus product. In the new form about 3/4ths of the phosphorus was available for phosphorus retention to experimental animals (rats and chicks), as compared with readily available phosphorus in a salt mixture. This partial unavailability was overcome by feeding the product at increased levels. This material may be a useful phosphatic feed supplement if it can be produced cheaply. B. H. Webb

703. The calcium, magnesium and potassium contents of the serum of ewes fed high levels of potassium. P. B. Pearson, J. A. Gray and R. Reiser, A. & M. College of Texas, College Station. J. Animal Sci., **8,** 1: 52–56. Feb., 1949.

Potassium bicarbonate included as approximately 5% of a ration of alfalfa hay and grain had no significant effect on the amount of calcium, magnesium and potassium in the blood serum of mature ewes. F. C. Fountaine

704. The influence of tocopherols upon the mammary and placental transfer of Vitamin A in the sheep, goat and pig. F. Whiting, J. K. Loosli and J. P. Willman, Cornell Univ., Ithaca, N. Y. J. Animal Sci., **8,** 1: 35–40. Feb., 1949.

Three prepartal rations furnishing respectively 12,000 I. U. Vitamin A, 80 mg. tocopherol, and 12,000 I. U. Vitamin A plus 80 mg. tocopherol daily were compared to basal rations for ewes, goats and sows.

Supplementing the prepartal rations with 12,000 I. U. of Vitamin A resulted in increased stores of Vitamin A in the livers of the newborn, and in the colostrum of all species studied. Addition of 80 mg. of tocopherol to the basal ration increased the liver stores of only lambs. Tocopherol supplements had no influence on the Vitamin A content of the colostrum. Combination of tocopherol and Vitamin A supplements had no effect on liver stores of the newborn or the Vitamin A content of the colostrum, as compared to rations containing only Vitamin A supplements.

F. C. Fountaine

Also see abs. no. 635, 721.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

705. A metabolic regulator in mammalian spermatozoa. H. A. Lardy, D. Ghosh and G. W. E. Plaut, Univ. of Wisconsin, Madison. Science, **109,** 2832: 365. Apr. 8, 1949.

Metabolic data, together with motility observations obtained on epididymal and ejaculated bovine spermatozoa lead to the discovery of a metabolic regulator. This substance is present in bound form in epididymal spermatozoa and apparently is liberated into the seminal fluid as a water-soluble conjugate from which the active form is released by mild alkaline hydrolysis. The substance also is liberated during prolonged storage of excised epididmides in the refrigerator. Aerobic fermentation by yeast is stimulated by the regulator, and this characteristic forms the basis for an assay, the details of which are to be reported elsewhere. The regulator is believed to be responsible for the high rate of respiration of ejaculated spermatozoa. The possibility is advanced that if liberation of the regulator could be prevented, or a method of counteracting its effects devised, viable spermatozoa could be preserved for longer periods. M. Loewenstein

706. Effects of dilution on motility of bull spermatozoa and the relation between motility in high dilution and fertility. P CHENG, L. E. CASIDA AND G. R. BARRETT, Univ. of Wisconsin, Madison. J. Animal Sci., **8,** 1: 81–88. Feb., 1949.

Six semen samples from each of 5 bulls were diluted at 1:10, and successively from 1:100 to 1:12,800 with 0.9 NaCl and with 0.08 *M* sodium citrate diluents. There was a progressive decrease in % of motile spermatozoa from low to high dilutions. Motility was not restored in spermatozoa reconcentrated by centrifuging. The addition of egg yolk to citrate buffer markedly increased the motility of spermatozoa in dilutions from 1:100 to 1:12,800. There was no significant correlation between motility and fertility when measured within bulls. F. C. Fountaine

707. Evidence of an inherited seminal character associated with infertility of Friesian bulls. J. L. HANCOCK, Wellcome Veterinary Research Station, Frant, Sussex. Vet. Record, **61,** 22: 308–309. May 28, 1949.

