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R. C. Foley

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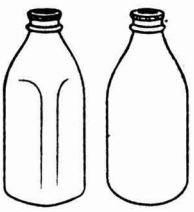

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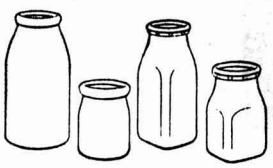

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A FILTRATION METHOD FOR DETERMINING SEDIMENT IN ROLLER PROCESS NONFAT DRY MILK SOLIDS

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INTRODUCTION

The relatively insoluble material in reconstituted roller process nonfat dry milk solids prevents the determination of sediment content, including scorched particles, by filtration through a standard lintine disc. The insoluble white protein material in reconstituted roller process nonfat dry milk solids appears as particles in suspension. These particles prevent filtration by covering the surface of the disc. As a substitute procedure, the tumbler method has been used. This method, as outlined by the American Dry Milk Institute (1), specifies that a reliquefied 25-g. sample shall be allowed to stand in a 20-oz. tumbler for 5 hr. After 5 hr., the amount of sediment on the bottom of the tumbler is visually compared with the ADMI Sediment Standards photoprint for the tumbler method.

Reports of work to establish a disc method for determining the sediment content, including scorched particles, of roller process nonfat dry milk solids were not found in the literature.

DEVELOPMENT OF TESTING PROCEDURE

These experiments were undertaken to develop a satisfactory filtration method for measuring the sediment, including scorched particles, in roller process nonfat dry milk solids. A preliminary investigation revealed that a 40 per cent sodium citrate solution, as outlined in "Standard Methods for the Examination of Dairy Products" (2) and such a solution in combination with a 10 per cent sodium hydroxide solution failed to give a filtrable solution of roller process nonfat dry milk solids. However, a filtrable solution of nonfat dry milk solids was produced by using a pepsin-hydrochloric acid solution. Investigation to determine the minimum digestion period, maximum temperature and minimum amount of solvent solution necessary to produce a filterable solution using 25-g. samples of roller processs nonfat dry milk solids, established the following testing procedure, hereafter referred to as the solvent-disc method, to be satisfactory:

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Mix 25 g. of sample with 100 ml. of pepsin-hydrochloric acid solution[1]; place in 45° C. water bath and hold 20 min.; add approximately 0.5 ml. of caprylic alcohol or diglycol laurate (defoaming agents); bring to a boil within 5 to 8 min. and filter immediately through a 1.25-in. lintine disc (waffle side up).[2] Rinse sample container with boiling, or near boiling, water and pass rinse through the filter disc. The temperature of the sample solution should not be allowed to drop prior to boiling or prior to filtering. In the absence of a standard more representative of the material in dried milks, the quantity of sediment, including scorched particles, in the sample is determined by comparing the disc with the USDA Sediment Standards for Milk and Milk Products.[3]

EXPERIMENTAL RESULTS

Spray process samples. In order to determine whether the regular disc method (reconstituted with warm water and filtered) and the solvent-disc method give a more accurate determination of sediment content than the tumbler method, the sediment content of 25-g. quantities of 40 samples of spray process nonfat dry milk solids was determined by the three methods.

Tumbler scores were recorded using the ADMI Sediment Standards[4] photoprint for the tumbler method and scored to the nearest tumbler represented on the photoprint. Discs were scored for total sediment to the nearest disc, using the USDA Sediment Standards.[5]

The results of tests of the 40 samples of spray process nonfat dry milk solids are shown in table 1.

The 12 samples having a no. 1 tumbler score had a sediment range by the regular disc method from 0.075 mg. to 1.00 mg. and by the solvent-disc method from 0.075 mg. to 0.5 mg. The 22 samples having a no. 2 tumbler score had a sediment range of 0.075 mg. to 2.50 mg. by the regular disc method and by the solvent-disc method 0.050 mg. to 1.00 mg.

Of the 40 duplicates 25-g. quantities, 20 showed slightly less sediment, and 19 showed the same amount of sediment, when filtered by the solvent-disc method as they did when they were filtered by the regular disc method. There were only four samples, however, that differed by more than 0.10 mg. of sediment. The results indicate that a slight loss of sediment may occur when the solvent-disc method is used instead of the regular disc method, but the loss is

[1] 10 g. of pepsin (powder 1: 3,000) dissolved in approximately 500 ml. of distilled water; 30 ml. of CP, HCl, 37%, specific gravity 1.1878 added and solution made up to 1,000 ml. with distilled water. Solution filtered. The pH of the solvent solution ranged from 0.5 to 0.7 during the experiment. Sample plus solvent pH ranged from 1.6 to 2.3.

[2] Aspirator was used in this experiment.

[3] The USDA Sediment Standards for Milk and Milk Products is made up of discs representing 0. mg.; 0.025 mg.; 0.050 mg.; 0.075 mg.; 0.10 mg.; 0.20 mg.; 0.30 mg.; 0.50 mg.; 1.00 mg.; and 2.50 mg.

[4] The ADMI Sediment Standards for the tumbler method as interpreted by using the USDA Sediment Standards would give the following approximate comparisons: Tumbler no. 1 = 0.075 mg.; no. 2 = 0.10 mg.; no. 3 = 0.3 mg.; no. 4 = 0.5 mg.; no. 5 = 1.00 mg.; no. 6 = 2.50 mg.

[5] See footnote 3.

TABLE 1

Comparative scores of 40 (25-g.) samples of spray process nonfat dry milk solids determined by the tumbler, disc and solvent-disc methods

Tumbler method	Disc method	Solvent-disc method
25-g. quantity	25-g. quantity	25-g. quantity
	(*mg.*)[a]	(*mg.*)[a]
No. 1		
(12 samples)	0.075	0.075
	0.075	0.100
	0.075	0.075
	0.075	0.075
	0.100	0.075
	0.100	0.075
	0.100	0.075
	0.100	0.100
	0.200	0.100
	0.300	0.200
	0.300	0.200
	1.000	0.500
No. 2		
(22 samples)	0.075	0.050
	0.075	0.075
	0.075	0.075
	0.075	0.075
	0.100	0.075
	0.100	0.075
	0.100	0.075
	0.100	0.075
	0.100	0.100
	0.100	0.100
	0.100	0.100
	0.100	0.100
	0.100	0.100
	0.200	0.100
	0.200	0.200
	0.200	0.200
	0.200	0.200
	0.300	0.200
	0.300	0.300
	0.300	0.300
	1.000	0.100
	2.500	1.000
No. 3		
(4 samples)	0.100	0.075
	0.300	0.200
	0.300	0.200
	0.500	0.200
No. 4	>2.500	>2.500
No. 6	>2.500	>2.500

[a] USDA Sediment Standards for Milk and Milk Products used.

not significant. The results also indicate that the two disc methods give comparable results and that both give a more accurate determination of sediment content than does the tumbler method.

Roller process samples. The sediment content of 25-g. quantities of 100 samples of roller process nonfat dry milk solids, representing the product from 16 different companies and 25 different plants was determined by use of the solvent-disc method and the tumbler method.

To determine whether the tumbler method shows the total sediment content of the sample, the following determinations were made: Each of the tumblers, after scoring, was siphoned off to within 0.5 in. of the bottom. It was found that the bulk of the insoluble white protein particles in reconstituted roller process nonfat dry milk solids remained in suspension in the upper portion of the tumbler. By siphoning off to within 0.5 in. of the bottom of the tumbler it was possible to remove the bulk of the insoluble protein material (other than sediment, including scorched particles). The top portion of each sample was mixed with 50 to 70 ml. of the pepsin-hydrochloric acid solution, after which the solvent-disc method was followed. By removing the bulk of the insoluble protein material, the bottom 0.5 in. of each sample could be mixed with 100 ml. of a hot, 10 per cent sodium citrate solution, brought to approximately 200 ml. with hot water and filtered (modified regular disc method). Sediment scores of the discs were determined for total sediment to the nearest disc by using the USDA Sediment Standards.

Results of tests of the 100 samples of roller process nonfat dry milk solids are shown in table 2. Only one of the 100 25-g. quantities was difficult to filter by using the established solvent-disc method. There is little correlation between the tumbler score and the quantity of sediment in the sample. As shown by the solvent-disc method, 45 of 76 samples that scored no. 1 or no. 2 by the tumbler method contained 1.00 mg. or more of sediment. In all tumbler scores, even no. 1 and no. 2, there were samples containing more than 2.50 mg. of sediment, as shown by the solvent-disc method. Table 2 also indicates that nearly all of the sedimenet content of the roller samples is found in the bottom 0.5 in. of the tumblers after the samples have been held for 5 hr. The ranges of sediment content of that portion of each sample which had settled to the bottom 0.5 in. of the tumbler as determined by a modified disc method was the same as that shown in 25-g. samples by the solvent-disc method. The small quantities of sediment found in the top portion of the tumblers indicate that a slight but insignificant amount of material is lost in the solvent-disc method. It is also indicated that most of the sediment settles to within 0.5 in. of the bottom of the tumbler but that the depth of vision is so limited that the sediment is not accurately determined.

To determine the depth of vision when scoring tumblers of roller samples, a red, glass-marking pencil was lowered to the bottom of tumbler samples until its point was in direct contact with the bottom of the tumbler. The pencil then was raised until the red point no longer was visible. The depth of vision as measured from the point of the pencil to the tumbler bottom was 1/16 to 1/8 of an inch.

To determine the extent that the pepsin-hydrochloric acid solution digests portions of the sediment, 10 samples of roller process nonfat dry milk solids were digested in this solution for 5 hr. at 45° C. The effect of this digestion is shown in table 3. Five hr. of digestion does not completely destroy the sedi-

TABLE 2

Comparison of scores of 100 (25-g.) samples of roller process nonfat dry milk solids determined by the tumbler method and solvent-disc method with the scores of the bottom 0.5 in. and top of the tumblers determined by the modified disc and solvent-disc methods

Tumbler method	Solvent-disc method	Modified disc method	Solvent-disc method
25-g. quantity	25-g. quantity	Bottom 0.5 in. of tumbler	Top of tumbler
	(*mg.*)[a]	(*mg.*)[a]	(*mg.*)[a]
No. 1			
(36 samples)	0.050	0.025	0.025
	0.075	0.050	0.025
	0.100	0.100	[b]
	0.200	0.200	0.025
	0.300	0.100	0.200
	0.300	0.200	0.100
	0.300	0.300	0.000
	0.300	0.300	0.025
	0.300	0.300	0.025
	0.300	1.000	0.025
	0.300	1.000	0.500
	0.500	0.300	0.100
	0.500	0.300	0.200
	0.500	0.300	0.200
	0.500	0.300	0.200
	0.500	0.500	0.025
	0.500	0.500	0.050
	0.500	0.500	0.100
	1.000	0.500	0.500
	1.000	1.000	0.025
	1.000	1.000	0.075
	1.000	1.000	0.075
	1.000	1.000	0.200
	2.500	2.500	0.075
	2.500	2.500	0.100
	2.500	2.500	0.100
	2.500	2.500	0.500
	2.500	2.500	0.500
	2.500	1.000	1.000
	>2.500	2.500	0.500
	>2.500	>2.500	0.500
	>2.500	>2.500	0.500
	>2.500	>2.500	0.500
	>2.500	>2.500	1.000
	>2.500	>2.500	1.000
	>2.500	>2.500	1.000
No. 2			
(40 samples)	0.100	0.100	0.000
	0.200	0.200	0.000
	0.200	0.300	0.000
	0.200	0.100	0.100
	0.200	0.200	0.000
	0.300	0.200	0.100
	0.300	0.300	0.000
	0.300	0.200	0.075
	0.500	0.300	0.100
	0.500	0.300	0.200
	0.500	0.300	0.200
	0.500	0.500	0.025

[a] USDA Sediment Standards for Milk and Milk Products used.
[b] Failed to filter.

TABLE 2—(*Continued*)

Tumbler method	Solvent-disc method	Modified disc method	Solvent-disc method
25-g. quantity	25-g. quantity	Bottom 0.5 in. of tumbler	Top of tumbler
	(*mg.*)[a]	(*mg.*)[a]	(*mg.*)[a]
	0.500	0.500	0.075
	0.500	1.000	0.000
	1.000	0.500	0.100
	1.000	0.500	0.100
	1.000	0.500	0.300
	1.000	0.500	0.500
	1.000	0.500	0.500
	1.000	1.000	0.000
	1.000	1.000	0.000
	1.000	1.000	0.025
	1.000	1.000	0.025
	1.000	1.000	0.075
	1.000	1.000	0.075
	1.000	1.000	0.100
	1.000	1.000	0.300
	1.000	1.000	0.300
	1.000	1.000	0.500
	1.000	2.500	0.025
	2.500	1.000	0.500
	2.500	1.000	0.500
	2.500	2.500	0.500
	2.500	2.500	1.000
	2.500	>2.500	0.200
	>2.500	1.000	1.000
	>2.500	>2.500	0.100
	>2.500	>2.500	0.300
	>2.500	>2.500	0.500
	>2.500	>2.500	0.500
No. 3 (15 samples)	0.500	0.500	0.200
	1.000	1.000	[b]
	1.000	1.000	0.100
	2.500	2.500	0.050
	2.500	2.500	0.075
	2.500	1.000	0.300
	2.500	2.500	[b]
	2.500	>2.500	[b]
	>2.500	>2.500	0.050
	>2.500	>2.500	0.075
	>2.500	>2.500	0.200
	>2.500	>2.500	0.300
	>2.500	>2.500	0.500
	>2.500	>2.500	1.000
	>2.500	>2.500	[b]
No. 4 (8 samples)	1.000	1.000	0.000
	2.500	1.000	1.000
	>2.500	>2.500	0.050
	>2.500	>2.500	0.075
	>2.500	>2.500	0.200
	>2.500	>2.500	0.300
	>2.500	>2.500	0.500
	>2.500	>2.500	0.500
No. 5 (1 sample)	>2.500	2.500	0.500

[a] USDA Sediment Standards for Milk and Milk Products used.
[b] Failed to filter.

TABLE 3

Effects of 5-hr. digestion at 45° C. on the sediment in 10 (25-g.) samples of roller process nonfat dry milk solids

Tumbler score[a]	Initial solvent-disc score	Disc score after 5 hours digestion[b]
	(*mg.*)[c]	(*mg.*)[c]
No. 1 (3 samples)	0.50	0.20
	1.00	0.30
	2.50	2.50
No. 2 (3 samples)	0.20	0.20
	0.50	0.50
	>2.50	2.50
No. 3 (2 samples)	>2.50	1.00
	>2.50	2.50
No. 4	2.50	0.50
No. 5	>2.50	2.50

[a] 25-g. quantities, ADMI tumbler method followed and ADMI tumbler standard used.
[b] Duplicate 25-g. quantities mixed with 100 ml. of pepsin-hydrochloric acid solution and placed in 45° C. water bath for 5 hr.
[c] USDA Sediment Standard for Milk and Milk Products used.

ment. Samples showing varying quantities of initial sediment still show sediment after 5 hr.

Microscopic examination. Microscopic examination showed the same types of sediment on the discs of spray process samples filtered by use of the regular disc method as those filtered by the solvent-disc method. Microscopic examination of roller process samples also showed the same types of sediment on the discs of the bottom 0.5 in. of the tumblers and discs of the 25-g. duplicate quantities filtered by the solvent-disc method.

An examination of 60 discs representing both spray and roller nonfat dry milk solids showed that in all instances, a very high percentage of the sediment content was made up of scorched and burned protein particles. After 5 hr. of digestion, microscopic examination revealed the same types of sediment to be present.

COLLABORATIVE STUDIES

Army Medical Center. To obtain further information and verification of results, a collaborative study was conducted with the Veterinary Section, Army Medical Laboratory, Army Medical Center, Washington, D. C. Forty-eight samples of roller process nonfat dry milk solids representing 44 different plants were tested for sediment content by using the tumbler and solvent-disc methods. The same testing and grading procedures as previously outlined were used. Results of tests of the 48 samples of roller process nonfat dry milk solids are shown in table 4. There is little correlation between the tumbler score and the quan-

TABLE 4

Comparison of scores of 48 (25-g.) samples of roller process nonfat dry milk solids determined by the tumbler and solvent-disc methods

No. of samples	Tumbler method 25-g. quantity	Solvent-disc method 25-g. quantity (*Mg.*)[a]						
		0.10	0.20	0.30	0.50	1.00	2.50	>2.50
9	No. 1	1	2	5	..	1	..	..
19	No. 2	..	..	..	5	9	3	2
7	No. 3	..	..	..	..	3	1	3
6	No. 4	..	..	..	..	1	2	3
2	No. 5	..	..	..	..	..	..	2
5	No. 6	..	..	..	..	..	1	4

[a] USDA Sediment Standards for Milk and Milk Products used.

tity of sediment in the samples. In tumbler score no. 1, there is one sample containing 1.00 mg. of sediment, and in tumbler score no. 2, 14 of the samples contain 1.00 mg. or more of sediment as determined by the solvent-disc method.

American Dry Milk Institute. To determine the practical application and the reproducibility of the solvent-disc method, the American Dry Milk Institute sponsored a project in which the Dairy Branch, Veterinary Section of the Army Medical Center Laboratory, American Dry Milk Institute Laboratory, and seven commercial laboratories cooperated. Samples for testing were collected by the American Dry Milk Institute and distributed to the participating laboratories. The authors tested 217 samples and approximately like quantities were tested by the other laboratories. At a meeting of representatives of the American Dry Milk Institute, the Army Medical Center and the Dairy Branch, the results of this project were presented. It was agreed that the results indicated that the solvent-disc method was accurate and reproducible.

SUMMARY AND CONCLUSIONS

A filterable solution of roller process nonfat dry milk solids was produced by using a pepsin-hydrochloric acid solution as a solvent. The established procedure of using 25 g. of sample mixed with 100 ml. of the solvent, heating for 20 min. at 45° C., bringing to a boil and filtering, produced satisfactory results with 99 out of 100 samples of roller process nonfat dry milk solids.

The application of the regular disc method and the solvent-disc method to 40 samples of spray process nonfat dry milk solids showed that the two methods gave comparable results on duplicate samples. The loss of sediment by digestion in the solvent-disc method was insignificant. Only four samples differed by more than 0.10 mg. in sediment content when tested by the regular disc and solvent-disc methods. Both disc methods gave a more accurate picture of sediment content than did the tumbler method.

There was little correlation between the tumbler scores of 100 samples of roller process nonfat dry milk solids and the sediment content of the samples. The 76 samples that scored no. 1 or no. 2 ranged in sediment content from 0.50 mg. to over 2.50 mg. Of these 76 samples, 45 contained over 1.00 mg. of sediment when scored by the solvent-disc method.

Microscopic examination of the discs revealed comparable types of sediment by all methods. Discs of samples of both spray and roller process nonfat dry milk solids showed that in all instances a very high percentage of the sediment content was made up of scorched and burned protein particles.

The solvent-disc (pepsin-hydrochloric acid solution) method has been found satisfactory for determining the sediment content, including scorched particles, of roller process nonfat dry milk solids by filtration through a standard lintine disc.

Collaborative studies with the Veterinary Section, Army Medical Laboratory, Washington, D. C., and in cooperation with the American Dry Milk Institute established that the solvent-disc method is accurate and reproducible.

ACKNOWLEDGMENT

The authors wish to express their appreciation for the technical assistance of H. A. Barron, Chemist, Dairy Branch Laboratory, PMA, Chicago, Ill.

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(2) AMERICAN PUBLIC HEALTH ASSN. Standard Methods for the Examination of Dairy Products, 9th ed., par. 9.17, New York, 1948.

THE EFFECT OF RAW SOYBEANS ON BLOOD PLASMA CAROTENE AND VITAMIN A AND LIVER VITAMIN A OF CALVES[1]

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In previous reports (7, 8) it was noted that cows receiving during the prepartal period a concentrate ration containing 40 per cent raw soybeans developed signs and/or symptoms of a vitamin A deficiency of greater severity than was expected on the basis of the carotene intake. Since Hilton *et al.* (1) had shown that the feeding of soybean hay to cows depressed the vitamin A of the butter, it was suspected that soybeans might also exert an influence on the vitamin A requirements of cattle.

The data presented herein are the results of an experiment designed to determine the possible effect of the feeding of soybeans upon the blood plasma carotene and vitamin A and liver vitamin A of calves on a known carotene intake. A brief resumé of the plasma and liver vitamin A data, but not the carotene data has been published (8).

EXPERIMENTAL

Four calves, two Ayrshires and two Holsteins, approximately 14 wk. of age were placed on a vitamin A deficient ration. They were considered to be depleted when the blood vitamin A had decreased to less than 5 γ per 100 ml. of blood plasma. This level was reached after they were on the vitamin A deficient ration for 35 days. Each calf then was given slightly more than the minimum requirement (4) or 32 γ of carotene per pound of body weight per day in the form of alfalfa leaf meal for the remainder of the experiment. At the end of 49 days, the calves were divided into two groups with a Holstein and Ayrshire calf in each group. Group I was continued on the basal ration and group II was changed to the test ration which contained approximately 30 per cent ground raw soybeans. This period of 170 days will be referred to as the test period. The rations are shown below:

Ingredient	Basal ration	Soybean ration
	(*lb.*)	(*lb.*)
Barley	240	100
Oats	180	180
Wheat Bran	180	180
Linseed meal	60	.....
Soybeans	.....	200
Salt	8	8

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1 Paper no. A294, contribution no. 2244 of the Maryland Agricultural Experiment Station.

Seventy-five grams of D-activated plant sterol were added to each ration. In addition, each calf received 1 lb. of skimmilk daily. Clean shavings were used for bedding and were kept in the feed boxes at all times as the source of roughage. No attempt was made in these studies to balance the fat content of the two rations. The calves were fed according to Morrison's standards for growing dairy cattle (6).

The calves were weighed at weekly intervals and the feeding was adjusted on the following day. The alfalfa leaf meal was analyzed for carotene at weekly intervals. Blood samples for the determination of blood plasma carotene and vitamin A were drawn at 7 a.m. once each week before the feeding of alfalfa leaf meal. Spinal fluid pressure measurements were made 33, 48 and 104 days after soybeans were added to the ration.

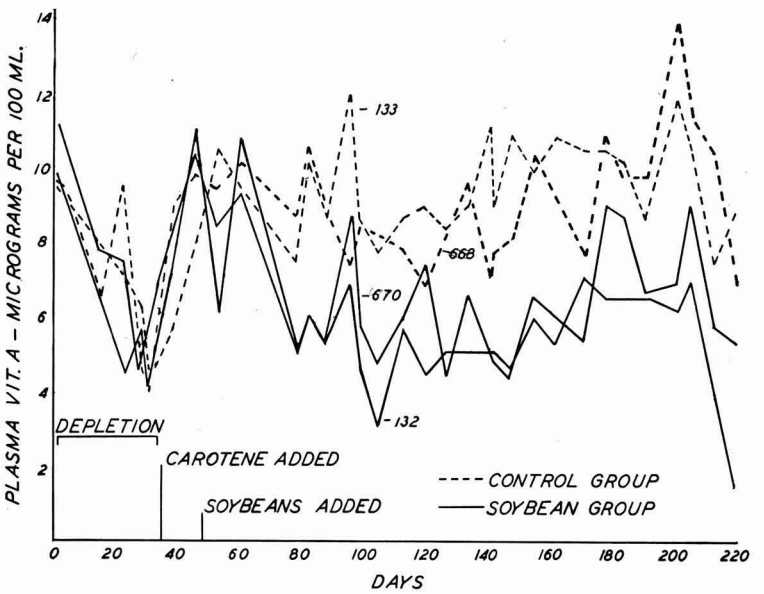

FIG. 1. The effect of the feeding of soybeans upon the plasma vitamin A of calves.

At the end of the test period the calves were slaughtered and the livers were mascerated and analyzed for vitamin A, using an unpublished method of H. G. Wiseman of the Bureau of Dairy Industry. A modification of the procedure of Moore (3) and of Kimble (2) was used for plasma vitamin A and carotene. The carotene content of the alfalfa leaf meal was determined by the method of Moore and Ely (5). The spinal fluid pressure technique was that of Sykes and Moore (10).

RESULTS

The plasma vitamin A and carotene are shown graphically in figures 1 and 2 for the depletion period, for the 2-wk. period of carotene addition without soy-

beans and for the remainder of the test period during which two of the calves received soybeans in the ration. It will be observed that both the plasma carotene and vitamin A values were lower in the two calves that received soybeans; the vitamin A values were affected much more than the plasma carotene. The

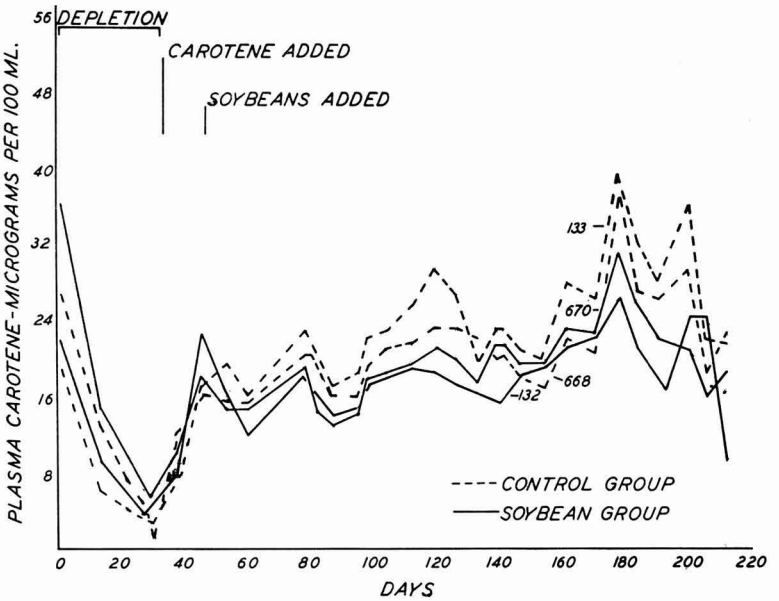

FIG. 2. The effect of the feeding of soybeans upon the plasma carotene of calves.

reason for the marked decline in blood plasma carotene and vitamin A at 220 days was not apparent. A new lot of alfalfa leaf meal was used at this time and it is possible that some error was made in the analysis of the meal or in feeding.