A morphological abnormality of the spermatozoa of 7 closely related Holstein-Friesian bulls, all of which had a very poor breeding record, is described. This abnormality appeared only after staining, and photomicrographs are shown of affected spermatozoa stained with iron haematoxylin where the abnormality appeared as a deeply stained area at the anterior pole of the head. Other semen characteristics, including motility ratings, density, viability at 4° C., and methylene blue reduction time, all were within the normal range on these bulls. The average percentage of affected spermatozoa ranged from 79 to 96%, and the bulls were used in 6 different herds on 108 females, none of which became pregnant. When these females were mated to known fertile bulls, clinical histories indicated they were of normal fertility. R. P. Niedermeier

708. An apparatus for the extraction of fertilized eggs from the living cow. L. E. ROWSON AND D. F. DOWLING. Vet. Record, **61,** 15: 191. April 9, 1949.

An apparatus that enables one to wash the ova from the uterine horn with a minium amount of irrigating medium is described in detail. It consists of a solid rubber tube about 30 in. long with 3 channels. Two of the channels act as a two-way catheter with 1 opening near the tip and the 2nd a few inches back. The 3rd channel opens into a 1 in. latex collar vulcanized to the tube just behind the 2nd opening of the two-way system. This collar can be inflated with air after the tube is in position and seals off the tip of the uterine horn. The technique of inserting this irrigating tube with the aid of a steel stilette, withdrawn after insertion, also is described. Epidural anaesthesia is suggested as being of help in controlling the operation. The name of a London firm now manufacturing this apparatus is given.
R. P. Niedermeier

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

709. Instrumentation for animal shelter research. C. F. KELLY, T. E. BAND AND C. LORENZEN, JR., U. S. D. A. Agr. Eng., **30,** 6: 297–304. June, 1949.

Research in animal shelters many times involves measurement of heat flow. Temperature and heat flow are so closely related that instruments for measuring one usually are useful for measuring the other. The estimation of heat loss rate by convection requires the knowledge of dry-bulb temperature of air and rate of air flow. Rate of heat loss by radiation involves the temperature of surfaces or the direct measurement of emission. Wet-bulb temperature of air is required in the estimation of total heat loss and moisture removal through ventilating systems. Instruments available are radiometers for measuring total solar and sky radiation and intensity of radiation from a surface; globe thermometer and Kata thermometer for measuring the combined effects of temperature, radiation and air flow; touch thermocouple for measuring the wet-bulb depression; and potentiometers for measuring and recording the thermoelectric effect of the preceding instruments.

The thermocouple is used in making surface temperature measurements. The diameter of the wire should be small; 30 or 36 gauge wire with a butt-welded junction is satisfactory. For surface temperature of swine and cattle a touch thermocouple has been used.

The wet-bulb temperature of air can be measured satisfactorily with a thermoelectric psychrometer which uses a single wet and dry junction of 36 gauge copper and canstantan. Dust from feed and floor make frequent changing of wicks necessary. Facial tissue and cigarette paper are satisfactory substitutes for absorbent cotton wicks and are easier to change.

Small heat flow meters fitted with handles can be used to measure heat flow by radiation and convection from walls, floors, ground or animals. The instrument may be cemented, screwed or taped to the surface being tested. When fitted with a suitable handle it can be held against the animal. With animals, the time the instrument is held against the animal should be sufficient to allow heat transfer rate to reach a steady state, but not so long as to materially affect the animal's subcutaneous circulation.

The flat plate radiometer is used for measuring the heat load on animals and structures from both the sky and ground, and for comparing the efficiency of shades in cutting off solar radiation. A device for calibrating small radiometers is described and a formula for determining the calibrating factor is given.

Potentiometers for general purpose work should be calibrated in millivolts and need not have an automatic cold junction compensator.

H. L. Mitten, Jr.

710. Milking barn. G. J. AND D. R. POLIVKA. U. S. Patent 2,472,122. 9 claims. June 7, 1949. Official Gaz. U. S. Pat. Office, **623,** 1: 128. 1949.

To facilitate machine milking of cows, the stalls of this milking barn are raised a few feet above the floor level. The cows enter the stalls from raised aisles. To conserve space and to provide convenience the stalls are set at an angle to the aisles. R. Whitaker

711. Teat cup. H. O. LINDREN (assignor to Aktiebolaget Separator Corp.). U. S. Patent 2,473,379. 1 claim. June 14, 1949. Official Gaz. U. S. Pat. Office, **623,** 2: 578. 1949.