TABLE 1

The effect of the feeding of soybeans upon the plasma carotene and vitamin A of calves

Ration	Calf	No. of analyses	Plasma carotene	Plasma vitamin A
			(γ/100 ml.)	(γ/100 ml.)
Control	133A	25	25.68 ± 8.33	9.60 ± 1.26
Control	668H	25	22.40 ± 6.83	9.13 ± 1.58
Soybean	132A	25	18.90 ± 4.93	5.64 ± 1.68
Soybean	670H	25	17.18 ± 5.22	6.46 ± 1.54

The average plasma carotene and vitamin A values during the test period are shown in table 1. The lower plasma carotene and vitamin A values of the soybean fed calves were found to be highly significant when compared to the control calves. The F values for between groups for plasma carotene and vitamin A

were 7.875 and 48.219, respectively, whereas, Snedecor's (9) value at the 1 per cent level is 6.90. The greater effect upon plasma vitamin A is reflected in a much larger F value for between groups for plasma vitamin A than for carotene. There was no significant difference between calves within groups for either plasma carotene or vitamin A.

The vitamin A content of the livers of the four calves at the end of the test period is shown in table 2. The difference is even more striking than in the case of plasma vitamin A, the two calves on the soybean ration having 0.72 and 2.75 γ of vitamin A per gram of liver and the two controls 9.07 and 9.22 γ per gram.

TABLE 2

The effect of the feeding of soybeans upon the liver vitamin A of calves

Ration	Calf	Weight of liver	Vitamin A in liver (wet basis)
		(*kg.*)	($\gamma/g.$)
Control	133A	3.0	9.07
Control	668H	3.5	9.22
Soybean	132A	3.7	0.72
Soybean	670H	3.5	2.75

Spinal fluid pressure measurements were made during the test period. These are recorded in table 3. It will be noted that all of the values are within the normal range as established by Moore *et al.* (4).

TABLE 3

Spinal fluid pressure of experimental calves

Ration	Calf	Spinal fluid pressure during test period		
		33rd d.	48th d.	104th d.
		($mm. H_2O$)	($mm. H_2O$)	($mm. H_2O$)
Control	133A	80	95	85
Control	668H	75	75	100
Soybean	132A	75	80	80
Soybean	670H	80	110	100

DISCUSSION

The marked depression in the plasma and liver vitamin A produced by the feeding of ground raw soybeans to calves on an adequate but low intake of carotene suggests that difficulties may be encountered in feeding soybeans to cattle that are on a relatively low carotene ration. Such conditions could be encountered when rather large amounts of soybeans are added to the ration of cattle receiving a rather poor quality timothy hay as the main source of carotene.

Squibb *et al.* (10) fed soybeans to cows and reported a depression in plasma carotene but only a slight depression in plasma vitamin A which was quite different from our earlier report (8) and from the data in this paper. However, their cows received relatively large and varying amounts of carotene and no attempt was made to determine the actual carotene intake.

No increase was observed in the spinal fluid pressure values. However, the low liver vitamin A values indicate considerable depletion. It appears that the experiment may not have been continued for a sufficient length of time or that the calves may have been a little too old at the beginning of the experiment for the effect of the vitamin A depletion to be refleced in an increase in spinal fluid pressure.

CONCLUSIONS

The addition of 30 per cent ground raw soybeans to the ration of two calves when compared with two control calves decreased both the plasma and liver vitamin A markedly. Plasma carotene also was decreased but not to as great an extent as plasma vitamin A. The spinal fluid pressure measurements were all within the normal range.

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A STUDY OF THE EFFECT OF NORDIHYDROGUAIARETIC ACID ON THE OXYGEN ABSORPTION OF THE PHOSPHOLIPID FRACTION OF MILK. I. THE EFFECT OF CONCENTRATION OF ANTIOXIDANT[1, 2]

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It has been shown by investigators (5, 10, 11) that the oxidation of the phospholipid fraction of milk is one factor responsible for the development of oxidized flavors in dairy products.

The role which each of the other constituents of milk fat has in the development of oxidized flavor is not entirely understood, even though considerable progress has been made in studying various aspects of the problem. This problem is complicated by the relationship between the oxidative changes which occur in the milk fat and the influence of other milk constituents, especially ascorbic acid.

In this investigation a study has been made of the effect of the antioxidant nordihydroguaiaretic acid (NDGA) on the oxygen absorption of the phospholipid fraction of milk.

EXPERIMENTAL METHODS

Isolation of the phospholipid fraction from milk. The milk product used for isolating the phospholipid fraction of milk was either dried, washed-cream buttermilk or condensed, washed-cream buttermilk.

The procedure for dried washed-cream buttermilk was as follows: (a) Milk produced by the University herd during the months of May and June was separated at 27° C. into skimmilk and cream. The cream tested approximately 45 per cent fat. The products were handled in stainless steel or aluminum equipment. (b) The cream was pasteurized at 66° C. for 30 min., cooled to 4° C. and held overnight at near this temperature. (c) The cream was diluted with four times its weight of distilled water at 4° C. and mixed well but gently. (d) The diluted cream was reseparated into cream containing approximately 33 per cent fat. This was called washed cream. It was cooled to 4 to 5° C. and held at this temperature for 12 hr. (e) The cream was churned at 10° C. and the buttermilk was saved. This was called washed-cream buttermilk. (f) The buttermilk was preheated to 49° C. and condensed in a 16–in. stainless steel vacuum pan at 26 to 27 in. of vacuum (43 to 49° C.) to as high a content of total solids as possible.

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1 The data published here are taken from a thesis presented by the senior author to the Faculty of the Graduate School, University of Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1950.

2 This investigation was aided by a grant from the Wm. J. Stange Co., Chicago, Ill.

3 Now at the University of Arizona, Tucson.

The extraction of the phospholipid fraction from the washed-cream buttermilk which had been dried by lyophilization was accomplished in the following manner: (a) The powder was extracted by shaking with ethyl ether. Eight extractions were made by shaking for 30 min. each time and allowing the ether layer to rise and then siphoning it off. The first extraction was made with 9.0 ml. of ether per gram of powder, the second, third, fourth and fifth each with 2.0 ml. per gram, and the remaining extractions with 1.5 ml. of ether per gram of powder. (b) The ethereal extracts were combined and the volume reduced *in vacuo* at 30° C. to one-tenth the original volume. (c) Four volumes of acetone were added at 4° C. and the mixture was allowed to stand at that temperature overnight. (d) The supernatant acetone-ether mixture was siphoned off. (e) The precipitate was washed six times by decantation, each time with six volumes of acetone at 4° C. (f) The phospholipid fraction was allowed to remain in acetone at 4° C. under an atmosphere of nitrogen until it was to be used. (g) Immediately before using, it was filtered by reduced pressure under a stream of nitrogen and the last traces of acetone were removed *in vacuo* at 20° C.

In the second procedure, the phospholipid fraction was isolated from condensed washed-cream buttermilk by, essentially, the procedure of Swanson and Sommer (9), except that NH_4OH was omitted from the extraction. The procedure was as follows: (a) To each liter of buttermilk was added 1 l. of 95 per cent ethyl alcohol, 1.6 l. of ethyl ether and 1.6 l. of petroleum ether. The mixture was shaken for 1 to 2 min. after the addition of each solvent and then it was allowed to stand. The supernatant liquid then was siphoned off. (b) The extraction was repeated with 1 l. each of ethyl ether and petroleum ether per liter of buttermilk. (c) The extracts were combined and the volume was reduced *in vacuo* at 30° C. to one-tenth the original volume. (d) Four volumes of acetone were added at 4° C., and the procedure was carried out from this point as in the case of the isolation from lyophilized buttermilk.

The supply of phospholipid material isolated from lyophilized buttermilk was depleted near the end of this study. Since more material was needed and in order to save time, the phospholipid was isolated directly from washed-cream buttermilk (9). The fractions isolated from the lyophilized buttermilk and from condensed buttermilk were of comparable compositions, as indicated by iodine numbers of 35.64 and 35.86, nitrogen contents of 1.70 and 1.73 per cent and phosphorous contents (2) of 3.81 and 3.75 per cent, respectively. The yields were also practically the same (table 1).

Measuring oxygen absorption. The first use of the Barcroft-Warburg apparatus for measuring oxygen uptake of fatty materials was made by Jany in 1931 (3). The measurement of changes in oxygen concentration in the atmosphere surrounding dairy products had been chiefly accomplished by using apparatus such as the Van Slyke manometer. Overman *et al.* (8) measured oxygen absorption in butter with a specially constructed manometer.

In this study, the amount and rate of oxygen absorption was measured with

TABLE 1

The yield and composition of the phospholipid fraction of milk isolated from lyophilized and condensed washed cream buttermilk

	Washed cream buttermilk	
	Lyophilized	Condensed
Wt. of milk used (*lb.*)	1962	2000
Fat (%)[a]	4.0	4.0
Wt. of washed cream (*lb.*)	217.0	257.0
Fat (%)[a]	35.0	35.0
Wt. of washed cream buttermilk (*lb.*)	128.0	156.0
Fat. (%)[b]	0.81	1.09
Total solids (%)[b]	2.25	2.19
Wt. of condensed washed cream buttermilk (*lb.*)	23.0	26.5
Fat (%)[b]	4.06	5.98
Total solids (%)[b]	10.08	12.10
Wt of lyophilized washed cream buttermilk (*g.*)	1090	.....
Fat (%)[b]	28.57	.....
Water (%)[b]	4.01	.....
Wt. of phospholipid fraction (*g*)	51.0	49.5
Nitrogen (%)[c, d]	1.73	1.70
Phosphorous (%)[c]	3.81	3.75
Hanus iodine number[c]	35.64	35.86

[a] Babcock method.
[b] Mojonnier method.
[c] Average of duplicates.
[d] Semi-micro Kjeldahl method.

a Barcroft-Warburg respirometer. Fifteen-ml. flasks with one side arm were used. All measurements were made at 50° C. A preliminary experiment showed that 1 gr. of phospholipid in an atmosphere of air did not give a satisfactory rate of oxygen absorption. For this reason, an atmosphere of oxygen (1, 4, 7) with a gassing manifold similar to the one described by Umbreit *et al.* (12) was used. Tank oxygen was allowed to flow through the flasks at a pressure of 8 lb. per in. for 3 min. The flasks then were sealed immediately with Apiezon Type N stopcock grease obtained from the James G. Biddle Co., Philadelphia, Pa.

The detection of the presence of oxidized flavor. Oxidized flavor was detected by combining the contents of the absorption flask with good-flavored homogenized whole milk containing 3.9 per cent fat. The contents were mixed with the milk at 32° C. in a Waring blendor for 15 sec. An amount of milk was used which gave an added phospholipid content of 0.04 per cent. The flavor was determined at the end of the reaction.

EXPERIMENTAL RESULTS

The oxygen absorption of the phospholipid fraction of milk with and without added copper and NDGA. In this and succeeding portions of the study, 0.5 g. of phospholipid was weighed directly into the absorption flask. The concentrations of the various solutions or suspensions were adjusted so that 3.0 ml. of liquid were added to the flask. The concentrations of the materials are expressed on the basis of the weight of the phospholipid. Copper was added as $CuSO_4$ at a concentration of 1.0 ppm., unless otherwise specified.

Figures 1, 2 and 3 show the oxygen absorption curves for the phospholipid fraction with and without added copper and with varying concentrations of NDGA. Figure 1 shows the oxygen absorption of the phospholipid with and without copper and NDGA. The induction phase of oxygen absorption in the case of the phospholipid fraction without NDGA is practically non-existent during the time interval of this experiment. In the presence of 0.005 per cent NDGA, the oxygen absorption was greatly retarded, even in the presence of 1.0 ppm. copper. In the absence of NDGA,, oxidized flavor was detected at the end of 300 min., while in the phospholipid containing NDGA it was absent.

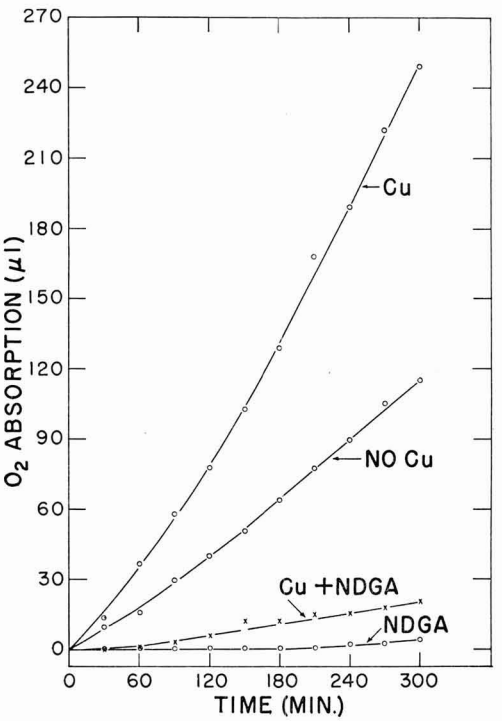

FIG. 1. The oxygen absorption of the phospholipid fraction of milk with and without 1.0 ppm. Cu and 0.005% NDGA.

Figure 2 shows the oxyen absorption curves for the phospholipid fraction in the presence of 1.0 ppm. copper and concentrations of NDGA up to 0.004 per cent. Concentrations of the antioxidant of 0.0001, 0.0002 and 0.0004 per cent have little, if any, antioxidant activity. The range of activity is somewhere between 0.0004 and 0.0001 per cent NDGA. Oxidized flavor was detected in the phospholipid containing no NDGA and also in that containing 0.0001, 0.0002 and 0.0004 per cent of the antioxidant.

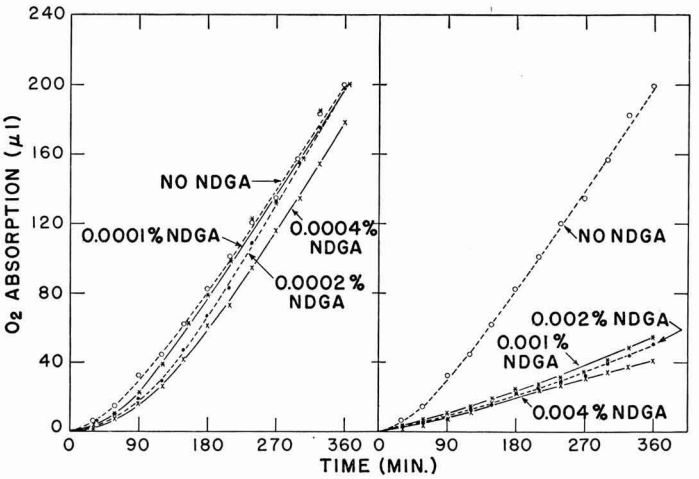

FIG. 2. The oxygen absorption of the phospholipid fraction of milk with 1.0 ppm. Cu together with NDGA at concentrations of 0.0001, 0.0002, 0.0004, 0.001, 0.002 and 0.004%.

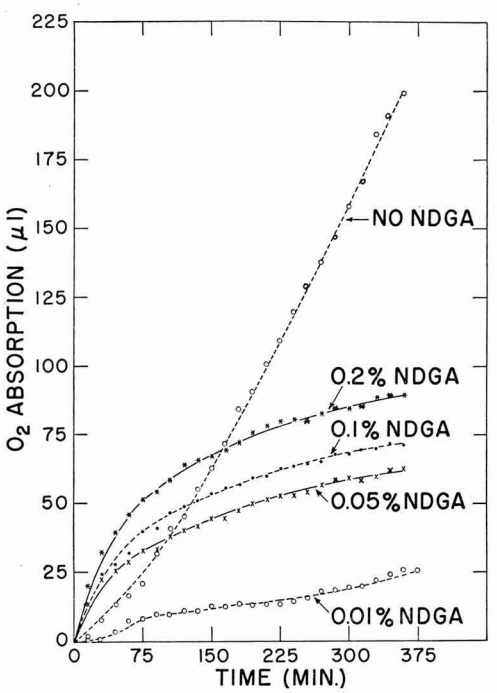

FIG. 3. The oxygen absorption of the phospholipid fraction of milk with 1.0 ppm. Cu together with NDGA at concentrations of 0.01, 0.05, 0.1 and 0.2%.

Figure 3 shows the oxygen absorption curves for the phospholipid fraction in the presence of 1.0 ppm. added copper and concentrations of 0.01, 0.05, 0.1 and 0.2 per cent NDGA. The curves for the concentrations of 0.05, 0.1 and 0.2 per cent indicate that oxygen absorption proceeded at an increasing rate at the beginning, followed by absorption at a decreasing rate. Lundberg *et al.* (6) observed that increased concentrations of antioxidants tended to accelerate the formation of peroxides during the early stages of oxidation. Because of increased oxygen absorption at the higher concentration of NDGA, a very slight oxidized flavor was detectable in the phospholipid containing 0.2 per cent NDGA when it was added to homogenized milk at a concentration of 0.04 per cent. No oxidized flavor was detected in the phospholipid containing 0.01, 0.05 and 0.1 per cent NDGA.

CONCLUSIONS

The optimum concentration of NDGA for the most effective antioxygenic action in the phospholipid fraction was from 0.001 to 0.01 per cent.

Concentrations of NDGA below 0.001 per cent were too low to be effective and concentrations of 0.05 per cent and above accelerated the oxygen absorption at the beginning of the oxidation reaction.

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A STUDY OF THE EFFECT OF NORDIHYDROGUAIARETIC ACID ON THE OXYGEN ABSORPTION OF THE PHOSPHOLIPID FRACTION OF MILK. II. THE EFFECT OF SYNERGISTS, METALLIC CATALYSTS AND pH[1, 2]

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In a study of the synergistic action of several materials, Clausen *et al.* (1) found that methionine and ascorbic acid were two of the most effective synergists. Citric acid is used commercially as a synergist with antioxidants. For this reason, it was thought to be important to study the synergistic action of methionine, ascorbic acid and citric acid with the antioxidant nordihydroguaiaretic acid (NDGA) in the phospholipid fraction of milk. At the same time it was determined whether or not NDGA alone or together with a synergist such as citric acid would retard oxygen absorption in the phospholipid fraction when the antioxidant and synergist are added to the system after the oxidation reaction has been allowed to proceed for a period of time.

Veno and Saida (6) made a study of the effect of several metallic "catalyzers" on the oxygen absorption of unsaturated oils. They studied vanadium, cobalt, nickel, copper, manganese, iron and lead, all as the linoleates. In this investigation the catalytic effect of copper, nickel, iron and cobalt were studied as the sulfates.

It has been shown that NDGA is not a very effective antioxidant in foods or materials which have an alkaline reaction (3, 4). There are no reports in the literature to show the effect of a low pH on the activity of NDGA. For these reasons, it was decided to study the effect of pH on the oxygen absorption of the phospholipid fraction in the presence of NDGA.

EXPERIMENTAL METHODS

The methods used in this study were the same as those used in the previous investigation (5). In the portion dealing with synergists and metallic catalysts, NDGA was added at a concentration of 0.0008 per cent. The synergists were added at a concentration of 0.01 per cent and the catalysts at 1.0 ppm. In the part of the study involving pH, the systems were buffered with McIlvane's standard buffer solutions (2) which were made with citric acid and sodium phosphate and with which it is possible to buffer in the range of pH 3.0 to 8.0. In this part of the study, 0.005 per cent NDGA was used. To study the retarding effect of NDGA and citric acid on oxygen absorption which has been allowed to proceed for a period of time, the antioxidant suspensions and citric

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[1] The data published here are taken from a thesis presented by the senior author to the Faculty of the Graduate School, University of Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1950.

[2] This investigation was aided by a grant from the Wm. J. Stange Co., Chicago, Ill.

[3] Now at the University of Arizona, Tucson.

acid solutions were placed in the side arms of the reaction flasks and then tipped into the phospholipid suspensions at the end of 1, 2, 3, 5 and 7 hr. NDGA was added at a concentration of 0.005 per cent and citric acid at 0.01 per cent.

EXPERIMENTAL RESULTS

The effect of synergists on the antioxygenic action of NDGA. Figure 1 shows the oxygen absorption curves of the phospholipid fraction with and without added copper and with the synergists methionine and citric acid. The results show that methionine had practically no antioxidant action while citric acid had a very slight antioxidant effect. In all cases, the phospholipid had a detectable oxidized flavor.

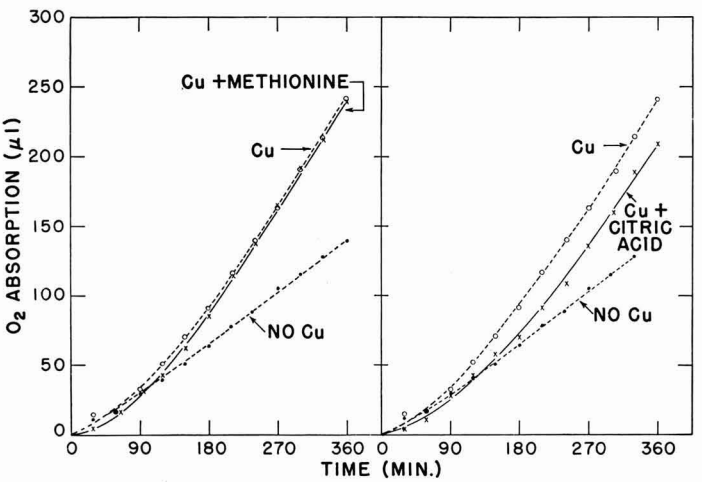

FIG. 1. The effect of 0.01% methionine and citric acid on oxygen absorption of the phospholipid fraction of milk with and without 1.0 ppm. added Cu.

Figure 2 shows the oxygen absorption curves of the phospholipid fraction with and without added copper and the synergist ascorbic acid. Included are the curves for the oxygen absorption of the phospholipid with and without copper, NDGA and the synergist methionine. Oxidized flavor was detected in the phospholipid fraction which contained added ascorbic acid but the flavor was not detected in that which contained added NDGA and methionine.

Figure 3 shows the oxygen absorption curves for the phospholipid fraction with and without added copper and NDGA and the synergists citric acid and ascorbic acid. At the end of 360 min., there was neither oxygen absorption nor detectable oxidized flavor in the phospholipid fraction containing copper, NDGA and either synergist.

The retarding effect of NDGA and citric acid on oxygen absorption which has been allowed to proceed for a period of time. The data shown in figures 4 and 5 represent the oxygen absorption curves for the phospholipid with NDGA

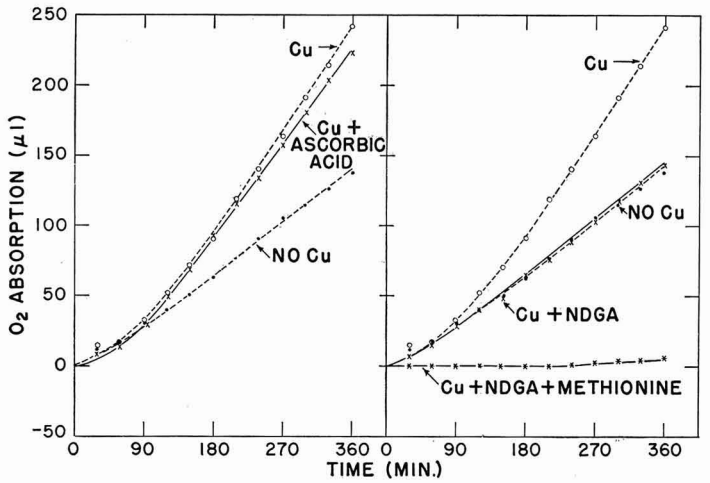

FIG. 2. The oxygen absorption of the phospholipid fraction of milk with 1.0 ppm. cu and 0.01% ascorbic acid and with 1.0 ppm. Cu, 0.0008% NDGA and 0.01% methionine.

and NDGA and citric acid added at the beginning (0 hr.) and at the end of 7 hr., respectively.The oxygen absorption curves for the phospholipid to which the antioxidant and synergist were added at the end of 1, 2, 3, and 5 hr. showed rates of change similar to those in figure 5. In each case, the break in the curve occurred in 30 to 60 min. after the addition of the NDGA and citric acid.

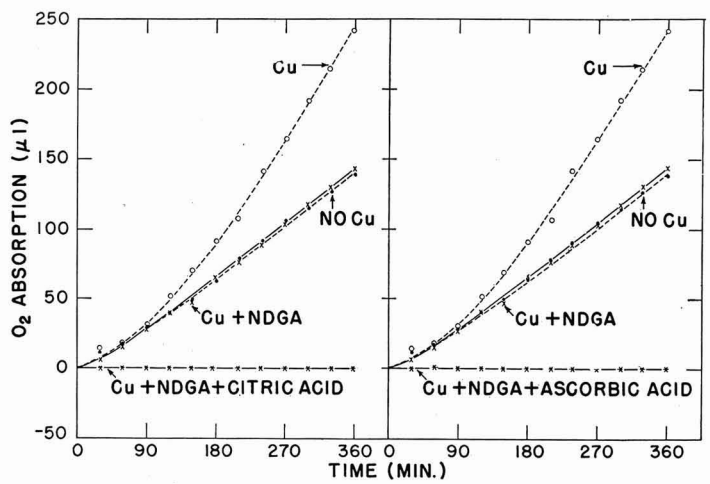

FIG. 3. The effect of 0.01% citric acid and ascorbic acid on the phospholipid fraction of milk with and without 1.0 ppm. Cu and 0.0008% NDGA.

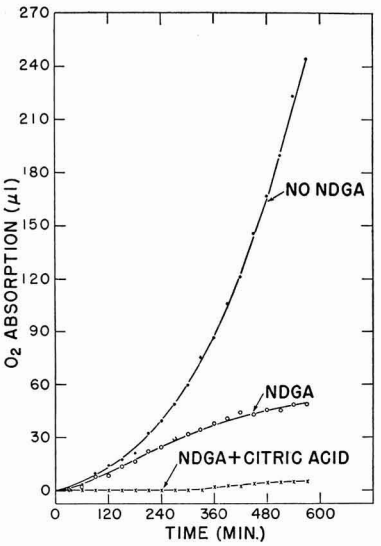

FIG. 4. The oxygen absorption of the phospholipid fraction of milk as influenced by the addition of 0.005% NDGA alone and together with 0.01% citric acid at the beginning of the oxidation reaction. (1.0 ppm. added Cu.)

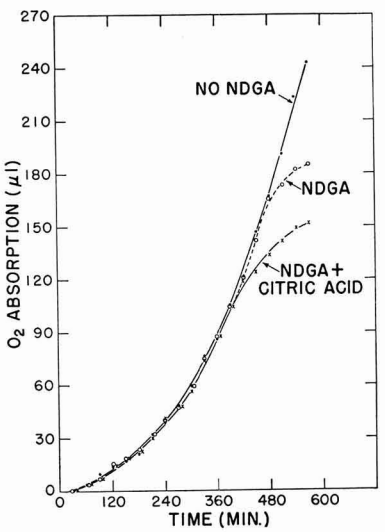

FIG. 5. The oxygen absorption of the phospholipid fraction of milk as influenced by the addition of 0.005% NDGA alone and together with 0.01% citric acid at the end of 7 hr. of the oxidation reaction. (1.0 ppm. added Cu.)

The results show that NDGA, alone or with the synergist citric acid, will retard oxidation which already is in progress. The phospholipid to which NDGA and NDGA and citric acid were added at the end of 5 and 7 hr. had a detectable oxidized flavor. Oxidized flavor was detected only slightly in the phospholipid to which the antioxidant was added at the end of 3, 5 and 7 hr. The phospholipid to which NDGA was added at 0, 1 and 2 hr. and to which both NDGA and citric acid were added at 0, 1, 2 and 3 hr. did not have a detectable oxidized flavor.

The effect of metallic catalysts on the antioxygenic action of NDGA. At the end of 360 min. the oxygen absorption values for the phospholipid containing added copper, nickel, iron and cobalt were 248.8, 241.5, 224.7 and 220.1 μl. respectively (figure 6). The similar values for the phospholipid containing

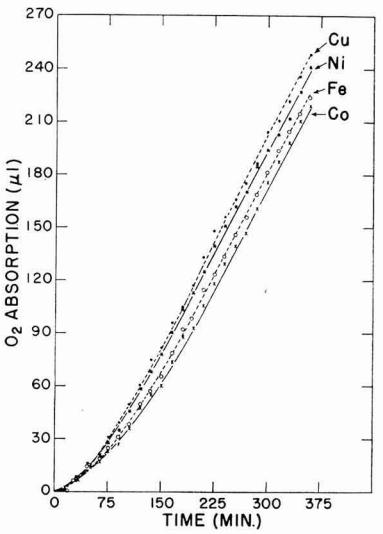

FIG. 6. The effect of 1.0 ppm. of the metallic catalysts copper, nickel, iron and cobalt on the oxygen absorption of the phospholipid fraction of milk.

added NDGA and the catalysts were 145.7, 142.3, 133.7 and 129.9 μl., respectively (figure 7). Oxidized flavor was detected in the phospholipid fraction in all cases. The flavor was more intense, however, in the phospholipid fraction which contained no NDGA.

The effect of pH on the antioxygenic action of NDGA. Figure 8 shows the oxygen absorption curves for pH 6.5, 7.0, 7.5 and 8.0. Of these hydrogen ion concentrations, NDGA was most effective at pH 6.5 and least effective at pH 8.0. The antioxidant being five times more effective at the lowest pH. Oxidized flavor was not detected in any of these phospholipid fraction.

Figure 9 shows the oxygen absorption curves for pH 3.0, 4.0, 5.0 and 6.0. These curves show that the antioxidant effectiveness of NDGA is even less

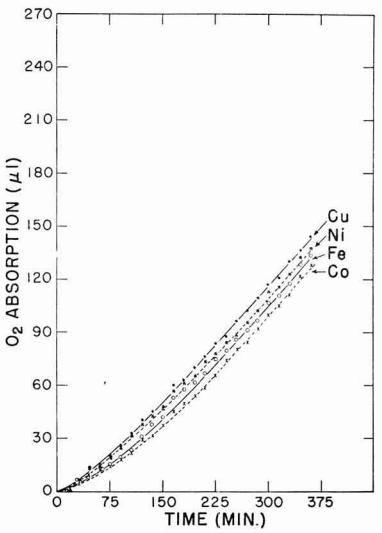

FIG. 7. The effect of 1.0 ppm. of the metallic catalysts copper, nickel, iron and cobalt on the oxygen absorption of the phospholipid fraction of milk in the presence of 0.0008% NDGA.

at pH 3.0 than at the more highly alkaline pH of 8.0 (figure 6). Oxidized flavor was present in the phospholipid buffered at pH 3.0 but it was absent in that buffered at pH 4.0, 5.0 and 6.0.

DISCUSSION

When used alone, ascorbic acid, methionine and citric acid were not effective in retarding the oxygen absorption of the phospholipid fraction. In the

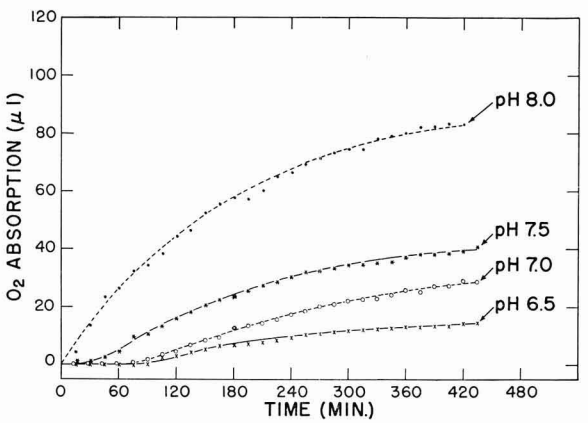

FIG. 8. Oxygen absorption of the phospholipid fraction of milk as influenced by pH and in the presence of 1.0 ppm. Cu and 0.005% NDGA.

presence of NDGA, all three were effective synergists. Citric acid and ascorbic acid were equally effective while there was an indication that methionine was slightly less effective.

NDGA will stop or retard oxidation which had been allowed to proceed for a period of time. An explanation of this phenomenon may be found in the chain theory of oxidation which states essentially that an activated molecule of auto-oxidizable substance and a molecule of oxygen unite with the formation of a peroxide and the liberation of energy. The newly formed peroxide molecule is able to pass this energy on to another molecule of the substance thus initiating a type of chain reaction. Thus, when a molecule of an antioxidant comes into contact with an activated peroxide molecule, the antioxidant takes up the energy and is itself oxidized. In this manner the velocity of the autoxidation is reduced and the reaction chain shortened.

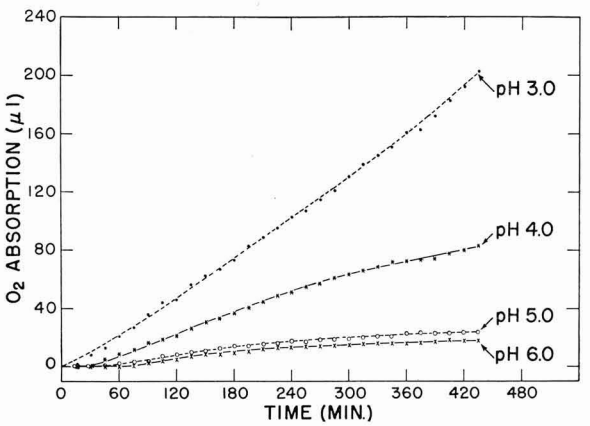

FIG. 9. The oxygen absorption of the phospholipid fraction of milk as influenced by pH and in the presence of 1.0 ppm. Cu and 0.005% NDGA.

Slight but definite differences were observed in the catalytic action of copper, nickel, iron and cobalt. These differences were apparent both in the presence and absence of NDGA. The order of greatest effectiveness was copper, nickel, iron and cobalt. This does not agree with the findings of Veno and Saida (6) who reported that cobalt was the most effective catalyst of the four metals studied. These workers used the catalysts as the linoleates, while in this study they were included as the sulfates.

The findings of other investigators (3, 4) were confirmed in regard to the effect of an alkaline pH on the antioxygenic effectiveness of NDGA. It was shown that NDGA is most effective at pH 6.5, while it is less effective at pH 7.0, 7.5 and 8.0. Furthermore, the effectiveness of the phenolic antioxidant NDGA is decreased at a low pH. Thus, at pH 3.0, NDGA showed very little antioxidant activity. The effectiveness was increased greatly as the pH was raised to 4.0, 5.0 and 6.0.

CONCLUSIONS

When used alone, methionine, ascorbic acid and citric acid were not effective in retarding the oxygen absorption of the phospholipid fraction.

With NDGA, methionine, ascorbic acid and citric acid were found to be very effective synergists.

NDGA alone or together with the synergist citric acid will retard oxidation which has been allowed to proceed for a period of time.

The order of greatest catalytic effect was copper, nickel, iron and cobalt, but the differences were slight.

The antioxygenic action of NDGA was greatest when the system was buffered at pH 6.5.

When the system was buffered at pH 8.0, the antioxygenic action of NDGA was decreased.

At pH 3.0, the antioxidant action of NDGA was greatly decreased.

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COMPARATIVE EFFICIENCY OF INTRACERVICAL AND INTRAUTERINE METHODS OF INSEMINATION IN DAIRY CATTLE[1]

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On a technical basis both intracervical and intrauterine methods of inseminating dairy cows have advantages and disadvantages as enumerated by Rowson (7) and Herrick (2). While technical factors may necessitate the use of one method in individual instances, the predominate method to be used should be determined by a comparison of breeding results. In a survey of inseminating techniques, Trimberger (9) concluded that the intrauterine method of breeding in which the inseminating tube could be guided through the cervix was the preferred technique. Raps (5), in 1948, found that the speculum was being used on less than 1 per cent of the cows artificially inseminated in Iowa. However, several artificial breeding associations in Missouri have made general use of the intracervical-speculum method and also the intrauterine method.

The data reported herein summarize a field comparison between the breeding efficiency of the two techniques.

EXPERIMENTAL

During a period of 3 mo. three inseminators working in one county and directly from a central bull stud inseminated part of the cows by the intracervical method, in which the inseminating pipette was passed through a speculum and the semen deposited in the posterior cervix; the remainder were inseminated by intrauterine insemination, in which the pipette was worked into the uterus by manipulation of the cervix *per rectum*. A total of 2,364 inseminations, 1,053 intracervical and 1,311 intrauterine, was made during the period.

To further test the effectiveness of the two insemination methods, the data were divided into first services and repeat breedings, since the reproductive ability of the first services group should be higher than that of a group of repeat breeders. Nothing but the number of previous negative services was known of the breeding history of the cows involved and all available data were used.

The data were analyzed for the efficiency of the two methods with different ages of semen. Most of the inseminations (62 per cent) were made with semen used within 12 hr. after collection and no semen over 60 hr. old was used.

All cows included in the study had been inseminated at least 150 days at the time of calculation of the percentage non-returns. In all comparisons between the two insemination techniques the Chi-square test for significance was used.

RESULTS

As shown in table 1, the tendency of the inseminators was to inseminate "repeat" cows by the intrauterine method. Semen was deposited in the uterus

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in 64 per cent of the "repeat" cows. It is obvious that this selective use of the intrauterine method introduced a bias in the result. On first services the difference of 5.0 per cent in assumed conception rate in favor of the intrauterine method was not statistically significant. The data revealed a highly significant increase of 8.9 per cent in percentage non-returns with the intrauterine method on "repeat breedings." Likewise, when first services and "repeat" inseminations were combined into total services the difference was significant.

When the data were analyzed by groups according to the age of semen used, the 5.0 per cent higher percentage non-returns, intrauterine over intracervical,

TABLE 1

Comparison of intracervical and intrauterine methods of inseminating dairy cows

Insemination Method	1st Services	Repeat Services	Total Services	Age of semen used (hr.)		
				< 12	24–36	48–60
Intracervical services	578	475	1053	637	378	38
% non-returns	46.7	49.8	48.1	52.4	42.3	34.2
Intrauterine services	478	833	1311	827	419	65
% non-returns	51.7	58.7	56.1	57.4	55.4	44.6
Difference[a]						
% non-returns	5.0	8.9[b]	8.0[b]	5.0	13.1[b]	10.4

[a] Intrauterine vs. intracervical
[b] $P < 0.01$

when fresh semen was used was not significant. With semen used 24 to 36 hr. after collection, the difference in percentage non-returns was 13.1 per cent, significant at the 1 per cent level. The number of cows bred with 48 to 60 hr. semen was too small for the difference of 10.4 per cent in percentage non-returns to be significant. The data showed a 10.1 per cent drop in assumed conception rate between fresh and 1-day-old semen when the intracervical method was used; there was only a 2.0 per cent decrease between fresh and 1-day semen with the intrauterine method. This decrease with day old semen was highly significant with the intracervical method and insignificant with the intrauterine method.

DISCUSSION

Some selective rebreeding of cows by the intrauterine insemination technique has altered the comparison between the intracervical and intrauterine method; nevertheless, in the six comparisons made, intrauterine breeding gave significantly better results in three cases and the trend was the same in those comparisons where the differences were not significant. The difference observed in these data is smaller than that observed by Lasley and Bogart (4) who reported a difference of 19 per cent in favor of the intrauterine method on 68 intracervical and 199 intrauterine inseminations with beef cattle. Holt (3) reported a first services conception rate of 33.4 per cent for intracervical insemination and 56.1 per cent for intrauterine insemination on 751 cows bred at random. Raps (5), in a field comparison of the effectiveness of the speculum and deep cervical or intrauterine techniques, found a 6.7 per cent advantage in the latter on first

services only. The difference in percentage non-returns to second services by the two methods was negligible (1 per cent). This is not in agreement with the present data, for here the difference on first services is not statistically significant, but the difference is significant on inseminations after the first. If the difference in efficiency between the two techniques is real, it could be expected that some cows that failed to conceive to an intracervical insemination would become pregnant to a subsequent intrauterine insemination. Herrick (2) found no significant difference in conception rate in the Iowa State College dairy herd when half of the herd was bred by depositing semen on the external cervical os, via a speculum, and half was bred by cervical fixation. Herman and Swanson (1) found no significant difference between the two methods of insemination in the Missouri Station Dairy Herd. All cows are inseminated at the middle of the estrus period, and the speculum method still is used routinely by beginning inseminators. These observations and the present data suggest that when reproductive factors are optima the two methods may be equally efficient.

In an *in vitro* study of sperm penetration of bovine vaginal-cervical mucus, Roark (6) observed changes in penetration rates during estrus. Penetration was maximal in mucus collected during full and late estrus. However, individual variations in penetration rates were observed. The range was from 0 to 6 mm. penetration per minute. Furthermore, while vigorous sperm from one bull's semen were unable to penetrate a sample of mucus, sperm of equal vigor from another bull's semen could penetrate the same mucus. His study suggests that, in some instances, the cervical mucus may be a barrier against the migration of sperm through the cervix.

Sergin *et al.* (8) concluded that the physico-chemical environment in the cervix makes this area more favorable for sperm survival than either the vagina or uterus. Sperm in the cervix served as a constant supply which passed into the uteri. A combination intrauterine-intracervical insemination technique has been used in which half of the semen is deposited in the uterine horn on the side of the functional ovary and the remainder in the middle of the cervix (10). The comparative efficiency of this technique needs further investigation.

It has been suggested that by use of the intrauterine technique the detrimental effect of an infected cervix may be by-passed. Passing a catheter through an infected cervix may, however, be a dangerous practice and the use of intrauterine insemination in this instance is questionable.

SUMMARY

A study of the comparative efficiency of intracervical and intrauterine semen deposition in inseminations with dairy cattle was made.

Percentage non-returns were significantly higher with the intrauterine method on total services and repeat breedings. The difference on first services was not significant.

With semen 24 to 36 hr. old, the intrauterine method showed a significant difference of 13.1 per cent greater non-returns for cows inseminated under field conditions.

Intracervical percentage non-returns were significantly lower with semen used 24 to 36 hr. after collection than with semen used within 12 hr. after collection; with intrauterine insemination the decrease was not significant.

The data indicate that as a general insemination technique intrauterine breeding gives better results than deposition of semen in the posterior cervix, via the speculum.

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THE RELATION OF SITE OF SEMEN DEPOSIT TO BREEDING EFFICIENCY OF DAIRY CATTLE[1]

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Site of semen deposit in relation to conception rate in dairy cattle has created much interest in recent years. Depositing semen in the uterus of the cow has become standard technique in many breeding associations throughout the United States. This practice has been adopted on the assumption that it is superior to vaginal or shallow cervical deposition of semen. Despite the vast amount of research on breeding efficiency of dairy cattle, few experiments have been conducted to determine the effect of depositing semen in various locations of the reproductive tract.

Lasley and Bogart (2) in a preliminary investigation involving 40 cows compared vaginal and cervical deposition of semen. Conception rates were 25.0 and 65.0 per cent, respectively, for the two treatments. In a later study, the same authors (2) reported that 59 per cent of the cows inseminated with the aid of the speculum conceived as compared to 78 per cent of those inseminated into the uterus by aid of rectal manipulation of the cervix. Holt (1) in comparing 377 intra-cervical and 374 intra-uterine inseminations, reported 33.4 and 56.1 per cent conception, respectively. Raps (3) in a study involving 2,712 first and second services compared deep cervical or intra-uterine with the speculum technique of insemination and reported 61.8 and 55.8 per cent conception, respectively. Salisbury and Van Demark (4) conducted two controlled experiments involving 936 services in which semen was deposited in the cervix, in the body of the uterus and in the horns of the uterus. Results obtained indicated that cervical deposition of semen was as satisfactory as the deposition of semen into the body or horns of the uterus. Preliminary results on several thousand inseminations indicated no advantage in depositing the semen beyond the cervix.

This investigation was conducted in an effort to determine the effect of different sites of semen deposit upon breeding efficiency of dairy cattle.

EXPERIMENTAL

The experimental design consisted of replications of the standard 4×4 Latin square. The experiment was initiated on November 1, 1949, and the last replicate was completed on March 31, 1950. Treatments used were as follows: A. Two-thirds of semen deposited in the body of the uterus and one-third in the second or third fold of the cervix. B. Semen deposited in the second or third fold of the cervix. C. Semen deposited in the body of the uterus. D. One-half of semen deposited in each horn of the uterus.

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[1] The data contained in this paper are from a thesis presented by the senior author to the faculty of the Graduate School, Louisiana State University, in partial fulfillment of the requirements for the degree of Master of Science.

Ten experienced technicians affiliated with the Louisiana Artificial Breeding Cooperative, Inc., were selected to perform the inseminations. In order to insure randomization of all treatments, duplicate booklets containing the order of semen deposits in the standard 4 × 4 Latin square were used by each technician.

Semen, which had been cooled gradually, was extended in 2.9 per cent citrate-sulfanilamide-yolk (1 part yolk to 3 parts buffer) at an average of 1:77 (1 part of semen to 77 parts of extender) with none greater than 1:100 (1 part of semen to 100 parts of extender). Semen was shipped 6 days a week to each technician, with a majority of the inseminations being made with semen less than 36 hrs. old. Approximately 1 ml. of extended semen was used for each insemination. The measurement of fertility was based on 60- to 90-day non-returns to first service. Treatment differences were tested by analysis of variance (5).

RESULTS AND DISCUSSIONS

Two thousand and fourteen first services were included in the experiment. Inseminations were made in various stages of estrus, although a majority was made in the middle and latter parts of the period. A summary of the conception rates for each inseminator on each treatment is presented in table 1. Simi-

TABLE 1

Mean breeding efficiency and total services of inseminators for each treatment (based on 60- to 90-day non-returns to service)

Inseminators	Treatments							
	A		B		C		D	
	Total Ser.	% N. R.	Total Ser.	% N. R.	Total Ser.	% N. R.	Total Ser.	% N. R.
1	16	43.7	15	73.3	15	60.0	16	56.2
2	46	41.3	47	40.4	46	41.3	47	51.1
3	115	68.7	115	60.9	114	70.2	117	67.5
4	35	68.6	33	69.7	35	77.1	35	71.4
5	46	63.0	47	70.2	47	68.0	49	57.1
6	51	60.8	50	58.0	48	50.0	50	44.0
7	28	67.8	27	66.7	28	78.6	28	85.7
8	76	61.8	75	64.0	78	64.1	78	65.4
9	50	60.0	48	50.0	49	73.5	50	62.0
10	40	72.5	43	39.5	40	62.5	41	65.8
Total	503		500		500		511	
Mean		62.4		58.4		64.8		62.6

larly, a summary of the conception rates for each bull on each treatment is presented in table 2. Fertility, expressed as percentage of conception, for treatments A, B, C and D was 62.4, 58.4, 64.8 and 62.6 per cent, respectively. The differences in fertility between treatments A and B, B and C, and B and D were 4.0, 6.4 and 4.2 per cent, respectively. However, the differences in fertility between treatments A and C, A and D, and C and D were negligible, as is shown by the treatment means. The differences between treatments were

TABLE 2

Mean fertility and total services of bulls for each treatment (based on 60- to 90-day non-returns)

Bulls	Treatments							
	A		B		C		D	
	Total Ser.	% N. R.	Total Ser.	% N. R.	Total Ser.	% N. R.	Total Ser.	% N. R.
1	35	62.8	25	32.0	27	63.0	28	35.7
2	20	60.0	21	90.5	21	80.9	27	74.1
3	26	38.5	26	38.5	27	51.8	30	43.3
4	36	69.4	48	54.2	52	63.5	47	63.8
5	31	83.9	38	65.8	31	71.0	32	65.6
6	18	61.1	14	71.4	22	63.6	22	63.6
7	21	66.7	16	50.0	13	48.5	11	45.5
8	4	50.0	3	00.0	3	66.7	3	100.0
9	6	50.0	11	63.6	12	33.3	10	100.0
10	11	54.5	20	55.0	9	44.4	9	77.8
11	13	53.8	13	53.8	11	45.4	15	60.0
12	15	73.3	8	72.7	8	50.0	11	63.6
13	4	50.0	4	75.0	6	66.7	7	42.8
14	5	80.0	7	57.1	11	63.6	9	55.5
15	16	68.7	14	57.1	22	77.1	19	63.1
16	19	31.6	14	57.1	15	66.7	10	60.0
17	27	40.7	24	45.8	24	79.2	23	69.6
18	22	81.8	20	65.0	19	73.7	22	59.1
19	25	72.0	37	67.6	33	72.7	29	79.3
20	21	66.7	22	73.3	19	89.5	20	70.0
21	15	40.0	16	62.5	17	58.8	17	64.7
22	15	80.0	19	52.6	17	64.7	22	68.2
23	5	40.0	4	25.0	5	40.0	4	25.0
24	15	80.0	13	76.9	14	78.6	11	81.8
25	40	57.5	32	37.5	30	36.7	32	53.1
26	17	70.6	19	68.4	15	80.0	17	47.0
27	4	75.0	1	100.0	4	100.0	1	100.0
28	4	100.0	4	25.0	2	50.0	5	60.0
29	4	50.0	5	100.0	2	00.0	3	66.7
30	0	00.0	1	100.0	4	100.0	4	100.0
31	4	75.0	0	00.0	1	100.0	2	100.0
32	2	50.0	1	100.0	2	100.0	2	50.0
33	1	00.0	0	00.0	1	100.0	4	50.0
34	2	50.0	0	00.0	1	100.0	3	100.0
Total	503		500		500		511	
Mean		62.4		58.4		64.8		62.6

not significant when tested by the analysis of variance; however, the differences between inseminators were significant at the 5 per cent level and between bulls at the 1 per cent level.

The treatment means show that breeding efficiency was slightly higher when semen was deposited in the body of the uterus, as compared to deposition in the uterine horns, the cervix or a combination of the uterus and the cervix. Due to the wide variations in conception rates existing between treatments and within treatments with respect to both inseminators and bulls, no practical significance can be attached to the differences in treatment means. These results are in agreement with those of Salisbury and Van Demark (4), who also found no significant differences between similar sites of semen deposit.

SUMMARY

A study involving 2,014 first services, four sites of semen deposition, ten technicians and thirty-four dairy bulls was made to determine the effect of different sites of semen deposit upon breeding efficiency of dairy cattle.

The differences beween treatments (sites of deposition) were not significant when tested statistically. However, the differences between bulls and between technicians were significant at the 1 and 5 per cent levels, respectively.

The treatment mean for semen deposited in the body of the uterus was slightly higher than when semen was deposited in the cervix, in each horn of the uterus or a combination of the body of the uterus and cervix. However, no significance is attached to these differences. This study is being continued.

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FEEDING DDT AND ALFALFA SPRAYED WITH DDT TO CALVES[1]

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Extensive use has been made of DDT in spraying forage crops, but no data have been available heretofore on the effects of feeding the sprayed crops to growing calves. Also, no information has been available on the amount of DDT stored in the body tissues of calves when they are fed DDT either as residue or the pure compound. Other investigators (2, 4, 9) have published limited data on the storage of DDT in the body tissues of sheep, cows and pigs that were fed this compound. This experiment was undertaken in order to obtain similar information with young dairy calves.

METHODS

On July 29, 1947, a field of alfalfa was sprayed with 0.6 lb. of technical DDT per acre. Representative portions of the field were cut on August 6, August 18 and September 3. Immediately after the hay was cut, it was mow-dried in the barn. The difference in cutting interval provided hays having varying concentrations of DDT residues and gave information on the amount of weathering of the residue on the standing crop. The alfalfa was baled after mow-drying, then stored and, as the calves were placed on the experiment, it was fed to them until they were 8 mo. of age.

All calves used were Jersey males. When 10 days of age, they were fed skimmilk, a carotene concentrate, a grain mixture and the assigned roughage. The amount of alfalfa consumed varied with each calf's appetite up to 3 or 4 mo. of age, after which time alfalfa consumption was limited to 1.5 lb. per 100 lb. of body weight. Skimmilk gradually was withdrawn from the diet beginning at 3 mo. of age, and after 4 mo. no milk was fed. After the calves were 3 mo. old, the total digestible nutrient intake was limited to Morrison's requirement.

The hay that was selected for use as a control was obtained on the market and fed to the calves in group 4 (see table 1). A preliminary sample did not contain DDT. However, on 12 out of 15 monthly composite samples, the analysis of this hay showed 1.4 to 21.3 ppm. of DDT present. Consequently, the calves in group 6, which were fed dried beet pulp and grain and no DDT, were used for control animals.

All calves were slaughtered at 8 mo. of age, except no. 2, which was slaughtered at 6 mo. of age. Various internal organs and endocrine glands were weighed at slaughter. Samples of tissue from liver, kidney, heart, intestine, brain, spinal cord, muscle, lung, lymph nodes, spleen, abomasum and pituitary

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gland were taken for histological study. Samples of kidney fat, body and mesenteric fat, liver and rib and loin meat were taken for DDT analysis. The kidney- and body-fat samples were analysed separately except for nos. 14 and 15, which were combined into one sample. The samples of rib and loin meat were taken to approximate the edible portion in the region of the 12th and 13th rib and first and second lumbar vertebrae.

At intervals during the experiment, hemoglobin, vitamin A, carotene, calcium and phosphorus concentrations were determined on blood samples of each calf. Body weight gains and general appearance also were noted.