A teat cup for milking machines consisting of a rigid shell containing a flexible inner liner which is caused to extend and contract by a pulsing source of vacuum applied through a tube separate from the milk collecting tube. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

712. Year 'round specialty. Anonymous. Ice Cream Trade J., **45,** 7: 44, 95. July, 1949.

Cake for the "ice cream 'n cake" roll is received in sheets 24 in. long from the Newly Weds Baking Co. Chicago plant. In the plant, pans are lined with sheets of white waxed paper $19 \times 24\frac{1}{2}$ in. and the cake inverted is placed in the pan on top of the paper. Ice cream from a continuous freezer is spread over the cake by means of a special spreading device; the pans pass on a conveyor to a table where the roll is formed; a stainless steel miter box and saw is used to cut the 24 in. roll into 6 uniform sections. Eight sections are packed into a carton. The Standard Drug. Co. of Cleveland, O., markets this item through its 53 outlets at 39¢ for a 4″ roll. Different flavor combinations are made, including vanilla ice cream and chocolate cake, and vanilla and strawberry ice cream with vanilla cake. W. H. Martin

713. A guide to cabinet sizes. Anonymous. Ice Cream Trade J., **45,** 7: 58. July, 1949.

A special chart has been prepared by the Ice Cream and Cabinet Section of the Air Conditioning and Refrigeration Machinery Association. It gives the dimensions of the 1949 conventional type cabinets manufactured by its members.

W. H. Martin

714. Golden State markets "Redi-Serv" for single-service at the dealer fountain. Anonymous. Ice Cream Trade J., **45,** 6: 42–43, 88. June, 1949.

In a search for controlled ice cream portions, the San Francisco Golden State Co., Ltd., started producing last May the factory made single-serv-ice ice cream item, Golden State "Redi-Serv." It is extruded from a freezer at 18° F. It is deposited in a solid mass and touches only the bottom of the cup, not the sides. This necessitates constant low temperature. The portions may be adjusted by the delivery tube. At present the size is 2.4 fluid oz. These cups are packed in 4 half-dozen layers separated by wax paper, sealed and turned over so the cups are upside down and the ice cream protected at all times when being dispensed.

The advantages of this "Redi-Serv" are many. The souffle cup which one pushes on the bottom to release the ice cream is faster, more sanitary, has no waste, fits standard cones and other dispenser equipment and gives the dealer controlled portions. This cup is sold to dealers for about 3.5 cents, making it possible for him to figure exactly his costs and is promoting a 10 cent sundae. Consumer reaction to texture and flavor has been excellent. W. H. Martin

715. Shifts in gallonage. C. E. FRENCH, Purdue Univ., LaFayette, Ind. Ice Cream Trade J., **45,** 7: 38, 39. July, 1949.

Since 1925 Pennsylvania and New York State have occupied the first 2 positions in total ice cream production. California, Illinois and Ohio are next 3 ranking states. Greatest gains have been made by North Carolina and Tennessee, which have moved up to 14th and 15th places from 24th and 26th places, respectively. Other southern states have moved up substantially.

W. H. Martin

716. A study of sales and profits in malteds at the retail level. Anonymous. Ice Cream Trade J., **45,** 6: 66, 79. June, 1949.

The *Fountain Service Magazine* made a recent survey throughout the country which showed that though the Midwest makes a richer malt, the East and West sell more and profit more due to their lower prices and sound merchandising technics. This survey also shows flavor preference

the country over the same, with chocolate rated first with 78%, vanilla next with 11%, strawberry with 6.6%, pineapple polling 3.1%, and all other flavors accounting for the remaining 1.3%.
W. H. Martin

717. Ice cream volume forges ahead. Anonymous. Ice Cream Trade J., **45,** 7: 52, 91. July, 1949.

The volume of ice cream made in May, 1949, was 55,770,000 gal., representing an increase of 3% over May, 1948. Pennsylvania and New York were 8 and 10% ahead of last year. Massachusetts, up 12%, Indiana 9%, Michigan 11%, Washington State up 31%, and Oregon up 24%, were other states showing substantial increases. Production for the first 5 mo. was 204,450,000 gal. or 3% below 1948. W. H. Martin

Also see abs. no. 674, 686, 687, 688, 689, 690.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

718. Tank truck hauling of milk from farm to plant. D. C. Lightner, Creamery Package Mfg. Co., Chicago, Ill. Milk Dealer, **38,** 8: 42–43, 98–100. May, 1949.

Tank truck hauling of milk from farm to plant is practiced on the West Coast, especially in California, where the milking herds are large. The Bryant and Chapman Dairy of Hartford, Conn., is successfully using the system with smaller herds. Before buying a tanker the processor should determine the number of producers on or near a good highway, accessibility of the milk-house for loading the tanker and to the highway, electric power for refrigeration, and whether or not the quality of the milk produced on a farm is sufficiently uniform so that it is safe to mix it with other milk. Methods of cooling and storing the milk on the farm are discussed. It is believed that this method of transporting milk from the farm will grow and spread and that it may, in time, revolutionize milk production by eliminating the small inefficient producer. The processors apparently like it because it gives them control of the quality of the milk and producers like it because they have control over weighing and the taking of samples. C. J. Babcock

719. Canned fresh milk is a fact. W. Rudolph. Am. Milk Rev., **11,** 5: 2, 3, 53. May, 1949.

A method for canning milk immediately after it is drawn from the cow and pasteurized is described as the Stambaugh-Graves method, named for the 2 men who developed the process. It includes drawing the milk by vacuum (milking machines) to a glass weighing jar, from whence additional vacuum draws it to a storage vat. The milk then is homogenized at drawing temperature, followed by preheating to 190° F. The next step is heating in a special-type exchanger to 260° F. for 19 sec., after which the milk is placed in lacquered cans. The filled cans are sterilized (no information on this process is given). Nitrogen gas is used to blanket milk that otherwise would be exposed to air. Economies in marketing milk are claimed if the pilot plant scale operation can be adapted to commercial scale production. Distribution to areas where milk is not readily available would be possible. D. J. Hankinson

720. Ready-to-use whipped cream in cans. Anonymous. Milk Dealer, **38,** 9: 46, 90–91. June, 1949.

The sale of whipped cream in single metal containers is producing a profit for a growing number of distributors of this product. Experiences in several markets are related. The mix is made up with cream, vegetable stabilizer, condensed skimmilk (for additional milk solids), sugar and pure vanilla for flavor. The mix is standardized to a 30% butterfat content and pasteurized the same as an ice cream mix is pasteurized. The whipped cream containers are a 12 oz. size and are filled with 7 oz. of mix. The can is then charged with a combination of nitrous oxide and carbon dioxide gas. C. J. Babcock

Also see abs. no. 651, 652, 658, 660, 670, 672, 679, 681, 682, 690, 691, 692.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

721. Use of L-thyroxine by mouth for stimulating milk secretion in lactating cows. G. L. Bailey, S. Bartlett and S. J. Folley. Nature, **163,** 4151: 800. 1949.

Daily oral dosages per cow of 25, 50, 100 and 150 mg. L-thyroxine and 1500 mg. iodo-casein resulted in a milk yield after 3 wk. of 1.6, 3.6, 5.9, 6.3 and 4.3 lb./cow/day. The heart rate increase after 3 wk. was 5.2, 8.7, 15.5, 21.5 and 11.2 beats/cow/min. The 150 mg. dosage appeared excessive as indicated by sweating of the cows after 2 wk. There is some indication that the published estimates of the thyroxine content of iodo-casein are in error or that the efficiency of oral utilization of thyroxine in iodo-casein is much lower than that of the hormone. The oral/parenteral ratio for L-thyroxine in the cow is about 16:1. R. Whitaker

722. Observations on the effects of prepartal and postpartal estrogens and progesterone treatment on lactation in the rat. S. M. Walker and J. I.

MATTHEWS, Washington Univ. School of Medicine, St. Louis. Endocrinology, **44,** 1: 8–17. Jan., 1949.