After this experiment was completed, two more calves were fed larger amounts of DDT in oil solution. These calves were raised to 80 days of age on a normal ration. From the 80th to the 256th day (when slaughtered) they were fed timothy hay and a grain mixture composed of 97 per cent corn, 2 per cent bonemeal, 1 per cent salt and 10 ml. of cod-liver oil daily. In addition, they were given DDT dissolved in corn oil at the rate of 100 mg. DDT per kg. of dry matter consumed. This was given once daily in a capsule. The ration fed these two calves was very low in protein. This type of diet has been shown to enhance the toxic effects of DDT in mice (7).

A sample of each of the hays fed was taken five times each week and the samples were combined by monthly periods. DDT analyses were made on these monthly composite samples by the total organic chlorine method (1). Occasionally, the Schechter-Haller colorimetric method was used as a specific check for DDT (8).

The preliminary treatment accorded the various calf meat and fat samples analysed for DDT deposits was as follows: The liver samples were ground and thoroughly mixed and the rib and loin cuts were boned out and thoroughly ground. In each case, a 50-g. sample was taken and mixed well to dryness with anhydrous Na_2SO_4. The dried samples then were placed in a Waring Blendor and thoroughly extracted four times with redistilled chloroform, each time decanting off the chloroform. The combined extract then was filtered through a 24-cm. folded filter paper before analysis.

Preliminary studies on body- and kidney-fat samples showed that when solid fat was used, the amount of liquid fat that could be rendered from the fat sample varied considerably, due to the varying amounts of connective tissue and water present. To put all the samples of fat on a comparable basis, they were rendered out and dried first and then 5-g. samples of pure fat were weighed out on an analytical balance.

All samples of meat and fat were analysed as outlined by Schechter *et al.* (8).

RESULTS

The results are given in table 1. As indicated by the monthly composite samples, the alfalfa cut 8 days after spraying averaged 39 ppm. of DDT when fed to the calves. The alfalfa cut 20 days after spraying averaged 11 ppm. and that cut 36 days after spraying averaged 9 ppm. The average DDT content of samples of the alfalfa bought on the market averaged 5.7 ppm.

TABLE 1

Showing DDT intake of dairy calves and the concentration of DDT in the body tissues

Calf no.	DDT intake			Average daily gain in weight	Concentration of DDT in ppm			
	Total intake	Ppm. of dry matter consumed	Daily intake as mg./kg. body wt.		Body fat	Kidney fat	Rib and loin meat	Liver
	(*g.*)			(*lb.*)				
	Group 1. Fed alfalfa cut 8 d. after spraying							
1	10.2	22.1	0.64	1.15	100.0	104.0	1.7	0.2
2	6.5	21.7	0.58	1.17	80.0	83.2	1.2	0.2
3	8.9	16.8	0.48	1.06	84.8	87.3	1.7	1.1
4	5.3	10.8	0.30	1.19	71.8	75.0	0.6	0.2
	7.7	17.8	0.50	1.14	84.2	87.4	1.3	0.4
	± 2.2	± 5.3	± 0.15	± 0.06	± 11.8	± 12.2	± 0.5	± 0.4
	Group 2. Fed alfalfa cut 20 d. after spraying							
5	3.7	6.8	0.20	1.26	8.1	10.0	0.0	0.0
6	2.6	4.8	0.10	1.24	23.0	23.5	0.2	0.0
	3.2	5.8	0.15	1.25	15.6	16.8	0.1	0.0
	± 0.84	± 1.4	± 0.07	± 0.01	± 10.5	± 9.5	± 0.1	
	Group 3. Fed alfalfa cut 36 d. after spraying							
7	2.7	5.3	0.16	1.14	9.3	10.2	0.0	0.0
8	1.5	3.2	0.08	1.03	4.4	6.1	0.0	0.0
9	2.2	4.3	0.12	1.29	3.4	4.2	0.0	0.0
10	2.1	4.1	0.12	1.25	4.2	7.0	0.6	0.0
	2.1	4.2	0.12	1.18	5.3	6.9	0.2	0.0
	± 0.5	± 0.9	± 0.03	± 0.12	± 2.7	± 2.5	± 0.3	
	Group 4. Fed alfalfa bought on the market (Commercial alfalfa)							
11	1.2	2.2	0.07	1.14	2.0	3.0	0.0	0.0
12	1.2	2.6	0.09	1.00	22.0	22.9	0.4	0.0
13	1.5	2.8	0.08	1.38	5.3	7.9	0.0	0.0
	1.3	2.5	0.08	1.17	9.8	11.3	0.1	0.0
	± 0.2	± 0.3	± 0.01	± 0.19	± 10.7	± 10.3	± 0.2	
	Group 5. Fed timothy hay and corn and DDT by capsule							
14	34.5	106.1	2.9	0.64	340.0		12.7	1.9
15	37.6	103.0	2.7	0.68	345.0		13.1	3.9
	36.1	104.6	2.8	0.66	342.5		12.9	2.9
	± 2.2	± 2.2	± 0.04	± 0.03	± 3.5		± 0.3	± 1.1
	Group 6. Fed dried beet pulp and grain.							
16	0.0	0.0	0.0	1.37	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	1.60	0.0	0.0	0.0	0.0
				1.49				
				± 0.16				

± values indicate standard deviation.

None of the calves showed any gross abnormal symptoms when consuming the alfalfa or the DDT dissolved in oil. The average daily gains of individual calves fed the various alfalfa diets ranged from 1.0 to 1.6 lb. The average daily body weight gains of the groups that were fed alfalfa hay were practically identical but slightly less than the two calves receiving beet pulp. However, by the "t" test at the 5 per cent level, group 1 (fed early cut alfalfa) gained less than group 6 (fed beet pulp). The body weight gains of the two calves in group 5 were low. This was to be expected when calves were fed a ration low in protein,

and cannot be attributed to the DDT intake. No indications of DDT toxicity were noted in these two calves.

Hemoglobin values for the calves as individuals or as groups were within the normal range. After 90 days of age, individual calves varied from 9.0 to 11.0 g. of hemoglobin per 100 ml. of blood, which is within the normal range found for calves in this herd. Plasma vitamin A and carotene values were normal and showed a normal increase as the calves increased in age. Plasma calcium and phosphorus values also were normal. All groups averaged between 9.3 and 11.1 mg. calcium per 100 ml. at the various ages, while plasma inorganic phosphorus averaged between 6.2 and 9.2 mg. per cent.

At slaughter time, group averages for ratios of body weight to liver, kidney, and thyroid weights showed no consistent or statistical differences. Sarett and Jandorf (6) stated that 0.07 per cent DDT in the diet of rats produced an increase in the relative size of the liver. The two calves in group 5 received only 0.01 per cent DDT in this ration, and their livers were the smallest of all individuals. They averaged 1.50 per cent of body weight, while those fed no DDT (group 6) averaged 1.78 per cent. The individual values for the calves fed the alfalfa diets varied from 1.60 to 1.99 per cent.

Liver tissues from all calves except nos. 4, 8 and 13 were examined histologically. At least two routine paraffin sections stained with hematoxylin and eosin and three frozen sections stained for fat were examined. None of the livers examined showed any degree of fatty change or any other histologic alterations that could definitely be attributed to DDT ingestion.[4] Liver cell alteration of the type reported to occur in rodents on low dosage levels of DDT was not seen in any of these animals. However, the characteristic liver cell damage caused by DDT in rodents (rats, rabbits, mice and guinea pigs) has not been found to occur in animals of a variety of other species that have been treated with this compound (5).

The intake of DDT and the storage of this compound in the fat, meat and liver are presented in table 1. The general tendency for the concentration of DDT in a specific body tissue to increase as DDT intake increased is evident.

The DDT intake, as ppm. or mg. per kg., of group 5 (fed DDT by capsule) was 5.7 times larger than that of group 1 (fed alfalfa cut 8 days after spraying), but the concentration in the fat was 4-fold greater. The concentrations found in liver and meat were seven and ten times greater, respectively. The DDT intake of group 1 was about three to seven times greater than those of groups 2, 3 and 4, while the concentrations found in the fat were about 5- to 15-fold greater.

Only an occasional trace of DDT was found in the meat samples of calves consuming 2.2 to 6.8 ppm. of DDT and none was detected in the liver of these animals, while the calves receiving 10 ppm. or more of DDT showed definite storage of DDT in meat and liver.

In all cases, the kidney fat contained a higher concentration of DDT than did fat taken from the mesentery or other portions of the body.

[4] The authors are grateful to H. R. Seibold, Pathological Division, Bureau of Animal Industry, U.S.D.A. for performing and evaluating the histological work.

DISCUSSION

The increased concentration of DDT found in the body fat of calves when intake was increased is in agreement with the results of Harris *et al.* (4) on sheep. However, at comparable intakes, the calves in this experiment had higher concentrations of DDT than Harris *et al.* found in sheep fed for 4 mo. The longer period during which the calves received DDT may be one explanation of this difference. Laug *et al.* (5) showed that the concentration of DDT in perirenal fat continued to increase for 23 wk. when rats were fed a diet containing 5 ppm. of DDT.

Because of the limited number of calves, this experiment does not give any definite information on the differences in DDT storage as affected by capsule or residue feeding, as noted by other investigators (3, 4).

In this investigation, only the liver tissue was examined histologically. From observations in studies with rats on low levels of DDT intake, this is the organ that is most likely to show changes due to DDT ingestion (5). However, in these calves fed DDT at 2.5 to 105 ppm., there were no detectable hepatic cell alterations similar to those that have been noted in rats when fed DDT at 5 ppm. or more (5).

The occurrence of DDT either as residue or as contamination in hay purchased on the market is worthy of note. Its presence was confirmed by the colorimetric method of analysis. Although the amount was small and variable, it was sufficient to allow definite storage of DDT in the fat of all calves fed the alfalfa purchased on the market. In contrast, the calves simultaneously fed only dried beet pulp and grain had no DDT in their body tissues. Other investigators have found DDT in the fat of control animals used in their experiments (5).

All meat and fat samples, except those of the two animals fed DDT by capsule, showed some off color when analysed by the colorimetric method, which is indicative of decomposition of the technical DDT. This probably was due to exposure to the elements in the field and to some further decomposition upon being metabolized in the plant or in the animal's body. The two primary decomposition products are 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene and bis-(*p*-chlorophenyl)acetic acid, both compounds giving a reddish cast to the characteristic blue color developed by the colorimetric procedure used.

Animals 14 and 15 were given capsules containing technical and some *p,p'*-DDT along with their feed. No off colors were evident in the analysis of the tissues of these animals due to the fact that the DDT was taken into the animal's body in capsule form, and, therefore, not subjected to partial decomposition while on the plants in the field.

SUMMARY

DDT in oil solution and alfalfa containing various amounts of DDT residue were fed to 15 dairy calves. The growth of these calves was not adversely affected by the amounts of DDT ingested. Hemoglobin, vitamin A, carotene, calcium and phosphorus concentrations in the blood also were entirely normal. No

histologic changes ascribed to DDT ingestion were found in the livers of these calves. DDT intake varied from 0.07 to 2.9 mg. per kg. of body weight or 2.2 to 106 ppm. of the dry matter consumed. The DDT feeding period ranged from 160 to 230 days in length. The storage of DDT in the body and kidney fat varied from 2.0 to 345 ppm. and the concentration in the fat was proportional to the DDT intake. When DDT intake exceeded 10 ppm. of the dry matter intake, DDT appeared in the meat of the calves.

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THE RELATION OF SURFACE GROWTH TO THE RIPENING OF MINNESOTA BLUE CHEESE[1]

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Many soft and semi-hard varieties of cheese commonly develop a sticky layer on the surface which usually is referred to as "slime." The microorganisms isolated from this mucilaginous layer have been assigned an important role in the curing of limburger (5) and brick cheese (6). Blue-veined cheese, such as Roquefort or Minnesota blue, also develop a characteristic surface growth.

The development of this slime seems to follow a pattern. During the first 3 to 4 wk., mold accompanies the development of a sticky surface growth. This gradually is replaced with a reddish orange, sticky slime (3, 4, 9, 14). The slime has been described as consisting of yeast, micrococci and rod forms of bacteria and mold (2, 8, 12).

The growth of the slime has been used as an index of proper ripening conditions (4, 12). It also has been observed that its presence is associated with the best grades of cheese (9). Thom (12) found enough paraffined cheese, without slime, that developed characteristic flavor to indicate to him that the slime was not an essential factor in flavor production. Other than the subjective work of Thom, no experimental data have been found in the literature to show the influence of the slime on the ripening of blue cheese. This investigation was undertaken to determine the influence of surface growth on the ripening of this cheese.

METHODS

Manufacture and ripening. The procedure followed for making the Minnesota blue cheese was that used by the Dairy Division of the University of Minnesota. Green cheeses from each of two lots were segregated into three groups and processed to produce various amounts of slime during the ripening period. The three groups were as follows: (a) a group on which no slime was permitted to develop, (b) a normally ripened group of cheeses and (c) a group on which slime was permitted to develop for a prolonged period. The no-slime group was prepared by waxing the cheese. Punching was done through the wax. The second group was permitted to slime for about 2 mo. in the cave (48 to 50° F.). The last group consisted of cheeses which were permitted to slime until they were 4 mo. of age. These groups will be referred to as "waxed," "normal" and "prolonged slime," respectively. The cheeses were scraped at intervals determined by the development of the slime. All cheeses were kept in the cave until removed to low-temperature (33 to 35° F.) curing rooms at 4 mo. of age. At this time all cheeses were wrapped in tinfoil.

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Sectioning the cheese for analysis. The cheeses were sampled in the manner of Langhus *et al.* (6). A 3-in. cross section was cut from the middle of the cheese; from this section three layers parallel to the surface were removed for analysis. The first or "edge" layer, consisting of the slime-free surface and the cheese beneath it, measured 0.125 in. in thickness. The second or "middle" layer consisted of the cheese 1 in. inward from the "edge" layer. The third or "center" layer was the section comprising the remainder. These portions were examined chemically and organoleptically initially and at 3, 6 and 9 mo. of age. The portions were analyzed in an attempt to ascertain if ripening had proceeded inward from the exterior of the cheese.

Measuring changes in the cheese during ripening. The simplified extraction-distillation method described by Smiley *et al.* (10) was used for the determination of the total volatile acidity of the cheese.

A Leeds and Northrup glass-electrode potentiometer was used to make the pH determinations on a mixture of 40 g. of cheese and 20 ml. of distilled water. This potentiometer also was used to determine the pH of the slime.

A modification of the Kjeldahl-Gunning-Arnold method (1) was used to determine the total nitrogen in 1 g. of well-mixed sample. The ammonia was distilled into 50 ml. of 2 per cent boric acid solution (7) and the mixed indicator suggested by Ma and Zuazaga (7) was used in titrating the ammonia against standard 0.1*N* HCl.

The amino nitrogen content of the cheese was expressed as a percentage of the total nitrogen. The following method was devised to produce a homogenous cheese mixture that could be handled efficiently in the micro-Van Slyke apparatus. Ten g. of well-mixed cheese were weighed into a 250-ml. beaker. This cheese was macerated with about 25 ml. of warm (50–55° C.) phosphate buffer (pH 6.6 and ionic strength of 0.1). After the mixture was homogenized twice in a single valve, hand homogenizer, sufficient additional buffer was used to rinse all equipment and to make the total volume up to 200 ml. in a volumetric flask after cooling to room temperature. The flask was shaken well and 2 ml. of the suspended, homogenous mixture were used to determine the amino nitrogen by the micro-Van Slyke gasometric method (13).

Moisture was determined according to the official A.O.A.C. method (1).

The method of Wilster *et al.* (15) was used for determining the amount of salt in the cheese. Because of the high salt content of the cheese, it was necessary to add 20 to 30 ml. of 0.1711*N* $AgNO_3$ solution.

The cheeses were examined organoleptically for flavor, body, texture, color, mold growth and color of mold. The cheeses were assigned letter grades of A, B, C and D with corresponding plus or minus scores for each letter on the basis of their merits when judged by the above-mentioned criteria.

Examination of the slime. Samples of the slime were taken from typical cheeses at approximately weekly intervals by scraping with sterile spatulas. The material was placed in sterile jars. One g. of the slime was suspended in 99 ml. of sterile distilled water by vigorously shaking for 1 min. in a dilution

bottle containing glass beads. Smears of the slime suspension were prepared by spreading 0.01 ml. measured by a Breed pipette, over an area of 1 cm.^{2}. These smears were stained by using the Hucker modification of the Gram stain and were examined under oil immersion.

RESULTS

Description and analysis of the slime—microscopic. An examination of the microorganisms present in the surface growth at various times showed that initially and until the first scraping, after 1 mo. in the cave, yeasts were the dominant microorganisms. Mold growth also was increasing at this time. After the first scraping, Gram positive cocci and short Gram positive rod-shaped bacteria became more prevalent, while at 2 mo. of age these forms were dominant.

Macroscopic examination. During the first 6 days in the cave, no perceptible growth appeared. By the 10th day a white slime appeared on the surfaces of the cheeses. This growth had a yeasty odor. It was present, along with visible spots of blue mold, until the first scraping after 1 mo. in the cave. Following the first scraping, the cheese surfaces became covered with a growth of reddish orange, sticky, putrid slime mixed with tufts of blue mold.

The cheeses again were scraped after 2 mo. in the cave. The reddish orange slime reappeared and by the third scraping (75 days) the slime had become heavy and sticky. At this time, the normal cheeses were waxed and stored in the cave. The reddish orange mucilaginous slime reappeared on the prolonged slime cheeses and was allowed to remain until the 118th day in the cave. The condition of the surfaces of the waxed, normal and prolonged slime cheeses at 118 days of age is shown on figure 1. The surfaces of the waxed cheese were still

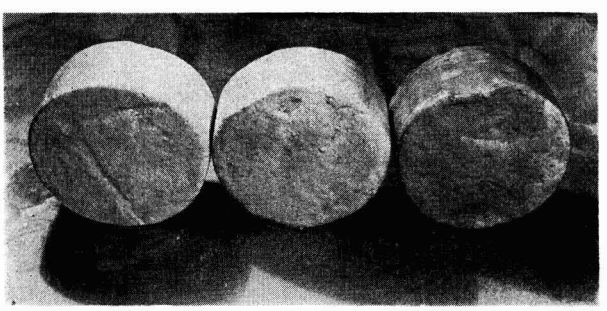

FIG. 1. (From left to right) Waxed, normal and prolonged slime cheeses at a cave age of 118 d. The waxed cheese is shown with the wax removed. The prolonged slime cheese is shown with the slime on it before scraping.

hard and firm. The normal cheese surfaces were softer and more pliable. The prolonged slime cheese is shown before final scraping. It had a softer surface than did the other two.

^pH values of the slime during cave ripening. The changes in the pH values of the slime taken from prolonged slime cheeses during cave ripening are

shown in figure 2. It may be noted that the reaction increased from pH 6.7 at 6 days in the cave to a high of pH 7.5 at 38 days. The slime remained at a pH 7.3 to pH 7.5 until after the reddish orange growth became dominant. At 60 days in the cave, the slime became more acid, and at about 75 days the pH was near neutrality and remained at this value until removal of the cheeses from the cave at 118 days.

Organoleptic examinations. There were no detectable organoleptic differences between the middle and center sections of the normal cheeses and the waxed cheeses when examined at 3 and 6 mo. of age. The edge portions of the normal cheeses, however, were softer than the waxed, were slightly reddish orange in color and, in general, were characterized by a nutty, bitter flavor.

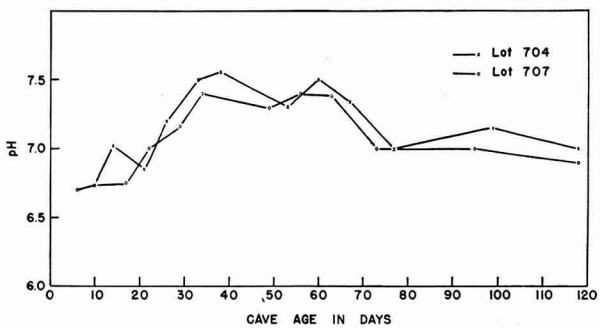

FIG. 2. Changes in pH of the slime from two lots of blue cheeses cave ripened for 118 d.

The prolonged slime cheeses had more body breakdown and a softer texture than the waxed or normal cheeses at 6 mo. of age. The reddish orange color had penetrated from the edge into the middle portions. The center portions were creamy in color, in contrast to the white of the waxed and normal cheeses. The main flavor criticisms of all portions of the prolonged slime cheeses were limburger, putrid and bitter.

At 9 mo. of age the center and middle portions of normal cheeses of lot 704 scored 0.5 to 1 grade higher than the waxed cheeses. The center and middle portions of the waxed and normal cheeses of lot 707 graded the same on flavor. A comparison of the edge portions of the waxed cheeses and the slimed cheeses, namely normal and prolonged slime, showed that the waxed cheeses had a normal blue cheese flavor, white color and relatively hard body, while the normal and prolonged slime cheeses had a buttery texture, slight orange color and a very putrid, limburger, bitter flavor. The normal and prolonged slime cheeses from both lots had more body breakdown and a softer texture than the waxed cheeses. It was evident from the visual examinations of the cheeses that the reddish orange color of the surface growth penetrated the cheese from the outside inward. This occurred only slightly in the normal cheeses, but it changed the whole appearance of the prolonged slime cheeses. Extensive or prolonged sliming was detrimental to the cheeses. The mold became brown in color. The color

of the cheeses changed from a white to a slight orange or cream. The surfaces were soft, making packaging difficult. The flavor definitely changed so that these cheeses had no score and were unmarketable.

It was the opinion of the judges that finer cheeses were produced under the normal slime conditions than were produced by waxing to prevent the formation of surface growth.

Surface growth and total volatile acidity. Figure 3 portrays graphically the influence of the waxed (no slime), normal slime and prolonged slime treatments on the total volatile acidity in portions of blue cheeses during the curing process. The edge portions of the normal and prolonged slime cheeses were much higher

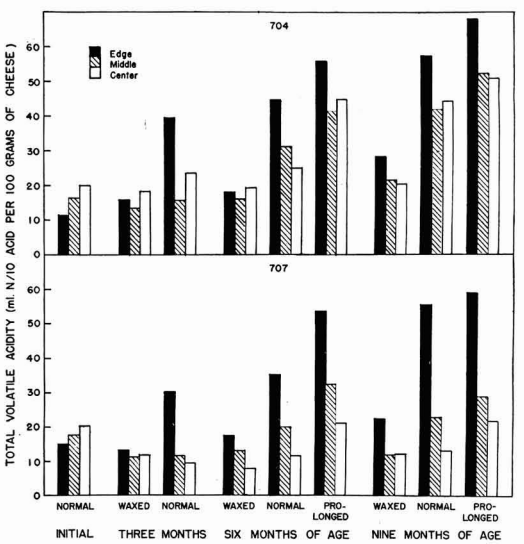

FIG. 3. The relation of waxed (no slime), normal and prolonged slime treatments to total volatile acidity in the edge, middle and center portions of blue cheeses from two lots during the ripening period.

in total volatile acidity than were the middle and center portions of the same cheese. The prolonged slime cheeses were higher in total volatile acidity than the normal cheeses and these in turn were higher than the waxed cheeses.

Surface growth and pH values. The influence of the waxed or no slime, normal and prolonged slime treatments on pH values in the various portions is presented in figure 4. In general, there was a gradation of pH values from a high at the edge portion to a low in the center portion of the slimed cheeses. The waxed cheeses did not show this trend but were practically constant in pH throughout each cheese.

Brine concentration. The brine concentration of the cheeses, that is, the amount of salt in the water of the cheese, was determined because of the effect of salt on the ripening process (11). The brine concentrations of the various

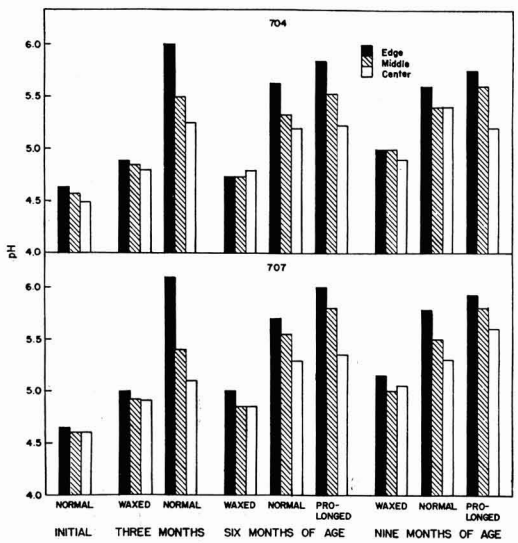

FIG. 4. The relation of waxed (no slime), normal and prolonged slime treatments to pH values in the edge, middle and center portions of blue cheeses from two lots during the ripening period.

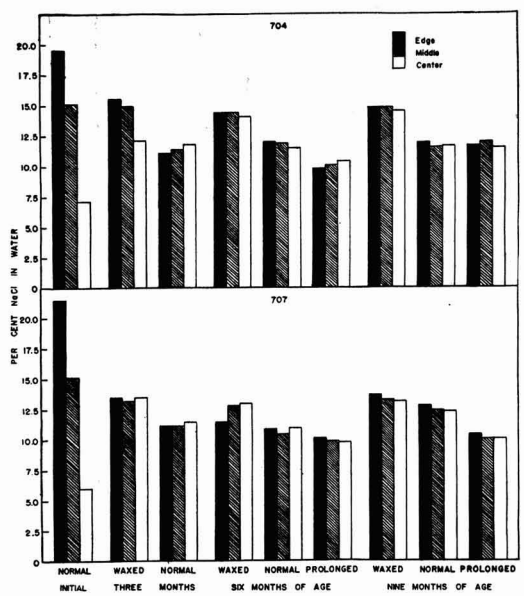

FIG. 5. Brine concentration in the edge, middle and center portions of waxed, normal and prolonged slime cheeses from two lots as determined at 3-mo. intervals during the ripening period.

portions of the cheeses are shown in figure 5. Initially, after salting, the cheeses had a much higher concentration of salt in the edge portion than the middle, and the middle portion was higher than the center portion. Even though the salting procedure was carefully controlled, the waxed cheese had a higher brine concentration than the other cheeses. It is doubtful that the higher brine concentration had a pronounced effect on ripening since the normal and prolonged slime cheeses of lot 704 at 9 mo. of age had essentially the same brine concentration, yet the prolonged slime cheeses were higher in total volatile acidity. The edge portions of all the cheeses had about the same brine concentration at 3. 6 and 9 mo. of age as the middle and center portions, yet the edge portions were higher in total volatile acidity and pH.

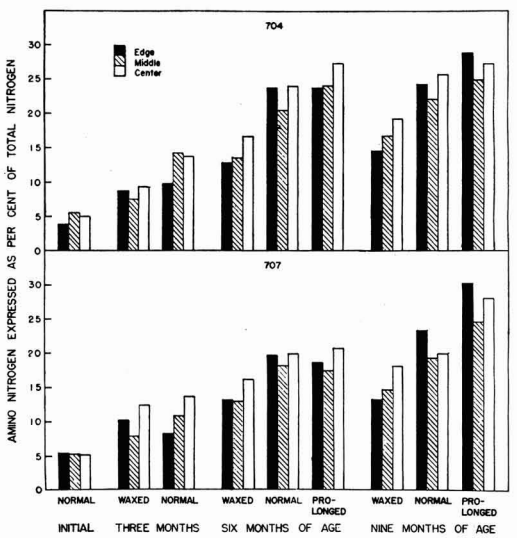

FIG. 6. The relation of waxed (no slime), normal and prolonged slime treatments to the percentage of amino nitrogen in the edge, middle and center portions of blue cheeses from two lots during the ripening period.

Surface growth and per cent amino nitrogen. The changes in the ratio of amino nitrogen to the total nitrogen of the blue cheeses during ripening are presented in figure 6. The percentage of amino nitrogen increased with the age of the cheeses. Here, as with the total volatile acidity and pH, the prolonged slime cheeses were the highest in percentage of amino nitrogen. In each age group the prolonged slime cheeses were the highest in per cent of amino nitrogen. The normal cheeses were next and the waxed or no slime were the lowest.

The organoleptic examination suggested that there was more protein degradation in the edge portions of the normal and prolonged slime cheeses than in the middle and center portions. The results of the amino nitrogen determinations

showed this trend in the prolonged slime cheeses of both lots and the normal cheeses 9 mo. old from lot 707, but the other cheeses did not show this trend.

It may be noted that the center portions generally were higher in amino nitrogen than the middle portions. The brine concentrations of the center portions were lower in the cheese initially and probably for a few weeks after placing them in the cave. At 1 mo. of age, the brine concentration had become approximately equal in all portions of the cheese. The higher concentrations of salt in the edge and middle portions during the early part of the ripening period might have retarded the microfloral activity, especially the mold. The microflora in the center may not have been retarded because of lower salt concentration; thus, the mold may have caused more proteolysis. This may account for the higher amino nitrogen percentages in the center portions over that of the middle portions. Also, the amino nitrogen values may not be a complete expression of the over-all protein breakdown.

DISCUSSION

The surface growth which developed on blue cheese was characteristic of Roquefort and blue cheese in so far as can be determined from the literature. The microscopic observations on the slime microflora corroborate the findings of Macy and Erekson (8), who reported that the development of yeasts in the slime of blue cheese was followed by a dominance of micrococci and rod forms of bacteria.

This work substantiates the statement of Matheson (9), regarding surface growth, that ". . . its presence is always associated with the best grades of cheese." The organoleptic results on the prolonged slime and normal cheeses, however, are in disagreement with the observations made by Evans (2), who observed no flavors in the outside layers more pronounced than the interior of the cheese. The effect of the surface growth on the blue cheese was most noticeable in the prolonged slime cheeses. The slime flavor and aroma were evident throughout these cheeses. Whether this putrid, limburger-like flavor came from the diffusion of the aroma inward and the absorption by the cheese, or was produced by enzymes from the surface microflora migrating inward cannot be definitely ascertained from the organoleptic examinations made in this study. However, due to the color changes and a softer texture in the prolonged slime cheeses, one might suspect migration of microorganisms or their enzymes from the surface inward. The prolonged slime cheeses had a higher percentage of amino nitrogen, a larger amount of total volatile acidity and a slightly higher pH when compared with the normal cheeses and the waxed cheeses. This indicates a definite effect of the prolonged slime treatment on the ripening process.

The normal cheeses showed the influence of the surface growth or conditions connected with the surface growth as measured by increased proteolysis, volatile acidity and pH over that of the waxed cheeses.

An analysis of the results of the total volatile acidity determinations has shown that there was an accelerated ripening in the edge portions of the slimed cheeses. The values obtained from the pH determinations showed evidence of

accelerated ripening proceeding from the surface toward the center. These results might be explained by the activity of (a) the surface microflora or (b) the increased activity of *Penicillium rogueforti,* or both.

On the other hand, evidence of progressive ripening from the surface inward could not be demonstrated by the volatile acidity determinations on the center and middle portions or by the amino nitrogen content of the various portions of the cheeses. This might have occurred yet could not be detected because of the activity of the mold and other microorganisms growing at the interior of the cheese.

Because of the pronounced effect of *P. roqueforti* on the ripening of this cheese, it is difficult to determine the influence of the surface microflora in producing the changes noted in the various portions of the cheese. Cheeses with normal surface growth, however, had higher total volatile acidity, pH and amino nitrogen, better body, texture and a slightly finer flavor than did the waxed cheeses. This is not meant to imply that good blue cheese cannot be made in the absence of slime.

SUMMARY AND CONCLUSIONS

Unripened cheeses from each of two lots were segregated into three groups and were processed to produce various amounts of slime during the ripening period. The three groups were: (a) a waxed group on which no slime was permitted to develop, (b) a normal group which was permitted to slime for the first 2 mo. in the cave, and (c) a prolonged slime group which was permitted to slime in the cave for the first 4 mo. of the ripening period.

Portions of the cheese were examined chemically and organoleptically, initially and at 3, 6 and 9 mo. of age to ascertain if ripening had proceeded inward from the exterior of the cheese. The chemical analyses included determinations of moisture, salt, total volatile acidity, total nitrogen, amino nitrogen and pH of the cheese. The surface growth was observed microscopically and described. Data have been presented indicating the relationship of the surface growth of Minnesota blue cheese or conditions under which it develops, to the ripening of this cheese.

The following conclusions may be drawn from the results secured:

The surface microflora of Minnesota blue cheese initially consists primarily of yeasts and some mold. Later cocci and rod forms of bacteria predominate.

The slime becomes alkaline during the ripening and the acidity of the cheese on which it develops is reduced.

There is accelerated ripening in the portions of blue cheese taken 0.125 in. inward from the surface, as determined by the criteria used in this study.

The activity of surface microflora or better ripening conditions at the surface or both increase the total volatile acidity, pH, and per cent of amino nitrogen and improve the body and texture of the cheese on which the surface growth develops.

Exposing the cheese to excessive or prolonged sliming is detrimental to the flavor, color of the cheese and the color of the mold in the cheese.

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EFFECT OF HIGH TEMPERATURE HEAT TREATMENT OF MILK ON THE AMMONIA CONTENT[1]

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The ammonia content of milk has been studied for many years, mostly in an attempt to correlate the amount of ammonia with the sanitary condition of raw milk. Limited attention has been given to ammonia production caused by high heat treatment, and no attempt has been made to relate this production to other changes such as flavor and color which result. In addition, it is likely that the methods used for measuring the ammonia in these earlier studies may not be as sensitive and accurate as more modern colorimetric procedures.

The effect of heating milk at various temperatures and for various times was studied by Kluge (2). Heating milk at 65° C. for 30 min. increased the ammonia content 0.06 mg. per cent; 71° C. for 1 min. had no effect; 85° C. for 1 min. increased the ammonia content 0.03 mg. per cent; boiling for 2 min. increased the ammonia 0.07 mg. per cent; and sterilization at 120° C. resulted in an increase of 0.98 mg. per cent. Others also have noted increases in the ammonia content of milk upon heating (3, 4).

The purpose of this study was to ascertain the effect of high heat treatment of milk, cream and rennet whey upon flavor, color and ammonia production and to determine the possible relationships among these changes.

EXPERIMENTAL

Procedure. Fresh, raw, mixed milk from the University herd was used. In the first phases, heating was accomplished in a boiling water bath. The milk was contained either in a 3-l., three-necked, round-bottom flask fitted with condenser, stirrer, thermometer and syphon for withdrawal of samples, or in sealed no. 2 Canco plain tin cans. When the milk was heated in the flask, about 20 min. were required for it to reach 96° C., whereas in sealed cans 10 min. were allowed. Samples were obtained after heating at 96° C. for 0, 1, 2, 3 and 4 hr., and were immediately cooled. Unheated control samples also were analyzed.

The method of Choi *et al.* (1) was employed for ammonia determination, except that larger samples were used and the apparatus and amounts of reagents adjusted accordingly. Briefly, the method was as follows: The proteins in 20 ml. of milk were precipitated with 20 ml. of 10 per cent sodium tungstate and 40 ml. of 0.5 N H_2SO_4. This mixture was filtered and 50 ml. of the filtrate

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(representing 12.5 ml. of milk) were pipetted into a 500-ml. Kjeldahl flask connected with steam generator and condensor. Twenty ml. of 8 per cent $Mg(OH)_2$ were added and steam distillation carried out at a rate sufficient to obtain 40 ml. of distillate in approximately 5 min. The receiver contained 10 ml. of 0.01 *N* H_2SO_4 to absorb the ammonia.

The distillate was treated with alkaline phenol and sodium hypochlorite, and then the ammonia was measured colorimetrically by means of a Cenco-Sheard spectrophotolometer, using 10-ml. tubular cells and a wave length of 633 mμ. This wave-length setting was established by transmission determinations. The quantity of ammonia corresponding to the observed transmission was obtained by reference to a standard curve which was prepared in accordance with directions of Choi *et al.* (1).

Preliminary trials were conducted to ascertain the reliability and accuracy of the method in the recovery of known amounts of ammonia added to the milk as $(NH_4)_2SO_4$. The results revealed that for concentrations of 1 to 6 mg. per cent of added ammonia, recoveries averaged approximately 93 per cent.

Effect of heating on the flavor, color and ammonia content of milk. A study was made of the flavor, color and ammonia content of milk heated in an open flask at 96° C. for 4 hr. The data presented in table 1 reveal that the heating

TABLE 1

Flavor, color and ammonia content of milk heated in an open flask

Time held at 95° C.	Flavor[a]	Color[a]	Ammonia content	Variation from control
(*hr.*)			(*mg. %*)	(*mg. %*)
Unheated	Normal	Normal	0.76	.....
0	Cooked 3	Normal	1.32	0.56
1	Cooked 3 Caramel 2	Brown 1	2.54	1.78
2	Cooked 2 Caramel 2	Brown 2	3.69	2.93
3	Caramel 3	Brown 3	4.60	3.84
4	Caramel 4	Brown 4	5.17	4.41

[a] Consensus of two judges, grading the intensity from 1 to 4.

for any one of the hour periods caused the production of ammonia and that the amount produced had increased markedly by the time the milk developed a caramel flavor and a brown color. Heating beyond the first hour resulted in increases in the amount of ammonia at an average rate of 0.96 mg. per cent per hour.

The heating of milk in closed cans yielded results somewhat similar to those obtained for the open-flask procedure, but with greater production of ammonia per unit of heating time (table 2). For example, the results in table 2 reveal an average hourly production of ammonia of 1.25 mg. per cent. The momentary heating produced a slight amount of ammonia (0.55 mg. per cent), whereas no observable production occurred for the same heat treatment when the milk was

TABLE 2

Flavor, color, and ammonia content of milk heated in sealed cans

Time held at 95° C.	Flavor[a]	Color[a]	Ammonia content	Variation from control
(*hr.*)			(*mg. %*)	(*mg. %*)
Unheated	Normal	Normal	0.98	.....
0	Cooked 3	Normal	0.98	0
1	Caramel 1 Cooked 2	Brown 1	2.44	1.46
2	Cooked 1 Caramel 2	Brown 2	3.61	2.63
3	Caramel 4	Brown 3	5.02	4.04
4	Caramel 4	Brown 4	5.96	4.98

[a] Consensus of two judges, grading the intensity from 1 to 4.

sealed. This difference may be due to the greater time required to bring the milk in the open flask to the specified temperature, thus increasing the exposure time of the milk to heat treatment.

Other trials were conducted in which samples were heated to 80, 90 and 95°C. for momentary and for 1-hr. holding periods. The results are presented in table 3. Although slight increases in ammonia were observed for all tempera-

TABLE 3

Ammonia content of milk heated momentarily and for 1-hr. periods at various temperatures

Temperature of heating	Momentary heating period		1-hr. heating period
	Increase in ammonia[a]	Intensity of cooked flavor[b]	Increase in ammonia[a]
(°*C.*)	(*mg. %*)		(*mg. %*)
80	0.02	2	0.20
85	0.05	3	0.54
90	0.05	4	1.06
95	0.32	4	1.49

[a] Ammonia content of the control samples averaged 0.36 mg. per cent for the momentary heating trials and 0.57 for the 1-hr. heating trials.

[b] Consensus of two judges, grading the intensity of the flavor from 1 to 4.

tures in the momentary heating trials, significant increases did not occur until the 95°C. temperature was used. Even for this higher heat treatment, far above the critical temperature for cooked flavor production, the ammonia produced amounted to only 0.32 mg. per cent. It would appear, therefore, that the cooked flavor may not be related consistently to ammonia formation, since this flavor may be produced without appreciable change in the ammonia content of the milk.

The data in table 3 also reveal that heating the milk for 1-hr. periods for any of the temperatures causes the production of ammonia. However, the amounts produced at 80 and 85°C. would indicate that little ammonia is produced at temperatures below 80°C., even when prolonged holding periods are used.

Heating cream and whey. Since, in earlier studies (5), it was observed that some of the heat-labile sulfur of milk was associated with the fat globule protein, it appeared desirable to ascertain if this protein material also contributed to the ammonia produced by heat. In addition, the role of casein was determined by measuring the ammonia produced by heating rennet whey.

TABLE 4

Effect of heat on the ammonia content of cream (20% fat) and rennet whey[a]

Time held at 95° C.	Cream (%)		Rennet whey	
	Ammonia content	Change due to heat	Ammonia content	Change due to heat
(*hr.*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)
Unheated	0.82	.....	0.69	.....
0	0.82	0	1.10	0.41
1	1.60	0.78	2.00	1.31
2	2.77	1.95	3.24	2.55
3	3.87	3.05	3.93	3.24
4	5.04	4.22	4.41	3.72

[a] Heated in sealed cans and agitated at 30-min. intervals.

Results for the heating of cream (20 per cent fat) and rennet whey are presented in table 4. These data, when compared to those for milk (table 2), reveal that the ammonia production is somewhat less in both the cream and the whey. There is no evidence to indicate that the protein associated with the fat is an unusual contributor of the heat-produced ammonia. The lower total production of ammonia from whey suggests that casein and other milk constituents removed by precipitation also may contribute to a slight extent, either directly or indirectly, to the ammonia produced by heat treatment.

In another series of trials, whey was homogenized with mineral oil to ascertain if the creation of new adsorption areas would increase the susceptibility of the proteins to heat, which would be reflected by increased ammonia formation. Results revealed no distinct differences between normal whey and whey-oil mixtures from the standpoint of ammonia production by heat.

SUMMARY

The application of the phenol-hypochlorite colorimetric procedure for determining ammonia revealed that, when milk was heated at 96°C., production of ammonia averaged 0.96 mg. per cent per hour for milk heated in an open flask and 1.25 mg. per cent per hour for milk heated in sealed cans.

The heating of milk under conditions to cause the milk to brown and to develop a caramel flavor resulted in increases in the ammonia content approximating 1.5 to 2 mg. per cent.

It is doubtful that the cooked flavor of milk may be correlated directly with ammonia production, although there was some indication that extremely shall quantities of ammonia were produced at 80°C. Certainly, a more sensitive method for ammonia measurement than the one used in this study would

need to be utilized to establish with certainty any relationship between the critical temperature for cooked flavor production and the simultaneous production of ammonia. Heating temperatures as high as 95°C. for momentary periods produced only small quantities of ammonia.

The non-casein nitrogen is the major source of ammonia produced by high heat treatment.

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THE RELATIONSHIP OF SEMEN PRODUCTION TO SEXUAL EXCITEMENT OF DAIRY BULLS[1]

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Present day practices for collecting semen from dairy bulls reflect considerable variation in the extent of sexual excitement attained by the bulls before they are allowed to serve the artificial vagina and ejaculate (8). Most of these practices and the information concerning their value have been passed from one breeding organization to another without objective evidence from controlled experiments as a basis for their evaluation.

If sexual excitement is one of the factors markedly influencing semen production of bulls, it may be subject to control and exploitation by those responsible for the collection of the semen. Both Anderson (2) and Lagerlof (5) state that in the bull willingness and ability to mate are not an indication of fertilizing capacity. McKenzie and Berliner (6) state that mating desire is no indication of the spermatozoa producing capacity of rams. Nevertheless, they have reported that the number of spermatozoa is less in the first ejaculates of rams that show a strong sexual impulse and mount ewes quickly than it is in subsequent ejaculates. Alifanov (1) has observed a correlation between sexual interest and spermatozoa production in the dog. Kirillov and Morozov (4) have reported a beneficial effect on the quantity and quality of ejaculates if the bulls have spent some time getting ready to mount. Anderson (2) also states that "better quality semen is said to be produced when the bull is held back for a little before being allowed to mount."

Mercier and Salisbury (7), working with dairy bulls, compared first and second ejaculates collected within a few minutes of each other and observed that the second ejaculates contained more motile spermatozoa. In their studies the first ejaculate was collected immediately after bringing the bull from the barn to the collection stall. Thus, the superiority of the second ejaculates over the firsts may have been to some extent a result of increased sexual excitement.

Hart *et al.* (3) have reported on two bulls that did not respond to hormone therapy for lack of libido but did respond to an estrous cow. They state that the presence of sufficient androgen alone is not conducive to sexual drive and normal copulatory behavior, but that stimulation from a cow is needed also.

In view of the lack of quantitative data in the literature pertaining to the relationship of sexual excitement to semen production, particularly in dairy bulls used for artificial breeding, the studies reported herein were undertaken. Spe-

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cifically, the object of the experiments was to determine whether or not induced and controlled levels of sexual excitement in dairy bulls at the time of semen collection would be accompanied by significant changes in semen production.

EXPERIMENTAL PROCEDURES

Two experiments were conducted with 11 bulls in each experiment. The bulls were selected from the temporarily inactive and the new bulls in the stud of the New York Artificial Breeders' Cooperative, Inc., and in each experiment were assigned at random into groups of five and six bulls each. The bulls were kept on one treatment for a period of 5 consecutive wk. and then changed to the other treatment. To avoid any bias between the observed treatments which may have entered the experiment as a result of the unequal numbers of bulls during the 5-wk. treatment periods, the semen criteria were analyzed by the method of least squares (11).

The procedure for accomplishing the increased sexual excitement of the bulls consisted of allowing the bulls to be near a non-estrous cow and restraining them for a period of 2 to 3 min. Then they were allowed to make a false mount, *i.e.,* they were allowed to mount but were not allowed to serve the artificial vagina. After this false mount they were allowed to serve the artificial vagina at the next attempt. Generally, the bulls ejaculated at this time. If they failed to do so they were allowed two more attempts.

The procedure for minimizing sexual excitement consisted of bringing the bulls directly from the barn to the collection stall, allowing them to mount immediately, serve the artificial vagina and ejaculate. No effort was made to restrain them. Occasionally a bull would not mount immediately or if he did mount he would not ejaculate. In those situations, which were infrequent, the semen was collected the first time the bull would mount and ejaculate. Insofar as it was possible to do so, all of the bulls on a particular experimental treatment (restrained or unrestrained) were brought to about the same level of sexual excitement.

The feeding and management of the bulls was the same for all and conformed to the routine for the entire stud. They were fed about 2 hr. before the time of semen collection, the latter being between 6 and 8 a.m. The bulls usually were exercised every other day, either on a mechanical exerciser for a period of 1 hr., which was equivalent to approximately 1 mi. at a slow walk, or in a dry paddock for periods varying from 7 to 16 hr. Both types of exercise were given at least 12 to 18 hr. prior to the time of semen collection.

The criteria used to evaluate the semen from the bulls were the volume (ml.) of semen per ejaculate, the per cent of motile spermatozoa as determined by microscopic examination (10) and the number of spermatozoa per milliliter of semen as determined with a Cenco photelometer according to the method of Salisbury (9).

RESULTS AND DISCUSSION

In table 1 are the least squares estimates of the treatment means for the semen criteria. During the periods when the bulls were restrained the means

TABLE 1

Least squares estimates of means for semen criteria (Based on 218 ejaculates from 22 bulls)

Criteria	Experiment			Mean difference between treatments for experiments 1 and 2 combined
	1	2	1 and 2 Combined	
Volume of semen per ejaculate (*ml.*)				
Restrained	4.5	4.2	4.3	0.64 ± 0.52[a]
Unrestrained	3.9	3.5	3.7	
Motile spermatozoa (%)				
Restrained	49	63	56	
Unrestrained	47	60	53	
Number of spermatozoa per ml. of semen ($\times 10^9$)				
Restrained	1.53	1.36	1.44	0.21 ± 0.15[a]
Unrestrained	1.25	1.21	1.23	
Number of live spermatozoa/ejaculate				
Restrained	3.34	3.89	3.62	1.14 ± 0.66[a]
Unrestrained	2.35	2.61	2.48	

[a] Denotes confidence limits at the 99% level.

were 4.3 milliliters of semen per ejaculate, 1.44×10^9 spermatozoa per milliliter of semen, 56 per cent motile spermatozoa and 3.62×10^9 motile spermatozoa per ejaculate. When the bulls were not restrained, the estimated means were 3.7 milliliters of semen per ejaculate, 1.23×10^9 spermatozoa per milliliter of semen, 53 per cent motile spermatozoa, and 2.48×10^9 motile spermatozoa per ejaculate. The differences between treatments were highly significant ($P < .01$) for all criteria except per cent motile spermatozoa. The increase in the numbers of motile spermatozoa per ejaculate is not interpreted as a reflection of increased spermatogenesis, but instead the increased sexual excitement before ejaculation makes it possible to obtain in each ejaculate a larger proportion of the spermatozoa that are being produced.

The practical application of these findings arises from the fact that when bovine semen is extended to a standard number of motile spermatozoa per unit volume of extender for use in artificial insemination, this increase in numbers of motile spermatozoa is equivalent to approximately 40 per cent more extended semen. Consequently, the number of sires needed to produce a particular volume of extended semen may be reduced. The reduction in the number of sires required by an artificial breeding organization makes possible not only a reduction in the total investment in sires but, what is more important, it makes possible more intensive selection of the sires that are to be used.

SUMMARY

In two experiments with 11 bulls each, restraining the bulls to induce sexual excitement was accompanied by highly significant ($P < 0.01$) increases in semen

volume per ejaculate and in numbers of spermatozoa per milliliter of semen. The number of motile spermatozoa per ejaculate was 41 per cent greater ($P < 0.01$). These results emphasize the practical importance of procedures designed to induce and control sexual excitement in dairy bulls at the time of semen collection.

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PAPER PARTITION CHROMATOGRAPHY OF THE FREE AMINO ACIDS IN AMERICAN CHEDDAR CHEESE

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Although early investigators have analyzed cheddar cheese for some of its protein decomposition products by standard chemical methods, it was not until recently that a more systematic study of the free amino acids in cheese was attempted.

VanSlyke and Hart (11) were among the first to investigate the nitrogenous compounds of American cheddar cheese. They were able to show the presence of tyrosine, histidine, lysine and traces of arginine and tyramine. In another investigation, Tuckey *et al.* (10) working with X-ray diffraction patterns, extracted and identified leucine, isoleucine, tyrosine and probably tryptophane from ripened cheddar cheese. In recent years, Harper and Swanson (7) using a microbiological method, have shown the presence of nine free amino acids in cheese. These were leucine, isoleucine, valine, glycine, proline, glutamic acid, aspartic acid, phenylalanine and lysine. Also, by means of microbiological assay, Reihard and Garey (9) showed the presence of a number of amino acids in both green and ripened cheese. Although definite progress has been made in this field, further research is necessary to attain a more complete picture of the free amino acids and amines in cheese and to evaluate their role in cheddar cheese ripening.

Paper partition chromatography was applied by the author to the determination of free amino acids and amines in ripened cheddar cheese with the hope that it might be possible to show the existence of more free amino acids and also hitherto unsuspected compounds.

METHODS

The two-dimensional, descending paper partition chromatographic method of Consden *et al.* (3) was employed but with minor modifications required to make it applicable to the study of cheese.

Preparation of cheese sample. Six g. of cheese were placed in a Waring Blendor with about 80 ml. of distilled water and the mixture blended for 1 min. The mixture was made up to 100 ml. with distilled water and heated to 75° C. with agitation for 5 min. and cooled to room temperature, after which it was filtered through Whatman no. 12 fluted filter paper.

To 25 ml. of the filtrate were added 75 ml. of 95 per cent ethyl alcohol; the solution was agitated and then allowed to stand for a few minutes until definite precipitation was observed. This mixture was filtered through Whatman no. 42 filter paper (11 cm.). Five ml. of the alcoholic filtrate were placed in a short, wide-mouth test tube and evaporated to dryness overnight in a vacuum desiccator. To the dried material in the test tube 0.3 ml. of distilled water was added

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and, with the aid of a rubber policeman, most of the material was mixed and dissolved.

Application of solution to filter paper. A large sheet of Whatman no. 1 (18.5 × 22.5 in.) filter paper was ruled with a pencil 2.5 in. from the edge down one of the short sides, turned through a 90° angle and ruled again down one of the long sides. At the intersection of these two lines a circle 0.75 in. in diameter was drawn.

The amino acid solution (0.3 ml.) was carefully and slowly applied to the circle with a 1-ml. Luer, Becton-Dickinson-type syringe, while the part of the paper containing the circle was placed directly over an illuminated microscope lamp for quick drying.

Placing filter paper in cabinet. After the area was dry, the filter paper was creased along the shorter pencilled line but about 2 in. from the edge. The edge of this creased line was placed in a pyrex glass trough containing a water-saturated solution of phenol.[1] Two troughs with a capacity of four papers were situated on wooden supports in a pine cabinet about 5 in. from the top. For this study the cabinets were made by the author and fashioned after those of Pratt (8) but were of double capacity. They were of 0.75 in. pine stock, glued and nailed with lapped joints. The outside dimensions were 30 × 30 × 20 in. Plate glass windows 25 × 16 in. were built into either side of the cabinet. The inner edges of the cabinet cover were lined with refrigerator-door, sponge-rubber strips and the cabinet interiors were coated with cheese paraffin to make them airtight and less subject to reagent corrosion. Heavy suitcase clamps were used to lock the cover to the cabinet proper.