Attempts were made to inhibit lactation in intact and in ovariectomized rats using the growth rate of nursing young as a measure of milk secretion rate. Treatment with 200 γ of estrone or diethylstilbestrol dipropionate daily begun on 2nd d. of lactation did not inhibit secretion in ovariectomized rats; 5 and 10 γ daily of estrone inhibited lactation in intact rats 10 to 12 d. after the beginning of treatment. Lactation inhibition induced by 1 mg. of diethylstilbestrol dipropionate, both in intact and ovariectomized rats, was accompanied by marked loss of weight of the mothers and was interpreted as a toxic effect. Prepartal injection of estrone did not prevent the initiation of lactation in either intact (25 γ and 100 γ) or ovariectomized (25 γ) rats; it did produce a delayed depression in intact rats. Daily prepartal treatment with progesterone, 2.5 mg. and 5 mg., neither prevented nor inhibited lactation. Simultaneous injection of estrone and progesterone, begun either prepartally or postpartally, did not prevent lactation but it did inhibit established lactation after 10 to 12 d. of treatment. The stimulation of mammary growth by the combined action of estrone and progesterone was thought to play a role in the inhibition of milk secretion in the rat. R. P. Reece

723. Effects of restricted feed intake in intact and ovariectomized rats on pituitary lactogen and gonadotrophin. J. MEITES AND J. O. REED, Michigan State College, East Lansing. Proc. Soc. Exptl. Biol. Med., **70,** 3: 513–516. Mar., 1949.

Forty-eight intact female rats (200 g.) and 35 rats (230 g.) which had been ovariectomized about a month previous were used in the study. They were divided into 5 groups: *ad libitum,* ¾, ½, ¼ and no-feed regimes. The unfed groups were killed at the end of 7 d. and all other groups at the end of 14 d. Assays showed a pituitary lactogen content in intact and ovariectomized rats on the ½, ¼, and no-feed regimes below that of the ¾ and full-fed controls. No change was observed in the gonadotrophic content of the pituitaries of either the intact or ovariectomized rats, regardless of the level of feed intake. In the intact, but not in the ovariectomized rats, restricted feed intake caused a marked reduction in the weights of the pituitary, thyroid and adrenals, except that on the no-feed regime adrenal weight was increased in both groups. R. P. Reece

724. The effects of estrogen on mammary structure of adrenalectomized and thiouracil treated castrate rats. R. F. JOHNSTON AND J. F. SMITHCORS, Michigan State College, East Lansing. Endocrinology, **43,** 4: 193–201. Oct., 1948.

Seventy-two albino rats were castrated at 3 wk. of age and placed into 8 groups. They received, *ad libitum,* a ration for laboratory animals and were maintained at constant temperature (76° F.) and humidity. Adrenalectomy, thiouracil treatment and estrogen treatment either alone or in various combinations were carried out when the rats were of similar age. Comparison on the basis of estrogen treatment was made on litter mates. Adrenalectomized rats received drinking water that contained 1% NaCl. Thiouracil was fed at the rate of 0.1% in the feed for 45 d. The estrogen diethylstilbestrol was injected subcutaneously at the rate of 10 γ daily during the last 10 d. of the experimental period. The rats were sacrificed the day after the last injection. The right abdominal mammary gland was removed, stained in toto with Harris's hematoxylin, and examined. At autopsy examination was made for the presence of cortical tissue. Thiouracil feeding resulted in a shortened and thickened mammary duct system. Estrogen and thiouracil treatment produced a mammary gland showing shortened and thickened ducts and considerable lobule-alveolar development. Rats that had been adrenalectomized for 55 d. had long atrophic ducts. Estrogen treatment and adrenalectomy produced mammary glands that were extensive in area and lobule-alveolar development greater than that of estrogen-treated, castrated rats. Adrenalectomy and thiouracil feeding resulted in mammary glands with a very short atrophic duct system. The addition of estrogen resulted in a mammary gland with a short thick duct system and considerably more lobule-alveolar development than any other group. R. P. Reece

725. Changes in the distribution and concentration of alkaline phosphatases in tissues of the rat after hypophysectomy or gonadectomy, and after replacement therapy. E. W. DEMPSEY, R. O. GREEP AND H. W. DEANE, Harvard Univ., Cambridge, Mass. Endocrinology, **44,** 1: 88–103. Jan., 1949.

It was shown that after either hypophysectomy or gonadectomy, phosphatase persisted in the mammary glands of rats, although in reduced amounts. It was thought, therefore, that the enzyme activity of the mammary gland does not depend completely upon the hormonal stimuli emanating from the hypophysis or ovaries.

R. P. Reece

Also see abs. no. 704, 733.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

726. Comparative nutritive value of butter and

vegetable fats under conditions of low environmental temperature. B. H. ERSHOFF, J. N. PAGONES AND H. J. DEUEL, JR., Emory W. Thurston Lab. and Univ. of Southern California School of Medicine, Los Angeles. Proc. Soc. Exptl. Biol. Med., **70,** 2: 287–290. Feb., 1949.

The nutritive value of fats was determined under the stress of low environmental temperature. Immature female rats were raised to maturity in a large walk-in refrigerator at a temperature of $2 \pm 1.5°$ C. and under standard laboratory conditions at an average temperature of $21 \pm 2°$ C. Purified rations were fed differing only in source of fat. The fats employed were cottonseed oil, corn oil, margarine fat and butterfat. Body weight gain was significantly reduced in all rats under cold room conditions. The gains in body weight on the various diets were not significantly different, either under cold room or room temperature conditions. R. P. Reece

Also see abs. no. 666.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

727. The relative growth of the thyroid gland in the bovine fetus. C. W. NICHOLS, JR., I. L. CHAIKOFF AND J. WOLFF, Univ. of California Medical School, Berkeley. Endocrinology, **44,** 6: 502–509. June, 1949.

Thyroid gland growth, in relation to body weight, body length and age, was investigated in the bovine fetus from 62 d. to term. Fetal age was calculated from 4 parameters: (a) body weight, (b) crown-rump length, (c) chest circumference, and (d) abdominal circumference. A total of 121 bovine fetuses was obtained from dams chiefly of the Hereford breed. The simple allometry equation $y = bx^k$ was found to fit the data for thyroid gland growth in relation to body weight, body length and age. The relative growth constant (k) for thyroid weight against body weight was found to be 1.0, thus indicating that thyroid weight in the fetus was nearly proportional to body weight within the limits of the empirical formula. Percentage growth rates were found to decrease with increasing age. R. P. Reece

728. The accumulation of thyroxine-like and other iodine compounds in the fetal bovine thyroid. J. WOLFF, I. L. CHAIKOFF AND C. W. NICHOLS, JR., Univ. of California Medical School, Berkeley. Endocrinology, **44,** 6: 510–519. June, 1949.

A total of 96 thyroids was obtained from fetuses ranging in age from 53 d. to term. The fetuses were obtained from dams chiefly of the Hereford breed. Representative portions of most of the thyroid glands were hydrolyzed on a steam bath for 12 hr. in 2N NaOH. A suitable aliquot of the hydrolysate was analyzed for thyroxine-like and non-thyroxine iodine. Measurable amounts of iodine first were detected in the fetal thyroid at 60 d. of age. The iodine content of the fetal thyroid increased steadily with increasing body weight, crown-rump length and calculated age; this increase in iodine content was greater than could be accounted for by mere increase in thyroid mass. The percentage of total iodine present as inorganic was similar to that observed in the adult thyroid gland. Thyroxine-like iodine increased steadily in the gland and the rate at which this fraction accumulated was shown to bear an exponential relation to body weight, body length and calculated age. R. P. Reece

729. Oral effectiveness of d,l-thyroxine in crystalline, monosodium and disodium forms. R. A. MONROE, AND C. W. TURNER, Univ. of Mo., Columbia. Am. J. Physiol. **156,** 3: 381–386. Mar., 1949.

An alkaline solution of thyroxine is more readily absorbed when given orally than is thyroxine when administered in a solid form. Earlier work in comparing the oral effectiveness of various forms of thyroxine has been confusing because within given experiments the salts and crystalline form of thyroxine have not always been compared in the same physical state. The present authors eliminated these solubility differences by administering crystalline and the mono- and disodium salts of d,l-thyroxine all in the solid state. Work was done on the chick.

Using a biological assay already described (Mo. Res. Bull. 392) these workers found the mono- and disodium thyroxine possessed equal oral effectiveness and both of these forms were twice as active as the pure crystalline form. Approximately 20% of the crystalline form was absorbed and 45% of each of the sodium salts was absorbed. V. Hurst

730. Antithyroid activity of ergothioneine. M. L. WILSON AND D. A. MCGINTY, Parke, Davis, and Co., Detroit, Mich. Am. J. Physiol., **156,** 3: 377–380. Mar., 1949.

Ergothioneine, the methyl betaine of mercaptoimidazole, is a constituent of normal blood. There has been some evidence to show that it possesses an antithyroid activity.