The edge of the paper was suspended in the phenol, the length of the paper hanging within the cabinet but over the edge of the trough. A period of 24 to 30 hr. at room temperature or until the phenol phase had almost reached the edge of the paper was used. When starting each phenol run, 1 ml. of 28 per cent NH_4OH was added down the side of the cabinet. The filter paper then was taken out of the phenol cabinet, dried at room temperature in a hood with a fan for from 4 to 18 hr., turned through a 90° angle and placed in a glass trough containing a water-collidine-lutidine mixture[2] situated in another cabinet of similar design to the first. After 48 to 60 hr. in the collidine cabinet, the paper was taken out and dried in a hood at room temperature.

Color development. The dry paper was completely sprayed under a hood with a ninhydrin-*n*-butyl alcohol solution[3] and placed in an oven for 6 min.

[1] Water saturated phenol solutions were produced by adding 300 ml. of distilled water to 453 g. of phenol crystals (c.p.). The mixture was heated to 66° C. and an additional 200 ml. of distilled water added. The mixture was cooled to room temperature, placed in separating funnels and allowed to remain until the two phases, phenol-saturated water and water-saturated phenol were clearly separated. The water-saturated phenol was drawn off when needed.

[2] Water-saturated solutions of collidine-lutidine were produced by mixing 500 ml. of gamma collidine (tech.) and 500 ml. of 2,4-lutidine (tech.) with 1,000 ml. of distilled water. The layers were allowed to settle for about 3 hr. before using the solution. Collidine and lutidine may be obtained from Koppers Co., Inc., Dairy Products Div., Pittsburgh, Penna.

[3] Ninhydrin-*n*-butyl alcohol solution: 1 g. of ninhydrin was dissolved in 1. l of warm *n*-butyl alcohol.

at 80 to 90° C. Identification of the colored areas which developed was made by placing the paper over a large X-ray type illuminator and comparing the areas against chromatograms of known pure amino acids or by consulting charts prepared by Dent (6).

RESULTS

Character of free amino acids in cheese. Chromatograms of 10 commercial cheddar cheeses showed on the average about 16 definite color areas for each cheese. The number varied to a certain extent with age, yet, surprisingly enough, the color spot pattern was similar with the major differences existing in the size and intensity of the individual color areas. However, in a number of cheeses the presence or absence of certain easily identifiable spots was noted.

The protein breakdown products which were interpreted from the chromatograms as being present in practically all cheeses analyzed in this series included leucines, methionine, valine, alanine, threonine, glycine, asparagine, glutamic acid, aspartic acid, proline, arginine, lysine, tyrosine, glutamine and phenylalanine. In some of the cheeses studied, tyramine, α-amino-*n*-butyric acid, serine and cystine as cysteic acid were observed. Histidine, though not too clearly outlined, was considered to be present in a few of the very sharp cheeses. In addition, it appears likely that γ-aminobutyric acid was present in a number of the cheeses. Tryptophane and histamine were not observed in any of the cheeses, although this does not mean they were not present in small concentrations.

A chromatogram of a cheddar cheese presenting all the amino observed has been reproduced in figure 1. This figure shows the location and relative intensity of concentration of the water-soluble nitrogenous compounds found in many of the cheeses analyzed. Those compounds that were observed in only some of the cheeses are encircled with a dotted line.

In a number of the cheeses studied, arginine and lysine were observed as separate areas; in others there was a blending of these two large areas. Consequently, these two acids are considered in combination and are referred to in the future as basic amino acids. In all cheddar cheese analyzed to date, a large yellow area between glutamic and aspartic acid has appeared, causing some distortion in regular shape on these areas. Neither the identity nor the significance of this yellow area is known.

Most of the amino acids and amines portrayed in figure 1 exhibited a purple coloration, but phenylalanine was greenish-black, tyrosine and tyramine were brownish-black, while a few such as glycine, were reddish. These variations from purple are due principally to the effect of pH on the individual amino acids. Some of the color areas may be canary yellow, as with proline, or orange, as with asparagine.

Identification of methionine, methionine sulphoxide, γ-aminobutyric acid and cystine. The area marked leucines-methionine (figure 1) is one of the largest and most intense in color. This was consistently true for all ten cheeses studied. As methionine is known to border the leucine and isoleucine area very closely, it was thought best to label this area as a combination. However, methi-

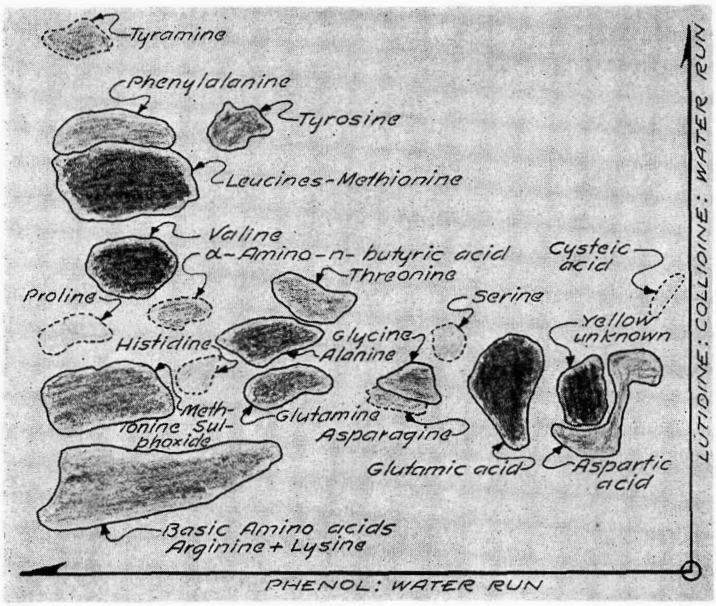

FIG. 1. A chromatographic view of the free amino acids, amides and amines in properly ripened commercial cheese. Composite from 10 cheeses. Solid lines on colored areas indicate presence of compound in all 10 cheeses. Broken area lines indicate presence in some cheeses.

onine, if present, can be identified in a separate run by converting it to methionine sulphone, using an oxidation technique involving H_2O_2 and ammonium molybdate (6). This new compound appears in a different position where it can be identified easily. The presence of methionine in cheese was confirmed by this procedure, but the new areas, although distinct, were quite small and low in color indicating that most of the leucine-methionine area is made up of leucine and isoleucine.

Methionine sulphoxide may appear as a purple area above arginine (figure 1) either because it exists in the biological fluid as such or because some of the methionine may be oxidized to this compound simply by being subjected to reagents such as phenol during the run. However, γ-aminobutyric acid also may occupy this area and, as Block (1) has stated that the latter compound may exist in cheese, an attempt was made to ascertain the true identity of this spot found in cheese. Hydrogen peroxide treatment with ammonium molybdate which would quantitatively transform methionine sulphoxide to its sulphone form did not completely erase this area. The technique of Crumpler and Dent (5), whereby $CuCO_3$ is used to tie up all the alpha amino acids as a copper complex but allow the gamma amino acids to go through, was applied. Using this treatment none of the usual colored spots appeared on the paper, with the exception of the area originally labeled methionine sulphoxide. This area was consider-

ably smaller than the original. As methionine sulphoxide would not show up under the conditions of this technique, it appears from this evidence that γ-aminobutyric acid also may be occupying this same area, thus confirming the observations of Block (1). The area was smaller in size after the oxidation treatment indicating some methionine sulphoxide probably also is present.

Cysteic acid shown on the chromatogram figure 1 was converted from cystine by the H_2O_2 technique suggested by Dent (6). Cystine ordinarily is decomposed by the reagents whereas cysteic acid is stable.

DISCUSSION

Data are presented to illustrate the character of water soluble protein decomposition products in commercial cheddar cheese. Attempts to relate flavor differences with compounds shown by this method will await the attainment of further information on the chemical constituency of cheddar cheese. Results obtained from this study make it apparent that paper partition chromatography can serve as a useful tool for analyses of cheese and other dairy products.

Before this investigation was initiated, it was feared that the salt concentration of the cheese might hinder proper analyses. Consden and Gordon (2) had pointed out that in some biological fluids of high salt concentration results were invariably poor and, as a consequence, they had suggested a desalting apparatus. In the case of these ten cheeses whose salt concentrations ranged from 1 to 2 per cent, no adverse effect was noted as clear chromatograms invariably were produced. This lack of deleterious effect may have been due to the high dilutions used.

It should be pointed out that the possibility exists in this type of analyses that a few of the areas designated as specific amino acids actually may represent another compound. In some instances one amino acid residing near another and having the same color may be confused with it or occasionally lower peptides may take the position on the paper usually occupied by an amino acid. Few workers have experienced any great difficulty in this respect for, with the small number of compounds that might be questioned, there now are available chemical confirmatory tests, marker tests and hydrolytic technics which help to establish identity.

Although peptides did not appear to be present on the chromatograms for the ten cheeses studied, one possibility of this sort might conceivably exist with the glutamine area. Dent (6) has stated that this area may be occupied by either glutamine or glycyl serine. An effective way to check this condition is to hydrolyze the solution at 100° C. for 24 hr. in the presence of 6*N* HCl to note if glycine and serine increased in concentration. Chromatograms obtained on cheddar cheese with and without hydrolysis indicated no increase in these two compounds, thus indicating that glutamine actually was present.

In the phenol cabinet, 1.0 ml. of 28 per cent NH_4OH was added to effect better separation of aspartic and glutamic acids. A few crystals of potassium cyanide also were placed in a petri dish at the bottom of the phenol cabinet to

minimize a pink discoloration on the filter paper which is considered due to phenol decomposition. In the collidine cabinet four drops of diphenylamine were added down the side of the cabinet with each run to bring about better separation of the phenylalanine. These measures were originally suggested by Consden *et al.* (2, 3).

A balance must be struck in analyses of this nature between obtaining color areas that are too large and congested and diluting to such a degree as to exclude many of the amino acids which may be present in lower concentrations. For the doubtful samples in this study this problem was resolved by running a chromatogram with a double concentration of cheese solution followed by one with a normal amount. The optimum amount for normal use in this work was found to be 5 ml.

SUMMARY

Paper partition chromatography was successfully used in the analyses of water-soluble protein decomposition products of a number of commercial cheddar cheeses.

These cheeses were found to contain leucines, methionine, valine, alanine, arginine, lysine, threonine, glycine, asparagine, glutamic acid, aspartic acid, phenylalanine, proline, tyramine, tyrosine, α-amino-*n*-butyric acid, serine, cystine and probably histidine and γ-aminobutyric acid. A number of the cheeses studied did not contain all these compounds.

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THE LIBERATION OF FREE AMINO ACIDS IN RAW AND PASTEURIZED MILK CHEDDAR CHEESE DURING RIPENING

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Observations reported in the preceding paper (6) on the distribution of free amino acids in commercial cheddar cheese showed the usefulness of paper partition chromatography when applied to analyses of water-soluble nitrogenous compounds liberated during cheese ripening. It seemed timely and opportune to study other important issues related to cheese ripening using this procedure.

Two topics which need investigation include the rate of production of free amino acids during cheese ripening and the possible differences existing in the character of amino acid and amine make-up of raw- and pasteurized-milk cheese. Recently Reihard and Gary (7), using microbiological methods, followed the production of a number of amino acids during ripening, but as yet no broad picture has been evolved. Certainly, little is known concerning differences in specific water-soluble nitrogenous compounds of raw- and pasteurized-milk cheese, although it is known that the water-soluble nitrogen generally is higher in the former. Dahlberg and Kosikowsky (2) showed that more tyrosine and less tyramine can be expected in pasteurized-milk cheese than in the raw-milk product.

This problem was studied and the results of this investigation are the basis for this paper.

METHODS

Paper partition chromatography as applied to the commercial cheeses in the preceding paper (6) was used. In addition, a rough quantitative procedure suggested by Block (1) was adapted, colored areas on the test paper being compared to colored chalk standard areas ranging from 0 to 5 in value. The colored areas then were cut out of the large filter paper and weighed. The weight multiplied by the intensity value gave an index, and these indices were compared to standard curves whose index values were obtained in the same manner but using highly purified known amino acids.

In this study, four cheddar cheeses were made in each lot. Three lots of cheese were made over a period of 6 mo., thus providing triplicate series of results.

The four cheeses in each lot varied as to whether cheese was made from raw or pasturized milk and as to whether the starter was a regular commercial *Streptococcus lactis* starter or a combination commercial *lactis*-DK (*Streptococcus faecalis*) starter. The amount of commercial starter used was 1.0 per cent for the pasteurized milk and 0.75 per cent for the raw milk. Combination *lactis*-DK was used in a 0.75 per cent + 0.75 per cent quantity for the other raw and pasteurized milk.

Milk from which the twelve cheeses were made averaged about 3.5 per cent fat and was of average commercial grade with an initial bacterial plate count of

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from 100,000 to 300,000 per milliliter. The milk was pasteurized at 162° F. for 16 sec. About 35 lb. of cheese curd were obtained from each lot of milk. The acidity of the curds just before milling fell very close to the 0.50 to 0.55 per cent range. After the cheeses were pressed overnight at room temperature they were vacuum canned and cured at 60° F. At 0-, 2-, 6-, 14-, 30-, 90- and 180 day intervals these cheeses were analyzed for their free amino acids and amines, using paper partition chromatography.

TABLE 1

Liberation of free amino acids during ripening of raw- and pasteurized-milk cheddar cheeses

Amino acid	Days							
	0	2	6	14	30	60	90	180
				(*Mg./g. cheese*)				
				Raw milk cheese (72949)				
Glutamic acid	0.14	0.22	0.23	0.36	2.28	4.93	> 6.06	> 6.06
Aspartic acid	0.01	0.10	0.09	0.15	0.26	0.56	0.82	1.21
Leucine-methionine	0.00[a]	0.22	1.12	0.51	0.63	2.46	> 6.06	5.42
Basic	0.00	0.17	0.15	1.57	1.73	5.38	> 6.06	> 6.06
Valine	0.00	0.10	0.13	0.24	1.25	3.37	5.00	5.07
Alanine	0.00	0.08	0.05	0.06	0.11	0.64	1.90	1.70
Glutamine	0.00	0.05	0.05	0.03	0.17	0.42	0.36	0.16
Serine	0.00	0.00	0.05	0.00	0.00	0.03	0.00	0.01
Phenylalanine	0.00	0.00	0.00	0.00	1.13	2.22	3.98	4.22
Tyrosine	0.00	0.00	0.00	0.00	0.11	0.18	0.40	1.06
Glycine	0.00	0.00	0.00	0.00	0.07	0.08	0.36	0.97
Threonine	0.00	0.00	0.00	0.00	0.17	0.54	0.40	0.60
Proline	0.00	0.00	0.00	0.00	0.00	0.11	0.24	0.25
Tyramine	0.00	0.00	0.00	0.00	0.00	0.51	0.75	1.43
α-amino-butyric acid	0.00	0.00	0.00	0.00	0.00	0.05	0.08	0.27
Cysteic acid	0.00	0.00	0.00	0.00	0.00	0.00	++	++
γ-amino-butyric acid } Methionine sulphoxide }	0.00	0.00	+	++	++	+++	++++	++++
Asparagine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
				Pasteurized milk cheese (72949)				
Glutamic acid	0.17	0.23	0.08	0.09	0.47	1.03	> 6.06	> 6.06
Aspartic acid	0.05	0.05	0.08	0.08	0.09	0.17	0.22	0.16
Leucine-methionine	0.00	0.06	0.18	0.18	0.64	0.95	3.19	3.19
Basic	0.00	0.19	0.33	0.00	2.36	5.83	> 6.06	> 6.06
Valine	0.00	0.06	0.01	0.06	0.54	3.29	3.94	2.06
Alanine	0.00	0.08	0.08	0.00	0.16	0.62	0.23	0.31
Phenylalanine	0.00	0.00	0.00	0.00	1.37	3.23	3.93	4.57
Tyrosine	0.00	0.00	0.00	0.00	0.40	0.97	0.91	1.14
Glutamine	0.00	0.00	0.00	0.00	0.14	0.58	0.25	0.27
Serine	0.00	0.00	0.00	0.00	0.06	0.27	0.33	0.91
Asparagine	0.00	0.00	0.00	0.00	0.00	> 6.06	> 6.06	> 6.06
Threonine	0.00	0.00	0.00	0.00	0.00	0.19	0.18	0.24
Glycine	0.00	0.00	0.00	0.00	0.00	0.08	0.27	0.33
Cysteic acid	0.00	0.00	0.00	0.00	0.00	0.00	++	++
γ-amino-butyric acid } Methionine sulphoxide }	0.00	0.00	0.00	0.00	0.00	++	+++	+++
Proline	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tyramine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
α-amino-butyric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] Notation 0.00 indicates inability to obtain detectable amount of particular amino acid at analytical period in question.

[b] As + signs increase concentration of amino acid increases.

RESULTS

Although the rate of production of free amino acids were followed through 180 days of ripening for all 12 cheeses in the three lots, table 1 lists only those results from one series and the data presented here are confined to raw and pasteurized milk cheeses made with regular commercial starter.

Free amino acids in cheddar cheese were evident surprisingly early in the ripening period, as shown in table 1. On the day of manufacture, only a few hours after the curds were produced, free glutamic acid and aspartic acid were noted in both raw and pasteurized milk cheese. At the end of 2 days there was a definite increase in the number of demonstrable free amino acids although in all cases the concentration was low. The number of demonstrable amino acids did not increase again until the 30th day of the ripening period, when a total of 12 areas representing amino acids were present for the raw milk cheese, whereas 10 areas were present for the pasteurized milk cheese. Phenylalanine may have been produced earlier but, because of its low sensitivity, high concentrations were required before it was observed. After the 60-day ripening period, a total of 16 amino acids, amides or amines were present for raw milk cheese, while 14 were observed in the pasteurized milk cheese. No further increase in numbers was noted from 60 to 180 days.

Glutamic acid, leucines, basic amino acids, valine and phenylalanine in all cheeses increased steadily in concentration during ripening until they were present in large quantities. In pasteurized milk cheese, beside the above, asparagine could be included. Certain other amino acids showed a much slower increase and a very small buildup over 180 days of ripening at 60° F. In raw milk cheese these included glutamine, serine, threonine, proline and α-amino-butyric acid, while for the pasteurized milk cheese aspartic acid, alanine, glutamine, threonine and glycine had not increased rapidly.

In the later stages of ripening, the raw milk cheese showed the presence of α-amino-butyric acid, tyramine and proline. None of these were observed in the pasteurized milk cheese with regular starter. On the other hand, definite quantities of asparagine were noted in the latter, while not being observed in the raw milk product.

For many of the amino acids the sharpest increase occurred between 30 and 60 days at 60° F., while for a number there was either a reduction or leveling off at 180 days.

Table 2 shows the free amino acid concentration of three lots of cheese, including raw and pasteurized samples, at the end of 90-days ripening at 60° F. The three cheeses, though not showing the same quantity of free amino acids, showed remarkably similar patterns.

Cheeses made with DK starter combinations closely resembled the pattern of amino acids exhibited by either its raw or pasteurized milk counterpart with regular commercial starter, although there was some variation in concentration of a number of amino acids. More cheeses of this nature must be studied to conclude whether these variations in concentrations are significant. However, in the case of tyrosine and tyramine, the differences were very significant. For pas-

TABLE 2

A comparison between the free amino content and flavor of raw and pasteurized-milk cheddar cheese from three different lots (Commercial lactic starter)
(All cheeses ripened 3 mo. at 60° F.)

Amino acid	Cheese 72949		Cheese 72849		Cheese 2491	
	(*Mg./g cheese*)					
	Raw	Past.	Raw	Past.	Raw	Past.
Glutamic acid	> 6.06	> 6.06	> 6.06	> 6.06	> 6.06	> 6.06
Aspartic acid	0.82	0.22	1.85	0.11	2.17	0.33
Leucines-methionine	> 6.06	3.19	4.54	1.66	4.32	2.43
Basic	> 6.06	> 6.06	> 6.06	> 6.06	> 6.06	> 6.06
Valine	5.00	3.94	5.59	1.37	4.70	4.97
Alanine	1.90	0.23	3.65	0.16	1.66	0.06
Glutamine	0.36	0.25	2.19	0.32	0.27	0.42
Phenylalanine	3.98	3.93	3.42	1.33	1.25	0.73
Tyrosine	0.40	0.91	0.34	0.86	0.31	1.35
Glycine	0.36	0.27	0.14	0.14	0.15	0.14
Threonine	0.40	0.18	0.33	0.19	0.35	0.23
Proline	0.24	0.00	0.91	0.00	1.06	0.00
Tyramine	0.75	0.00	0.87	0.00	1.40	0.00
α-amino-butyric	0.08	0.00	0.08	0.00	0.18	0.00
Asparagine	0.00[a]	> 6.06	0.00	2.43	0.00	> 6.06
Serine	0.00	0.33	0.00	0.00	0.11	0.13
Cysteic acid	++[b]	++	+++	++	0.00	0.00
Methionine sulphoxide } γ-amino butyric acid }	++	+++	+++	++	++	+
	39.0	40.0	38.0	39.5	38	39.0
Flavor	sl. unclean sharp	medium	sl. bitter sl. fermented sharp	medium	unclean sharp	sl. acid med. +
	29.0	29.5	27.5	29.0	29.5	29.5
Body	sl. open		open gassy	sl. open		

[a] Notation 0.00 indicates inability to obtain detectable amount of particular amino acid at analytical period in question.
[b] As + signs increase concentration of amino acid increases.

teurized milk cheese with DK starter no tyrosine was observed on the paper, whereas large areas of tyramine were noted after 60 days. Where no DK starter was used in the pasteurized milk, the tyrosine area was high after 60 days and no tyramine was apparent in the cheese. These findings are in agreement with the earlier work of Dahlberg and Kosikowsky (2) on the relationship existing between concentrations of tyramine present in cheese and the activity of specific types of bacteria during ripening.

DISCUSSION

One of the more interesting observations of this study was the rapidity of the development of free amino acids in cheddar cheese. Even a few hours after manufacture at least two free amino acids could be observed. It may be that these acids were residual from the milk itself. Storrs (8) has stated that at least one free amino acid, tyrosine, is present in fresh milk in traces. However, on the

second day the presence of four additional acids further supported the evidence that rapid development of free amino acids occurs in young cheddar cheese.

From these results it might be possible to make the generalization that as total free amino acids increased, the flavor of the cheese increased and that for many amino acids their sharpest increase in concentration coincided with optimum flavor and body. Yet the rapid production of some acids and the slow production of others over the entire ripening period, with the prospect that some may decrease in concentration after ripening reaches a certain point, makes it hazardous to evaluate the role of free amino acids in the development of typical cheddar flavor. It seems apparent that more total free amino acids are produced in raw milk cheese than in the pasteurized milk product, which is in agreement with the work of Reihard and Garey (7), but at the same time the level of certain specific amino acids appears to be higher in the latter. This experimental work was not designed for such flavor evaluation and possibly further research especially pointed for this objective may supply the necessary information.

Although histidine and histamine were not observed with these cheeses, this does not preclude the fact that they may be present. Histidine was considered to have been detected in a small number of cheddar and foreign types of cheeses studied in earlier work but because of its low sensitivity might not appear in many cheddar cheeses. Dent (4) states that histidine is detectable in about ten times the amount required for alanine, while eight times the amount of histamine are required when compared to alanine. Even with these lower sensitivities, it is apparent that when histamine or histidine are present in cheddar cheese their concentration must be quite low. It also is considered that those compounds declared not to have been observed in the pasteurized-milk cheese under study might be found if a large number of pasteurized milk cheeses were analyzed. An excellent sample is in the case of tyramine which exists in extremely low concentration in many pasteurized milk cheeses. Yet it has been shown previously (2) that for a few cheeses of this type, especially those of high cheddar flavor, the tyramine concentration may be high.

Asparagine appears to be more characteristic of pasteurized-milk cheese than of raw-milk cheese but probably does not exist in as large amounts as indicated by these results. The reason for this anomally is that asparagine rests so closely to glycine that it is almost impossible not to have a larger error than exists with many of the other amino acids. This greater error would extend only to its quantitative aspects and not to its identification, since the asparagine spot is brownish-orange while that for glycine is purple. The significance of large amounts of asparagine in pasteurized-milk cheese but not in raw-milk cheese is not known. Apparently, specific agents or enzymes destroyed by the heat were not available to convert this compound to such amino acids as aspartic. Raw milk cheese, on the other hand, contain much greater amounts of aspartic acid than pasteurized milk cheese.

Although the quantitative method was rough and the results subject to considerable experimental error, it seems reasonable to assume, in light of the earlier information on cleavage products liberated during ripening, that many changes

are occurring throughout the entire cheese ripening period. With the advent of newly released substrate or bacterial enzymes, some of the accumulated free amino acids may be converted to other compounds at various stages of ripening. The leucine-methionine area, from all available evidence, apparently is made up of high concentrations of leucine and of low concentrations of methionine. The latter amino acid is considered by Dent (3) to be a source of α-amino-butyric acid, a compound found in raw milk cheese. Decarboxylation of glutamic acid by specific bacteria is stated by Virtanen *et al.* (9) to result in γ-amino-butyric acid in legumes. This may explain the leveling-off or decrease in concentration in cheese of some of the amino acids during the time of ripening.

Quantitative results obtained in this study were of the same order of those obtained by Harper and Swanson (5), whose results were obtained using microbiological methods. The quantitative aspects of the sulphur containing amino acids and γ-aminobutyric have not been fully evolved and results are presented as relative amounts.

SUMMARY

A study was made of the rate of production of free amino acids, amides and amines in American cheddar cheese from the date of manufacture over a ripening period of 180 days at 60° F. Paper partition chromatography was used throughout and the compounds were determined using a rough quantitative procedure. In addition, differences in the character of the amino acids between raw- and pasteurized-milk cheese were noted.

The appearance of many free amino acids was very rapid in both raw- and pasturized-milk cheeses. At the end of 2 days at 60° F., 40 per cent of the number of free amino acids that were found in the cheese had appeared. Glutamic acid, leucine-methionine, basic amino acids, valine, and phenylalanine reached high concentrations in the raw milk cheese over the 180-day ripening period, while for the pasteurized milk cheese glutamic acid, basic amino acids, phenylalanine and asparagine increased to high proportions. On the other hand, glutamine, serine, threonine, proline and α-amino-butyric acid were found in low concentrations in raw milk cheese while aspartic acid, alanine, glutamine, threonine and glycine developed in low concentrations in the pasteurized product.