The present authors compared the antithyroid activity of ergothioneine to thiouracil in the rat and monkey. Measurements in the rat included thyroid weight, thyroid I concentration, and the absorption of radioiodine by the thyroid glands.

In the monkey the absorption of radioiodine by the thyroid was studied. The ergothioneine, in large dosages, exhibited no antithyroid activity. V. Hurst

731. Effects of alloxan administration in the calf. E. L. McCandless and J. A. Dye, Cornell Univ., Ithaca, N. Y. Am. J. Physiol., **156,** 3: 355–360. Mar., 1949.

Four Guernsey bull calves, 2–3 wk. of age, were used in this experiment. Following a control period, the animals were injected intravenously with varying dosages of alloxan monohydrate (Eastman) in a 5% aqueous solution. Diabetes did not develop in these calves as measured by blood glucose. The beta cells of the islets of Langerhans, usually destroyed in other species by alloxan injections, were not injured in 2 of the 3 calves which survived the experiment. Severe renal damage was present in all animals. V. Hurst

732. Pancreatic diabetes in the calf. E. T. Cook, J. A. Dye and E. L. McCandless, Cornell Univ., Ithaca, N. Y. Am. J. Physiol., **156,** 3: 349–354. Mar., 1949.

Three male calves, 2–3 wk. of age, were used in these experiments. Preliminary determinations were made of blood and urine glucose, urinary nitrogen and urinary ketone bodies. The diet consisted of whole milk and this was supplemented with pancreatin (Merck) following pancreatectomy at 5–7 wk. of age. Pancreatectomy resulted in hyperglycemia which varied in direct proportion according to food intake. After the animals were fasted, hypoglycemia occurred more rapidly and was more extreme in depancreatized animals as compared to the controls.

Although both fed and fasted normal calves exhibited traces of glucose in the urine, the depancreatized calves showed high urine glucose levels which, however, fell sharply following fasting. Urinary nitrogen in both normal and depancreatized animals increased when they were fed, but following fasting the urinary nitrogen increased in the controls whereas it declined in the depancreatized calves. The increase in endogenous glucose from protein is slight in the depancreatized animals as compared to the controls, since the amount of nitrogen found in the urine is an index of the amount of gluconeogenesis taking place.

Gluconeogenesis can account for only a small portion of the hyperglycemia produced in the depancreatized calf, and the chief factor in producing diabetic hyperglycemia in the calf is decreased glucose utilization. Diabetes did not greatly increase the fat metabolism and glucose tolerance was lowered both by fasting and by pancreatectomy. V. Hurst

733. Vitamins A and C concentrations in the blood plasma of ewes, their milk, and in the blood plasma of their lambs. A. L. Pope, P. H. Phillips and G. Bohstedt, Univ. of Wisconsin, Madison. J. Animal Sci., **8,** 1: 57–66. Feb., 1949.

No significant drop in blood plasma Vitamins A and C immediately before or following parturition was noted in 18 grade ewes maintained on practical rations of alfalfa-brome grass hay and grain concentrate. Blood plasma levels of both these vitamins were highest during lactation. Blood plasma Vitamin A of lambs at birth averaged 6 γ/100 ml., increased to 20 γ with 30 hr. after birth, and ranged from 20–33 γ/100 ml. in the 13 wk. period following. Blood plasma Vitamin C was low in lambs at birth, decreased in the subsequent 4 d., then increased to a normal range.

Colostrum contained from 6 to 7 times as much Vitamin A as later milk. The Vitamin C content of colostrum did not vary from that of normal milk. No carotene was found in the plasma or colostrum of the ewes. F. C. Fountaine

734. Transmethylation of guanidoacetic acid in beef liver autolyzates. T. L. Sourkes. Cornell Univ. Arch. Biochem., **21,** 2: 265–272. Apr., 1949.

Beef liver blended in an ice water-toluene mixture and autolyzed at 20° C. for 20 hr. contains an enzyme system capable of forming creatine from guanidoacetic acid in the presence of methionine. Both ATP and oxygen are necessary for the synthesis of creatine. Boiled liver juice has an activating effect while sodium taurocholate and creatine inhibit the transmethylation of guanidoacetic acid. H. J. Peppler

735. A polysaccharide related to the blood group substances and its reaction with borate. I. A study by electrophoresis. L. E. Krejci, L. Sweeny and C. A. Zittle, Biochemical Research Foundation, Newark, Del. Arch. Biochem., **22,** 2: 253–361. June, 1949.