Proline, tyramine and α-amino-butyric acid were detected in raw-milk cheese but not in the pasteurized-milk cheese. Asparagine was present in large quantities in pasteurized milk cheese but was not observed in raw milk cheese.

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GENETIC CORRELATIONS BETWEEN FIVE BODY MEASUREMENTS, WEIGHT, TYPE AND PRODUCTION IN THE SAME INDIVIDUAL AMONG HOLSTEIN COWS[1]

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The progress made when selecting for two or more characteristics depends in large part on the actual intensity of selection, the heritabilities of these characteristics and the genetic correlations between these characteristics in the same individual. The present study was an attempt to estimate various genetic correlations in Holstein cows and to investigate the commonality of these correlations.

Genetic correlations are not to be confused with phenotypic correlations. The latter are the net results of genetic correlations and, of similarities of the environment which affect both characteristics. By genetic correlation is meant here the correlation between the sets of genes which affect two characteristics on the same animal. The operations by which the genetic correlations are estimated here pick up only the average or additive effects of these sets of genes plus a bit of their epistatic effects, since only these contribute to the likeness between daughter and dam which was the basis of these estimations. The rest of the epistatic effects and the dominance effects might have been picked up if the estimates could have been based on a sufficiently large population of identical twins but such was not available. Similarity of environment merely means that an environmental circumstance happening to an individual may well have had similar or opposite effects on two or more characteristics. Indeed, it would be rare that an environmental circumstance which had a major effect on one part of the body would fail to have at least secondary effects on other parts, since the animal is a physiological unit to a considerable extent and there is so much interaction and coordination between different parts and organ systems. The internal environment or physiological balance of the animal would be a big factor in this.

Genetic correlations can be caused by linkage of genes, manifold effects of genes (pleiotropy) and different intensities or directions of selection in the non-interbreeding subgroups of a population. Linkage can be an important cause of genetic correlations only in a population where either the coupling or repulsion phase of the double heterozygote is far more abundant than the other. Such a condition would persist for only a few generations after a cross because, in a freely interbreeding population, the coupling and repulsion phases of the double heterozygote tend rapidly to become equally frequent. Different intensities or directions of selection in the non-interbreeding subgroups of a population would tend to make different groups of genes rare or abundant in the different subgroups. These group differences in gene frequency would contribute to genetic

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correlations when the whole population is considered as a unit, neglecting differences between the subgroups, but would not cause genetic correlations within subgroups, each considered as a unit by itself. Cases of this have recently been emphasized by MacArthur (13) and Mather and Harrison (14). Manifold effects of genes would be a cause of genetic correlations, regardless of the type of population encountered, provided the population is not homozygous for the genes with manifold effects.

Much of the published work showing genetic correlations between characteristics has involved a qualitative and a quantitative characteristic. Lindstrom (9, 10) and Green (5). In these cases the genetic correlations were attributed to the linkage of a qualitative gene with quantitative genes, but the results could have been due to the manifold effects of genes.

Why would one expect some genes to have manifold effects? Grüneberg (6) reasons that some genes would have manifold effects because the number of observable characteristics in an organism is infinite, while the number of genes is limited. It follows that many (perhaps most) genes must affect not merely one organ or characteristic but several at a time. The term "pleiotropism" or "pleiotropy" has been coined to cover this diversity of action of a single gene. Grüneberg proposes three ways in which a gene may produce manifold effects: (a) A gene may affect two characteristics directly but in independent ways. (b) A gene may affect two characteristics in essentially the same way; its primary product may affect them alike. (c) A gene may affect one characteristic directly and this characteristic will affect another characteristic. Grüneberg calls the first method of action genuine pleiotropy and the other two spurious pleiotropy. Dobzhansky and Holz (2) point out that, since the primary effects of no genes are known, it is futile and not pertinent to classify pleiotropy as genuine or spurious. Regardless of whether pleiotropy is genuine or spurious, genes do contribute to genetic correlations whenever the same genes affect two or more characters.

Some evidence on the manifold effects of single genes has been reported by Dobzhansky (1). This evidence was contested by Schwab (16), but by subsequent experiments Dobzhansky and Holz (2) gave strong evidence to support their earlier findings. Lately, Russell (15) has presented a case of one gene having manifold effects. It seems evident that some genes do have manifold effects, thus causing genetic correlations.

Hazel (8) obtained estimates of genetic correlations between score, weight and productivity in swine by correlating the phenotypic expressions of one character in one animal with the phenotypic expression of another character in a closely related animal. By this method he found a genetic correlation of 0.519 between the score and weight and correlations of zero between weight and productivity and score and productivity. Using Hazel's method and single production records, Tyler and Hyatt (18) found the genetic correlation between milk and fat production to be 0.85, that between milk and per cent fat to be -0.20 and that between butterfat and per cent butterfat to be 0.26. The corresponding phenotypic correlations were 0.93, -0.14 and 0.23.

SOURCE OF DATA

The data for the present study were taken from the Iowa State College Holstein herd over the period from 1932 to late in 1945. All 187 daughter-dam pairs that had records of body measurements, weight, type ratings and milk and fat production at 3 yr. of age were included in the study. The 187 daughters were by 22 different sires and from 180 different dams. Seven of the dams had two daughters by the same sire.

The production records were on a 2X, 243-day,[3] mature-equivalent basis, and the record which began nearest the third birthday was used. The type ratings usually were done by a group of three men; the type rating given was a weighting of the three independent ratings. Type ratings were divided into 18 categories by dividing each official class into three subclasses of low, middle and high. The classes were assigned numerical values from 0 through 17 in order to express them quantitatively. The weights were single weighings, recorded to the nearest 2 lb. In five cases the cows were very near calving, but no correction was made for this. Each body measurement, wither height, chest depth, body length, heart girth and paunch girth, was the average of three independent estimates. A description of these measurements, how they were taken and the errors which influence them was given by Touchberry and Lush (17).

ANALYSIS OF DATA

The first problem is to estimate the genetic correlations between the various characteristics measured. Obviously, the correlations between the phenotypic expressions of the characteristics on the same animal cannot be used, for they contain an environmental component as well as the genetic component. Hazel (8) has suggested the use of close relatives to avoid the effects of the common environment on characteristics of the same animal.

Figure 1 diagrams by path coefficients the correlations between wither height (X) and chest depth (Y) of a dam and the same two measurements on her daughter. In the figure, E_x, E_y, E'_x, and E'_y are the sum of all the factors except the genic ones affecting the two body measurements. G_x and G'_x are the genic values for wither height in the dam and her daughter respectively, and G_y and G'_y are the corresponding genic values for chest depth. The paths, g_x and g_y are the square roots of the heritability of wither height and of chest depth. U is that part of the dam's total genic value which affects her genic value for wither height independently of her genic value for chest depth. V is that part of the dam's total genic value which affects both her genic value for wither height and her genic value for chest depth, and W is that portion of the dam's total genic value which affects her genic value for chest depth independently of her genic value for wither height. U', V' and W' are the same portions of the total genic value in the daughter. The portions of total genic value U, V and W are independent of each other; therefore, it is reasonable to assume that U is independent of V' and W', V is independent of U' and W' and W is independent

[3] This was used instead of the usual 305-day record in order to avoid variations from or corrections for early rebreeding.

of U' and V'. As the animals are dam and daughter, U and U', V and V', and W and W' would be correlated, and these three correlations are shown in figure 1.

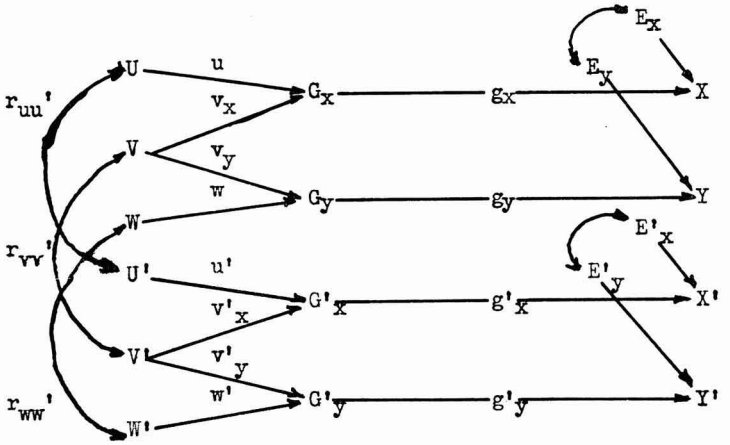

FIG. 1. Path coefficient diagram of the genetic correlation between wither height and chest depth using a dam and her daughter.

Examining figure 1, it is seen that the product of the paths v_x and v_y is the correlation between G_x and G_y and the product $v'_x v'_y$ is the correlation between G'_x and G'_y. The problem now is to find the two correlations $v_x v_y$ and $v'_x v'_y$ from the phenotypic characteristics X, Y, X' and Y'. We can safely assume that $v_x = v'_x$, $v_y = v'_y$, $g_x = g'_x$, $g_y = g'_y$, $u = u'$, $w = w'$ and that $r_{uu'} = r_{vv'} = r_{ww'}$. With these identities it can be shown that

$$\sqrt{\frac{(r_{XY'})\ (r_{X'Y})}{(r_{XX'})\ (r_{YY'})}} = v_x v_y. \tag{1}$$

The genetic correlation is now expressed in terms of quantities which can be measured. Formula (1) is the same as that derived by Hazel (8) and may be expressed in terms of covariance or, in certain types of data, preferably in regressions.

The correlations between the nine variables in the dams and the nine variables in the daughters, required when formula (1) is used, were computed on an intra-sire basis so as to avoid any environmental trends that might occur over the period of years. These intra-sire correlations are given in table 1 and were used in estimating the genetic correlations. The 187 dam-daughter pairs, with the daughters coming from 22 different sires would normally give 164 degrees of freedom for the correlations in table 1, but seven of the dams had two daughters by the same sire. This reduces the intra-sire degrees of freedom for the correlations from 164 to about 158. Lush (12) gives a formula which applies to such cases. The standard errors of the correlations in table 1 would be of the order of 0.07 or 0.08 but the correlations were carried to the third

TABLE 1

The intra-sire correlations between the nine characteristics measured on different but related (dam and daughter) animals

Measurements on the dam		Measurements on the daughter								
		A	B	C	D	E	F	G	H	I
Wither height	A	0.363	0.318	0.281	0.255	0.144	0.212	−0.009	0.087	0.033
Chest depth	B	0.298	0.401	0.269	0.301	0.134	0.191	−0.006	0.043	−0.033
Body length	C	0.239	0.246	0.288	0.223	0.090	0.187	−0.045	−0.101	0.084
Heart girth	D	0.182	0.287	0.001	0.307	0.180	0.198	0.016	0.034	0.019
Paunch girth	E	−0.006	0.103	0.014	0.140	0.133	0.078	−0.009	0.028	0.050
Weight	F	0.154	0.198	0.196	0.224	0.148	0.185	−0.022	0.026	0.023
Type rating	G	0.107	0.127	0.174	0.050	0.069	0.109	−0.085	0.139	0.158
Milk production	H	−0.122	−0.107	−0.037	−0.173	−0.180	−0.187	−0.219	0.127	0.098
Fat production	I	−0.106	−0.111	0.014	−0.148	0.114	−0.107	−0.186	0.438	0.175

Correlations $\geqq 0.16$ are significant at the 0.05 level of probability.
Correlations $\geqq 0.21$ are significant at the 0.01 level of probability.

decimal in the subsequent calculations, lest errors from dropped decimals should accumulate to become important.

Nine estimates of heritability can be made from the correlations on the diagonal from the upper left to the lower right hand corner of table 1 by doubling the nine correlations. The average inbreeding coefficient of these animals was approximately 0.045, so the average relationship would differ little from one-half. By doubling the correlations the four body measurements, wither height, chest depth, body length and heart girth have heritabilities of 0.73, 0.80, 0.58 and 0.61, respectively. The four characteristics, paunch girth, weight, milk production and fat production, have heritabilities of 0.27, 0.37, 0.25 and 0.35, respectively. The first four characteristics given above are largely measures of skeletal size and are little influenced by ordinary variations in environment. Paunch girth and weight are considerably influenced by stage of pregnancy and of lactation.

The intra-sire correlation of −0.085 between the type ratings of dam and daughter is not significant and could be due to sampling errors or to errors in making the type ratings. Harvey (7) found a heritability of 0.14 of intra-herd deviations in type based on nearly 3,000 daughter-dam pairs in the Jersey HIR. Tyler (19) found a heritability of 0.30 for single type ratings. His estimate was based on 3,738 daughters out of 1,601 dams and thus his estimate was subject to less sampling error. Nevertheless, the −0.085 remains the best estimate of the intra-sire correlation between the type ratings of dam and daughter in these data. Besides sampling errors, selection could be a factor affecting this correlation. If overdominance were real and the poorest dams had been culled, a nega-

tive heritability could result. As this seems unlikely in the present data, the negative correlation was given a value of zero in the succeeding analyses. In this quantity of data all these estimates of heritability have standard errors of the order of 0.15 or less. Hence, their exact magnitudes are not closely established, yet there seems no reason to suppose that they are biased.

Using the appropriate correlations from table 1 in formula 1, estimates of the genetic correlations were made and these estimates are given in table 2. As

TABLE 2

The phenotypic (top figure) and the genetic (bottom figure) correlations between the nine characteristics

Characteristics		B	C	D	E	F	G	H	I
Wither height	A								
Phenotypic		0.738	0.670	0.634	0.274	0.534	0.135	0.021	−0.009
Genetic		0.807	0.801	0.646	0.313	0.698	0	−0.081	−0.145
Chest depth	B								
Phenotypic		.....	0.712	0.810	0.425	0.665	0.183	0.015	−0.009
Genetic		.....	0.758	0.838	0.514	0.715	0	−0.142	−0.069
Body length	C								
Phenotypic		.....	.....	0.583	0.404	0.701	0.151	0.022	−0.098
Genetic		.....	.....	0.555	0.179	0.831	0	−0.317	0.150
Heart girth	D								
Phenotypic		.....	.....	.....	0.607	0.808	0.210	−0.083	0.086
Genetic		.....	.....	.....	0.788	0.883	0	−0.351	−0.278
Paunch girth	E								
Phenotypic		.....	.....	.....	.....	0.843	0.208	−0.002	−0.026
Genetic		.....	.....	.....	.....	0.685	0	−0.584	0.496
Weight	F								
Phenotypic		.....	.....	.....	.....	.....	0.229	−0.044	−0.080
Genetic		.....	.....	.....	.....	.....	0	−0.526	0.235
Type rating	G								
Phenotypic		.....	.....	.....	.....	.....	.....	0.182	0.258
Genetic		.....	.....	.....	.....	.....	.....	0	0
Milk production	H								
Phenotypic		.....	.....	.....	.....	.....	.....	.....	0.871
Genetic		.....	.....	.....	.....	.....	.....	.....	0.707
Fat production	I								

Phenotypic correlations $\geqq$ 0.15 and 0.20 are significant at the 0.05 and 0.01 levels of probability, respectively.

an example of the calculations, the genetic correlation between wither height and weight is derived by substituting the appropriate figures from table 1 in formula (1), thus:

$$\sqrt{\frac{(0.212)\ (0.154)}{(0.363)\ (0.185)}} = 0.698.$$

This figure is found in line *A*, column *F*, of table 2. When one figure in the numerator was negative and the other positive, the arithmetic mean of the two figures in the numerator was used in place of their geometric mean to avoid the difficulty of the square root of a negative number. When both figures in the numerator were negative, the geometric mean was used just as when all the figures were positive except, of course, the genetic correlation was given a nega-

tive sign. All genetic correlations involving type were given a value of zero. In doing this, little information was lost for only four of the 17 intra-sire correlations involving type were significant, and the correlations in the numerator of the formula for estimating genetic correlations usually were of opposite signs. Despite their insignificance, the intra-sire correlations between the type of the dam and the body measurements, weight and production of the daughter are all positive while those between the type of the daughter and the body measurements, weight and production of the dams are consistently negative and much smaller in absolute size. If one assumes a small positive phenotypic correlation and a zero or very small genetic correlation between type and the other characteristics, then the negative heritability of type could partly explain the small negative correlations between the type of the daughter and the other characteristics on the dam, and at the same time the small positive correlations between the type of the dam and the other characteristics on the daughter could exist. If the figure obtained by Harvey (7) or Tyler (19) is used in place of the – 0.085 as the correlation between the type of dam and the type of daughter, illogical values result when estimating the genetic correlations involving type.

Perhaps the best estimates of the genetic correlations involving either H or I alone are zero, as they were derived from intra-sire correlations which could have large sampling errors and since the two estimates of the same intra-sire correlations usually were of opposite signs. The milk and butterfat production of the dam are consistently negatively correlated with the body measurements and weight of the daughter, while the production characteristics in the daughter are consistently positively correlated with the body measurements and weight in the dam; the latter correlations are smaller in absolute size in 10 of the 12 cases. The reason for the consistent difference in these correlations is not apparent.

This leaves the 15 genetic correlations between wither height, chest depth, body length, heart girth, paunch girth and weight and the one between milk production and fat production as perhaps the only ones that are reliable estimates of genetic correlations. The ten genetic correlations between wither height, chest depth, body length, heart girth and weight were based on four positive significant intra-sire correlations in all cases, except one which was based on four positive correlations but only three of the four were significant. These 10 genetic correlations agree rather closely with those which can be derived from Gowen's work (4). The five genetic correlations involving paunch girth and the five characteristics above usually were based on but one significant intra-sire correlation, but the other three intra-sire correlations involved usually were near the level of significance and only one was negative. The genetic correlation between milk production and fat production was based on two positive significant intra-sire correlations and two positive intra-sire correlations that were just under the level of significance.

The phenotypic correlations between the nine variables on the same animals were intra-sire correlations. These phenotypic correlations are given in table 2 along with the corresponding genetic correlations. Considering the 16 genetic correlations which seem reliable and comparing them with the corresponding

phenotypic correlations, it is seen that the genetic correlations generally are larger, as would be expected. The four phenotypic correlations between body length and heart girth, body length and paunch girth, paunch girth and weight, and milk production and fat production are larger than their corresponding genetic correlations. In these cases, especially in the last two, one would expect high positive environmental or physiological correlations or both, so the results are not surprising. Fat is a constituent of milk, so the correlation between the two might just be termed largely automatic. The phenotypic correlations between body length and heart girth and body length and paunch girth are unlikely to be influenced by environment, as body length is almost purely a skeletal measurement and would be affected very little by condition or differences in fatness.

GENERAL, GROUP AND SPECIAL GENETIC SIZE FACTORS

On observing the genetic correlations it seems that there must be a portion of what was called V or V' in figure 1 which is common to all of the five body measurements and weight. This portion will be known from now on as the general genetic size factor, Z, which is diagrammed in figure 2 along with

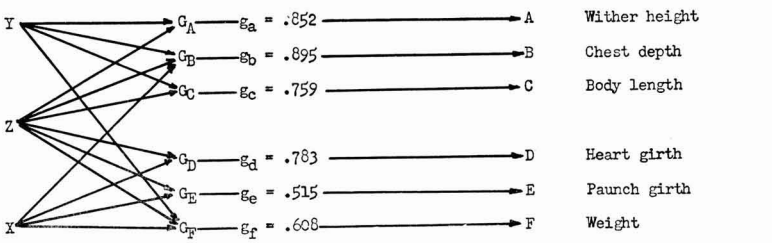

FIG. 2. Path coefficient diagram of the general and group genetic size factors. X—Flesh group genetic size factor. Y—Skeletal-weight group genetic size factor. Z—General genetic size factor. g_i—Square roots of the heritabilities.

skeletal and flesh size factors Y and X. The problem of determining to what extent Z determines the genic values of the six characteristics is solved by path coefficients as developed by Wright (20). Approximations to the least squares solution of the paths from the general genetic size factor to the six genic values are derived first. Then these values are adjusted to give the results more biological meaning. The values of the paths are given in table 3. Part of the solution

TABLE 3

The paths from the general and group genetic size factors

	Z_i		Y_i	X_i
	Least squares	Adjusted	Least squares	Least squares
Wither height	0.825	0.732	0.597	.....
Chest depth	0.913	0.810	0.337	0.217
Body length	0.794	0.705	0.452	.....
Heart girth	0.878	0.780	.....	0.668
Paunch girth	0.551	0.489	.....	0.656
Weight	0.937	0.832	0.322	0.369

TABLE 4

Solution for the general genetic size factor

Genic values	Genetic correlations	Z_iZ_j	$\triangle_{ij}$	$^rG_i\ G_j \cdot Z$	$Z_i'Z'_j$	$\triangle'_{ij}$	$^{r'}G_i\ G_j \cdot Z$
$G_A\ G_B$	0.807	0.753	0.054	0.234	0.593	0.214	0.535
$G_A\ G_C$	0.801	0.655	0.146	0.425	0.516	0.285	0.590
$G_A\ G_D$	0.646	0.724	−0.078	−0.290	0.571	0.075	0.177
$G_A\ G_E$	0.313	0.454	−0.141	−0.300	0.358	−0.045	−0.076
$G_A\ G_F$	0.698	0.773	−0.075	−0.378	0.609	0.089	0.235
$G_B\ G_C$	0.758	0.724	0.034	0.135	0.571	0.187	0.450
$G_B\ G_D$	0.838	0.801	0.037	0.187	0.632	0.206	0.562
$G_B\ G_E$	0.514	0.503	0.011	0.033	0.396	0.118	0.231
$G_B\ G_F$	0.715	0.855	−0.140	−0.978	0.674	0.041	0.127
$G_C\ G_D$	0.555	0.697	−0.142	−0.488	0.549	0.006	0.013
$G_C\ G_E$	0.179	0.437	−0.258	−0.509	0.345	−0.166	−0.268
$G_C\ G_F$	0.831	0.744	0.087	0.410	0.586	0.245	0.622
$G_D\ G_E$	0.788	0.484	0.304	0.763	0.381	0.407	0.745
$G_D\ G_F$	0.883	0.822	0.061	0.363	0.648	0.235	0.675
$G_E\ G_F$	0.685	0.516	0.169	0.579	0.407	0.278	0.575

Z_iZ_j—Products of the various pairs of paths (Least squares values).
$\triangle_{ij}$—Difference between the genetic correlation and the calculated genetic correlation.
$^rG_iG_j \cdot Z$—Partial correlations between the various G_i for a constant Z.
The primes denote the adjusted values.

for the general genetic size factor is given in table 4. Column 1 of table 4 repeats the genetic correlations from table 2, while column 2 gives the corresponding genetic correlations obtained as the products of the least squares values of the two particular paths concerned. Column 3 shows the residual genetic correlations, derived by subtracting the figures in column 2 from the corresponding figures in column 1. Several large negative residuals are found in this column. Since the correlations between the various G_i have been derived and the paths from Z to the various G_i have been calculated and are the correlations between Z and the various G_i, partial correlations between the various G_i can be found by the following formula:

$$^rG_iG_j \cdot Z = \frac{^rG_iG_j - Z_iZ_j}{\sqrt{(1-Z_i^2)(1-Z_j^2)}} = \frac{\triangle_{ij}}{\sqrt{(1-Z_i^2)(1-Z_j^2)}}.$$

These partial correlations are given in column 4 of table 4 and are independent of Z, or one might visualize them as the correlations between the various G_i in a hypothetical population where Z would be constant. From the size of the partial correlations, it is evident that some of the negative residuals are unduly large. No plausible biological reason appears for these negative residuals unless excessive growth in one part has automatically interfered with growth in another, and this does not seem likely; hence, it is assumed that these large negatives result mainly from minimizing the sum of the residuals. That is, more has been forced into Z than really belongs there. Since the method has unduly maximized the paths from Z to the various G_i, the paths must be reduced enough to get rid of the negative residuals.

The six cases involving negative residuals were used to determine how much to reduce the paths to remove the negatives in column 3. The six genetic corre-

lations from column 1 of table 4 were added and used as the numerator of the fraction, $\frac{3.106}{3.941}$, whose denominator was the sum of the six corresponding correlations in column 2 of the same table. The square root of this fraction, 0.888, is the factor which multiplies the least squares values of the paths to reduce them sufficiently to eliminate the large negative residuals. These reduced paths are called the adjusted paths and are shown in column 2 of table 3. If we multiply the correlations of column 2, table 4 by the fraction $\frac{3.106}{3.941} = 0.788$, we get the adjusted correlations of column 5, table 4, which is the same as getting the various products of the adjusted paths. By subtracting the adjusted correlations from the genetic correlations of column 1 of table 4, we get a new set of residuals which are given in column 6. Among these are only two negative residuals, and these are relatively small; besides, they occur in cases where the genetic correlations were based on only one significant intra-sire correlation. The factor 0.788 thus has effectively eliminated the negative residuals and the adjusted paths are used as the estimates of the paths from the general factor Z to the various G_i.

If we now examine the positive residuals in column 6 of table 4, several possibilities of grouping are apparent. Among these, one way seemed more satisfactory than the others; it would group G_B, G_D, G_E, and G_F into a flesh group and G_A, G_B, G_C, and G_F together in a skeletal-weight group. The main fault with this grouping is that the small residual correlation, 0.041, between chest depth and weight is included in both groups.

The paths from these two group factors to the various G_i were solved just as the Z_i were solved, and the least squares values of these paths are given in table 3. Tables 5 and 6 give part of the solution for the paths from the two genetic

TABLE 5

Solution for the skeletal-weight group, genetic size factor

Genic values	Residuals	Y_iY_j	$\triangle_{ij}$	$rG_iG_j \cdot Y$
G_A G_B	0.214	0.201	0.013	0.017
G_A G_C	0.285	0.270	0.015	0.021
G_A G_F	0.089	0.192	−0.103	−0.136
G_B G_C	0.187	0.152	0.035	0.041
G_B G_F	0.041	0.109	−0.067	−0.076
G_C G_F	0.245	0.145	0.100	0.118

TABLE 6

Solution for the flesh group, genetic size factor

Genic values	Residuals	X_iX_j	$\triangle_{ij}$	$rG_iG_j \cdot X$
G_B G_D	0.206	0.145	0.061	0.084
G_B G_E	0.118	0.143	−0.025	−0.034
G_B G_F	0.041	0.080	−0.039	−0.043
G_D G_E	0.407	0.438	−0.032	−0.056
G_D G_F	0.235	0.247	−0.012	−0.017
G_E G_F	0.278	0.242	0.036	0.052

group factors. Observing the small residuals in the third column of these two tables and the small partial correlations in the fourth column, it appears that no adjustment of the least squares values of the paths to get rid of large negative residuals is necessary.