A polysaccharide isolated from calf intestinal mucosa reacts with borate solutions to form diol-borate compounds of increased acidity and optical activity. The electrophoretic mobility of the polysaccharide, serologically related to the blood group A substance and known to contain L-fucose and D-galactose, was determined in both borate-free solutions and in solutions of buffer salts wholly or partially replaced by borate-boric acid mixtures. Increases in mobility paralleled a sharpening of the electrophoresis boundaries. The concentration of diol-borate is dependent upon the concentrations of both polysaccharide and borate ions. The degree of ionization of the diol-borate compounds is affected by pH.

H. J. Peppler

736. A material in bovine stomachs related to blood group B substance. S. M. BEISER AND E. A. KABAT, Columbia Univ. and Presbyterian Hospital, N. Y. J. Am. Chem. Soc., **71,** 6: 2274. June, 1949.

As determined by hemagglutination-inhibition tests, substances with either blood group A, B, O, AO or BO can be obtained from different individual bovine stomachs. Analysis of purified substances reveals 5–7.2% nitrogen, 51–60% reducing sugar (as glucose after hydrolysis), 23–34% hexosamine and 1.5–5.2% methylpentose. Except for their higher content of methylpentose, hog and human substances have a similar composition. Blood group B activity was 1–5% the activity of B substance from human saliva or horse stomach. Extensive but incomplete cross reactions occurred between bovine B substances and anti-horse B, showing a higher capacity to precipitate anti-B than would be expected from observations of the hemagglutination-inhibition test. H. J. Peppler

737. The fractination of bovine serum proteins by electrophoresis-convection. J. R. CANN, R. A. BROWN AND J. G. KIRKWOOD, Calif. Inst. of Technol., Pasadena. J. Am. Chem. Soc., **71,** 5: 1609–1614. May, 1949.

The applicability of the electrophoresis-convection technique as a tool in the fractionation of naturally-occurring inhomogeneous proteins was demonstrated by the partial fractionation of bovine serum. γ-Globulins of 96% purity and β-globulins of 71% purity were separated from the fresh serum of a Hereford cow. Considerable separation of γ_1- and γ_2-globulin was also obtained. The electrophoresis-convection method is considered to be a fractionation tool of great importance, because large quantities of materials can be fractionated with high efficiency in a single run with relative ease of manipulation and economy of time. H. J. Peppler

Also see abs. no. 635.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

738. Report on sediment tests in milk and cream. C. R. JOINER, Food and Drug Admin., Federal Security Agency, St. Louis, Mo. J. Assoc. Offic. Agr. Chemists, **32,** 2: 324–330. 1949.

A modified method for the preparation of standard sediment discs using cow manure, garden soil and charcoal is proposed. It gave reproducible results with a given sediment mixture. Differences in appearance of pads prepared from sediment mixtures from different sections of the country were encountered. F. J. Babel

739. Report on DDT as spray residue on foods. R. H. CARTER, Bur. of Entomology and Plant Quarantine, Agr. Res. Admin., USDA, Beltsville, Md. J. Assoc. Offic. Agr. Chemists, **32,** 2: 353–359. 1949.

Two methods were recommended to be adopted tentatively for determination of DDT residues in plant and animal materials: (a) determination of total organic chlorine content by the sodium and isopropanol method, (b) colorimetric determination based on the nitration of the compound and development of a blue color by sodium methylate. A procedure is given for extraction of DDT from milk samples before using the regular procedures. F. J. Babel

740. Semi-micro phenol coefficient methods for testing quaternary ammonium disinfectants. G. S. WARNER AND M. J. PELCZAR, JR., Univ. of Md., and L. S. STUART, Production and Marketing Admin., USDA, Washington, D. C. J. Assoc. Offic. Agr. Chemists, **32,** 2: 401–408. 1949.

A semi-micro phenol coefficient method for determining germicidal potency of quaternary ammonium compounds is described. The method makes use of trypticase broth. Results show the minimum lethal concentration found by the semi-micro procedure was considerably lower than when the A.O.A.C. method was used. Critical quaternary ammonium germicide concentration killing times could be established more easily by the semi-micro procedure than by the A.O.A.C. technic. F. J. Babel.

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