Table 7 gives the proportion of the genetic variance of the various G_i attributed to the general and group genetic size factors. These proportions are de-

TABLE 7

Proportion of the variance of the various genic values attributed to general, group and special genetic size factors

Genic values	Z_i^2	Y_i^2	X_i^2	Special
Wither height	0.536	0.356	.....	0.108
Chest depth	0.656	0.114	0.047	0.183
Body length	0.497	0.204	.....	0.299
Heart girth	0.608	.....	0.446	0.000
Paunch girth	0.239	.....	0.430	0.331
Weight	0.692	0.104	0.136	0.068

rived by squaring the paths from the sources to the various G_i. For example, the adjusted path from Z to G_A is 0.732 and the square of this path is 0.536; or 53.6 per cent of the variance of G_A is attributed to the general genetic factor. The figures in the last column of table 7 are the proportions of the genetic variance that can be attributed to special genetic factors which are not included in the general and group genetic factors. They are derived by subtracting the sum of the figures in the same line in columns 1, 2 and 3 from one. The sum of the figures in the line with heart girth is 1.054. The excess of 0.054 is attributed to sampling errors and to the method which has maximized the paths and included the small residual correlation between chest depth and weight in both the skeletal and flesh groups. The paths from the general genetic factor were adjusted but those from the group genetic factors were not. If the paths from the flesh group genetic size factor are adjusted to get rid of the small negative residuals shown in table 6, then the general genetic size factor and the flesh group genetic size factor account for 100.1 per cent of the variance of G_D. The excess of 0.054 certainly is not real and must be attributed to sampling error and methods of calculation.

An estimate of the environmental correlations between the various phenotypes could be made by subtracting the sum of the products of the sets of paths joining two phenotypes from the corresponding phenotypic correlations given in table 2. For example, the genetic correlation contributes $(0.515)\cdot(0.608)\cdot(0.685)$ to the phenotypic correlation between weight and paunch girth. That leaves 0.629 as the environmental contribution. This in turn yields 0.92 as the correlation between the environment which affects weight and the environment which affects paunch girth. Presumably this is one of the largest environmental correlations, since obviously a circumstance such as stage of pregnancy would affect both these characters strongly and in the same direction. On the basis of these environmental correlations, the importance of general, group and special environmental factors could be estimated.

DISCUSSION

It is apparent that the genic values of the five body measurements and weight are interwoven and show much dependence on each other. This is strong evidence of the manifold effects of genes which affect size in general. If a breeder were selecting for heavy weights he would automatically increase all five body measurements, *i.e.*, he would increase size in general. Biologically this is what we would expect, for it is hard to visualize a large animal that is not large in all of these respects. An animal disproportionately large or small in one or two of these characteristics would be an oddity.

Milk and fat production are highly correlated genetically, as is almost inevitable and automatic since the fat is a constituent in the milk, but on the basis of the present data the two seem to be genetically independent of the body measurements and weight. Indeed, the present statistically insignificant figures indicate the genetic relation between milk production and size to be negative if they are taken at their face value. This fact is disturbing in that it seems somewhat contradictory to other studies, particularly those concerning weight and the production of milk and fat. The production records here were age-corrected, and this would have had a tendency to remove such of the phenotypic correlation between weight and production as came from a correlation between age and weight (Gaines, 3). It does not seem likely, however, that this would remove completely the genetic correlation between weight and production nor that it would obscure the correlations between production and the five body measurements. With the relatively small number of daughter-dam pairs the sampling errors could mask these correlations. If the genetic correlations between production and the measures of size were really large, they would surely have shown up more clearly in these data.

Since the intra-sire correlation between the type of the dam and type of the daughter was -0.085 and was inferred to be zero in the further computations, little can be said about any genetic correlations between type and the other variables in these data. Suppose one takes Harvey's (7) estimate of 0.14 as the heritability of type ratings and his estimate 0.18 as the genetic correlation between type and production of milk; then consider the heritability of milk production as 0.25 as found in the present data. With these figures the correlation between the type rating and the genic value for milk production would be $(\sqrt{0.14})\cdot(0.18)$ and the correlation between milk production and the genic value for milk production $\sqrt{0.25}$. Since progress in selection is proportional to the correlation between the criterion and the genic value for which we are selecting, type rating as a criterion of selection for milk production would give only $\frac{(\sqrt{0.14})\,(0.18)}{\sqrt{0.25}}$ or 0.14 as much progress per unit of time as production records would. If the heritability of type ratings was 0.25, the type rating would be about 0.18 as efficient as the production record. Hence, in selecting for production, type rating would be of only a little value as a criterion of selection; however, type is of considerable economic value to some breeders and should not be overlooked in dairy cattle breeding.

SUMMARY AND CONCLUSIONS

In this study 187 Holstein dam-daughter pairs were used; the daughters were by 22 different sires. On each animal the wither height, chest depth, body length, heart girth, paunch girth, weight, and type rating at 3 yr. of age and the milk and fat records for the lactation beginning nearest the third birthday were used.

The heritability of each characteristic and the phenotypic and genetic correlations between the different characteristics were derived on an intra-sire basis. Four of the characteristics, wither height, chest depth, body length and heart girth, had heritabilities of 0.73, 0.80, 0.58 and 0.61, respectively, while heritabilities for paunch girth, weight, milk production and fat production were 0.26, 0.37, 0.25 and 0.35. The first group included characteristics that are primarily measures of skeletal size and presumably would be less influenced than the characteristics in the second group by such variations in environment as occur within a reasonably well managed herd. The various genetic correlations between the five body measurements and weight were relatively large and were proportioned among a general size factor, a skeletal factor and a flesh factor. From 67 to 100 per cent of the variance of the genic values of these six characteristics was determined by these three groups of size factors; the balance was determined by special size factors.

Some single genes do affect several quantitative characteristics. This is especially true in the case of the five body measurements and weight, all of which are measures of size. Consequently, a breeder selecting for large size in any one of these six measures also would increase the size of the other five. The genetic correlation of 0.71 between milk production and fat production gives evidence that there are single genes affecting both characteristics, as is almost inevitable. By selecting for one we would automatically be selecting for the other, but with a lesser pressure. These data offer no evidence that size and type are genetically positively correlated with production and, indeed, point in the opposite direction as concerns size and milk production, but the age corrections would have indirectly removed such correlation as exists because of a positive correlation between age and size.

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SUBSTITUTES FOR FLUID MILK IN FEEDING DAIRY CALVES[1]

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A vast amount of research during the past 60 yr. has demonstrated the difficulty of entirely replacing fluid milk in the ration of dairy calves. Various investigators (5, 6, 7, 11, 13) have successfully replaced a part of the milk with combinations of various plant and animal by-products. Since whole milk usually is expensive, a maximum reduction in the amount used for rearing calves is desirable at times. A major problem in replacing fluid milk appears to be that of furnishing adequate energy for normal growth without causing diarrhea.

Many ingredients have been incorporated into milk-substitute mixtures. The main criteria for selecting the various feedstuffs used have been their nutritive value for other species and their relative cost and availability.

This paper reports a series of studies designed to formulate mixtures capable of satisfactorily replacing fluid whole milk early in the life of the dairy calf.

EXPERIMENTAL PROCEDURE

Male and female calves of the Holstein breed were used in these experiments. The animals were housed in individual pens and bedded with wood shavings. In addition to the substitute, which was added to warm water and fed as a gruel, according to the schedule in table 1, all calves received a good grass-legume

TABLE 1
Schedule for feeding milk substitutes

Age of Calf	Substitute	Water[a]	Milk	Frequency
	(*lb.*)	(*lb.*)	(*lb.*)	
0–6 d.	(With dam 2 or 3 d., then whole milk)			
7–10 d.	0.25	2.0	2.5	Twice daily
11–14 d.	0.50	3.5	1.0	Twice daily
3rd to 5th wk.	0.60	5.0	None	Twice daily
6th wk.	0.60	6.0	None	Twice daily
7th wk.	0.50	6.0	None	Twice daily
8th wk.	0.40	6.0	None	Once daily
9th wk.	None	Ad lib.	None	....

[a] Water also was available free choice to each calf.

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TABLE 2

Composition of milk substitutes (lb.)

Ingredients	Formula No.											
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*12*	*13*
Dried skimmilk	50.00	50.00	50.00	50.00	30.00	30.00	15.00	.....	15.00	20.00	50.00	50.00
Dried whey[a]	30.00	10.00	10.00	10.00	25.00	30.00	45.00	60.00	45.00	27.338	10.00	20.00
Ground beet pulp	.....	20.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Banana meal[b]	.....	.....	20.00	.....	.....	.....	.....	.....	.....	.....	.....	.....
High fat soya flour	.....	.....	.....	20.00	.....	.....	.....	.....	.....	.....	.....	.....
Solvent extracted soya flour	.....	.....	.....	.....	.....	.....	.....	.....	.....	15.00	.....	.....
Apple pomace	.....	.....	.....	.....	10.00	10.00	10.00	10.00	.....	.....	.....	.....
Linseed oilmeal	.....	.....	.....	.....	.....	10.00	10.00	10.00	10.00	.....	.....	.....
Distiller's dried solubles	.....	.....	.....	.....	.....	.....	.....	.....	15.00	20.00	10.00	10.00
Dextrose	7.758	7.758	7.758	7.758	10.958	9.738	9.738	9.738	.....	.....	9.75	9.75
Oat flour	5.00	5.00	5.00	5.00	15.00	5.00	5.00	5.00	12.338	.....	5.00	5.00
Red dog flour	.....	.....	.....	.....	.....	.....	.....	.....	.....	15.00	.....	.....
Blood flour	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	10.00	.....
Dried brewer's yeast	4.90	4.90	4.90	4.90	4.90	4.90	4.90	4.90	.....	.....	4.90	4.90
Irradiated yeast	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Stabilized vitamin A[c]	2.20	2.20	2.20	2.20	4.00	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Trade minerals[d]	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.04	0.04	0.04
$CaHPO_4$	.....	.....	.....	.....	.....	.....	.....	.....	2.30	2.30	.....	.....

[a] Generously supplied by Western Condensing Co., Appleton, Wis.

[b] Generously supplied by United Fruit Co., N. Y.

[c] The stabilized Vitamin A feed in the first five mixtures contained 2,000 USP units/g. That used in the remaining mixtures contained 20,000 USP units/g. Both products were purchased from Distillation Products Inc., Rochester, N. Y.

[d] The trace minerals consisted of 56.57 parts ferric citrate, 19.73 parts cupric sulfate, 21.59 parts manganous sulfate, and 2.11 parts cobaltous chloride. This mixture supplied approximately 20 mg. of iron, 10 mg. of copper, 10 mg. of manganese, and 1 mg. of cobalt/lb. of feed.

mixed hay and a dry concentrate mixture. The control calves were fed according to the limited whole milk dry starter method as described by Norton and Eaton (11). The feeding of the substitutes have made possible a saving of about 300 lb. of whole milk per calf with this procedure.

The ingredients contained in the various mixtures and the chemical composition of the mixtures are presented in tables 2 and 3, respectively.

TABLE 3

Chemical analyses of the milk substitutes

Mixture no.	Moisture	Protein	Ether Extract	Crude Fiber	N.F.E.	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
1	6.66	25.11	0.87	1.07	58.45	7.84
2	6.09	22.23	0.49	3.47	60.71	7.01
3	6.16	22.68	1.28	1.85	61.48	6.55
4	6.14	30.88	4.98	1.86	49.38	6.76
5	6.77	18.94	1.36	3.10	63.31	6.52
6	10.14	22.63	1.14	3.79	54.66	7.64
7	10.93	20.17	1.12	3.90	55.64	8.24
8	8.79	17.39	1.11	4.01	59.91	8.79
9	7.98	21.31	2.30	2.13	55.38	10.90
10	9.63	26.49	2.74	3.42	47.88	9.84
11[a]	7.17	17.81	0.61	0.35	61.68	12.38
12	6.95	34.11	0.40	6.04	42.09	9.86
13	10.54	23.87	2.05	2.14	42.20	3.60

[a] Generously provided by Western Condensing Co., Appleton, Wisconsin. This product is composed of (1) dried whey product, (2) dried whey fermentation solubles (Ribolac), (3) dried milk albumen, and (4) not more than 1.5% of a vitamin supplement consisting of vitamin A, D and wheat germ oil.

Three growth trials were completed. The first involved 85 calves and provided information on nine substitute formulas. There is some lack of justification for considering this as a single trial. The calves were not all on experiment at the same time, but rather were placed on test as space became available over a period of about 18 mo., from September, 1948, to April, 1950. However, it is believed that for all practical purposes the results are indicative of the comparative value of the different mixtures. In the second trial mixture 10, a relatively inexpensive mixture, was compared to a commercial product, no. 11. Thirty calves were used in this trial. The third trial involving 56 calves compared mixtures 10, 11, 12 and 13 with whole milk as a control. The average daily gain to 8 wk. of age was considered the best measure of the value of a substitute under the experimental procedure used, since after this age and when

the calves were fed only hay and concentrates *ad libitum* they all gained at approximately the same rate, regardless of the mixtures fed during the first 8 wk. The digestibility of certain of the mixtures and of several of the carbohydrates used also were measured.

RESULTS AND DISCUSSION

Growth and feed consumption data for the three trials are summarized in table 4.

TABLE 4

The average daily gains and the total hay and starter consumed per calf to 8 wk. of age

Mixture no.	No. of calves	Av. initial wt.	Av. daily gains	Feed consumed	
				Hay	Starter
		(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
	Trial I				
1	10	89.2	0.75±.05	14.6±1.8	56.5± 3.1
2	5	88.8	0.71±.07	9.8±2.9	57.6± 2.2
3	10	89.0	0.79±.08	12.3±2.0	62.1± 5.0
4	5	87.2	0.39±.06**	5.5±1.0	40.6± 4.2
5	10	94.9	0.75±.05	26.0±2.6	58.9± 4.4
6	10	97.4	0.90±.07	23.8±5.3	78.4± 7.6
7	10	109.6	0.79±.06	28.6±3.3	67.3± 3.4
8	5	105.2	0.57±.07*	23.5±7.9	71.1± 1.3
9	9	99.2	0.58±.12*	30.8±4.7	47.1± 2.9
Control	10	100.4	0.79±.04	30.8±5.5	35.0± 4.5
	Trial II				
10	10	94.9	0.88±.13	32.1±6.1	78.1± 9.5
11	10	94.9	1.11±.04	22.0±4.1	90.6± 6.6
Control	10	93.6	1.16±.05	27.9±4.9	78.7± 6.1
	Trial III				
10	8	92.6	0.85±.09	11.8±3.0	71.8±12.7
11	15	96.4	0.97±.06	22.9±5.0	78.3±10.4
12	8	92.3	1.01±.07	33.1±8.7	65.8±12.5
13	8	90.1	1.01±.06	22.6±6.2	60.4± 8.0
Control	16	94.4	1.10±.05	24.3±8.0	69.7± 6.4

* Significantly lower than controls (odds, 19: 1).
** Significantly lower than controls (odds, 99: 1).

Trial 1: Mixture 1, composed largely of milk by-products (table 2), permitted approximately the same growth as the control group fed whole milk. It was ideal from the standpoint of suspension in a gruel. There were no serious cases of scours; however, the animals invariably voided moderately loose feces. Cost was the chief criticism of this mixture.

Beet pulp, which contains pectin, was included as 20 per cent of mixture 2 because pectin has been shown to aid in controlling diarrhea in rats (9). This mixture promoted fair growth, but it was extremely difficult to grind the beet pulp fine enough to give the substitute good mixing properties. The beet pulp did not have any beneficial effect upon diarrhea and it appeared to affect palatability adversely.

Mixture 3 contained 20 per cent of banana meal, another material of considerable pectin content, which has shown favorable effects on intestinal function (1). This meal was made by drying and grinding the entire banana. Mixture 3 proved very palatable and promoted growth comparable to the control calves. The banana meal appeared to be helpful in preventing scours. This product is not available in quantity at the present time, but promises to be of some value for calf feeding if and when it becomes available. However, digestion studies indicated that calves 3 to 4 wk. old were able to utilize only 50 per cent of the dry matter present in a mixture containing 50 per cent banana meal.

A high-fat soya flour (unheated) containing 22 per cent fat comprised 20 per cent of mixture 4. It was hoped that an increase in energy intake would improve weight gains. However, the mixture caused severe diarrhea in four of the five calves at about 4 wk. of age, and they did not respond to treatment. Two of the calves showed lacrimation typical of vitamin A deficiency. Plasma vitamin A and carotene levels for calves on this mixture were 8.4 and 9.4 as compared to 12.5 and 20.2 μg. per 100 ml. for similar calves on mixtures 1, 2 and 3, respectively. It is not clear just how this drop in blood plasma vitamin A was brought about, but it might well have been a direct result of the diarrhea. On the other hand, it also is possible that something in the soya flour interfered with the vitamin A metabolism, as has been suggested by other workers (14,15).

Dried whey normally is in less demand for human consumption than dried skimmilk, and therefore a comparison was designed to determine the extent to which dried whey could replace skimmilk in the substitute. Both of the products studied contained 50 to 55 per cent of lactose, although whey generally is higher in lactose than is dried skimmilk. From reports in the literature concerning the laxative nature of excess lactose (3), it might be supposed that high levels of either whey or dried skimmilk might induce scouring. A recent paper by Daniel and Harvey (2) indicates that the soluble salts of whey also increase the tendency toward diarrhea. Mixtures 6, 7 and 8 were formulated to contain 30, 45 and 60 per cent of dried whey, respectively, replacing in each case an equal amount of dried skimmilk. Each mixture contained 10 per cent of dried apple pomace, included for the purpose of controlling diarrhea (8). Mixture 6, containing the lower level of dried whey, permitted the most rapid gains. The incidence of scours increased with the level of dried whey. Only five calves were started on mixture 8; they all scoured spasmodically and grew poorly.

During the course of the experiments, 10 to 15 per cent of the calves developed a striking dropping away behind the shoulders. Some showed pronounced crookedness of the rear legs. Others were beset with both of the deformities. The large amount of vitamin D included in the diet would appear to remove deficiency of this vitamin as a possible cause of the abnormality. Calcium phosphate was added to mixtures 9 and 10 in amounts to meet the recommended daily intakes of the Committee on Dairy Cattle of the National Research Council, 1945 (10). The added mineral did not correct the abnormal

backs and legs. Mixture 9 differed from previous mixtures, too, in that it contained 15 per cent of distiller's solubles and did not contain brewer's dried yeast. One calf on this mixture died and the other nine gained an average of only 0.58 lb. per day for the first 8 wk.

Trial 2: The purpose of this trial was to compare the most economical mixture, no. 10, with a commercial product, mixture 11. The latter was almost entirely milk solids (see footnote, table 3). The performance of calves on mixture 10 was less satisfactory than on 11. One calf died early in the trial. Autopsy revealed severe broncho-pneumonia and malnutrition. A second calf that finished the trial actually lost weight during the 8-wk. period. The growth of calves on this mixture was extremely variable. Mixture 11 promoted growth approximately equal to the control group. Calves receiving mixture 11 voided very loose, dark colored feces. However, this looseness did not seem to interfere with growth of the calves. The color of the feces apparently is the result of iron contamination of the commercial whey product during the manufacturing procedure. Digestion studies on mixture 11 have shown very good utilization of the product by 3-wk.-old calves (table 7).

Trial 3: This study involved the same mixtures as trial 2 with the addition of mixtures 12 and 13. Mixture 12 is very similar in composition to formula III studied by Williams and Knodt (16). Growth on mixtures 11, 12 and 13 (table 4) was practically the same as that of the calves receiving whole milk. Mixture 10 again proved less satisfactory.

There are certain features about these studies which are difficult to explain in a fully satisfactory manner. For example, none of the calves fed mixtures 1 to 9, nor the milk-fed controls in trial 1, gained as rapidly as calves on similar mixtures in the later trials 2 and 3. We believe that environmental or management factors of which we were not aware rather than differences in the nutritive value of the feeds may be responsible. Therefore, comparisons between the trials appear to be less reliable than between mixtures within any one trial. Another question requiring further research to explain is why mixture 11, composed largely of whey products gave less diarrhea and better growth than mixture 8 which contained 60 per cent of dried whey. Studies are being continued in an attempt to explain these questions.

Most of the calves fed the milk substitutes were normal in appearance except perhaps for slightly rougher hair coats than calves fed whole milk. Calves on the substitutes generally gained less weight than those on whole milk from the first to the fourth week of age (fig. 1). After that age, as they consumed more hay and grain, the calves grew as fast as those fed milk, and at 4 to 6 mo. of age there was no clear difference between the animals. A number of heifer calves reared on substitute mixtures were kept for replacements in the experimental herd. All of these heifers have grown normally and produced in a satisfactory manner in their first lactations. Thus, it apppears clear that several of the mixtures used in these studies gave acceptable results as replacements for most of the fluid whole milk in rearing calves.

Digestion studies. During the growth studies, digestion trials were carried out with certain of the substitute mixtures. In these balance trials the calves were allowed a constant amount of the feeds during a 10-day preliminary period and a collection period of similar length. In the first trial calves were

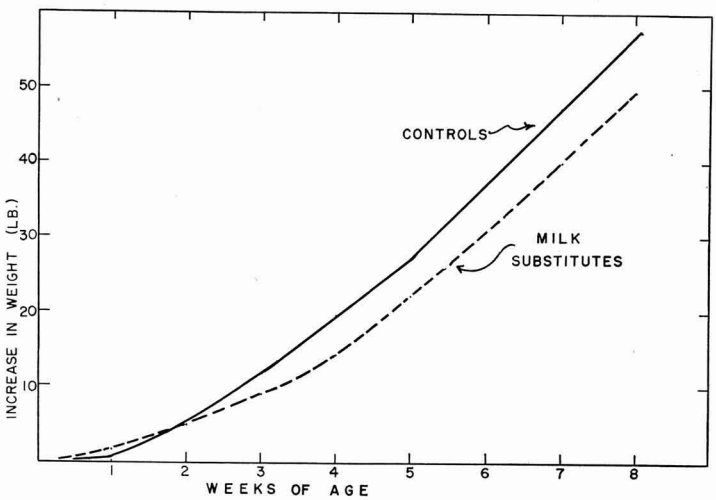

FIG. 1. Comparative increase in weight of 36 milk-fed control calves and 114 calves fed milk substitutes. (Animals from mixtures 4, 8 and 9 not included.)

fed mixtures 2 and 5. Hay and starter were fed as in the growth experiments. Collection of feces was started at 5 and again at 8 wk. of age. The feed intakes and the digestion coefficients observed are shown in table 5. These data show

TABLE 5

The average feed intakes and digestion coefficients for calves fed milk substitutes

Mixture no.	Calves		Daily feed intakes			Digestion coefficients					
	No.	Age	Substitute	Hay	Starter	Dry matter	Protein	Fat	Fiber	N.F.E.	Cellulose
		(*wk.*)	(*lb.*)	(*lb.*)	(*lb.*)	(%)	(%)	(%)	(%)	(%)	(%)
2	2	5	1.0	2.2	4.4	77.0	66.8	47.4	44.6	87.6	50.0
	2	8	1.0	4.4	8.8	80.7	72.9	74.2	50.5	88.8	57.4
5	2	5	1.0	3.3	4.4	79.3	67.8	68.7	45.1	88.8	52.4
	1	8	1.0	4.4	6.6	87.9	83.5	84.2	66.5	93.4	71.2

that both of the mixtures were highly digested to approximately the same extent. All nutrients were more fully digested by the calves at 8 wk. of age than they were at 5 wk.

In the second trial calves were changed to the diet to be studied after 1 wk. on whole milk. Following a preliminary period of constant feed intake, fecal

collections were made for 7 days starting at 21 days of age. The calves were fed only the diets being studied, no hay or starter being allowed. The digestibility of glucose, sucrose and corn starch was compared with banana meal and mixture 11, previously studied. The diets contained carbohydrate, 32.538 per cent; dried skimmilk, 50.0; casein, 10.0; dried brewer's yeast, 4.9; irridiated yeast, 0.10; dicalcium phosphate, 2.30; vitamin A supplement, 0.22; and trace minerals, 0.042 per cent. The chemical composition of the mixtures is shown in table 6.

TABLE 6

The chemical composition of the rations

Ration containing	Percentage composition					
	Dry matter	Protein	Fat	Fiber	N.F.E.	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
Banana meal	91.0	18.8	1.5	3.4	58.7	7.6
Glucose	93.3	27.4	0.7	...	48.5	6.7
Sucrose	96.6	26.8	0.2	...	63.9	5.7
Starch	87.5	27.4	0.7	...	55.4	4.0

TABLE 7

The influence of various carbohydrates upon the digestibility of rations by dairy calves

Ration containing	No. of calves	Digestion coefficients			
		Dry matter	Protein	Fat	N.F.E.
		(%)	(%)	(%)	(%)
Glucose	2	88.3	79.8	....	92.6
Sucrose	2	85.9	75.1	....	93.4
Starch	2	80.7	74.8	....	85.8
Mixture #11	2	88.7	68.1	42.8	96.0
Banana meal	2	51.0	53.0	58.0	54.8

The results (table 7) show that the starch was less well digested than glucose or sucrose, or than mixture 11 (largely whey products). The banana meal diet showed the lowest digestibility of all these mixtures. This was somewhat surprising in light of the satisfactory growth responses of calves fed mixture 3 which contained 20 per cent of banana meal. Calves made average daily gains to 8 wk. of age of 0.80 lb. on glucose, 0.70 lb. on sucrose and 0.61 lb. on the starch diets. These limited data suggest that large amounts of raw starch would be a less desirable source of energy than sugars for very young calves, in agreement with the recent report of Flipse *et al.* (4).

SUMMARY

Studies involving 185 Holstein calves were carried out to formulate substitute mixtures which would satisfactorily replace fluid whole milk in the ration of dairy calves after 10 to 14 days of age. Approximately normal rates of

growth were obtained with mixtures containing large amounts of dried milk solids. In general, however, calves raised on the substitutes were rather rougher in appearance from the third to the seventh week of age than calves fed whole milk. As an average, the rates of growth were somewhat slower on the substitutes during the first month than on milk, but the differences largely were overcome by the time the calves were 4 to 6 mo. of age.

Mixtures containing 20 per cent of high-fat soya flour, or dried beet pulp, or as much as 60 per cent of dried whey resulted in slower growth rates and more digestive disturbances than the other mixtures studied. Banana meal and apple pomace appeared to reduce the incidence of scours; however, all of the mixtures studied caused more diarrhea than fluid whole milk.

In limited digestion studies, dried whey, glucose and sucrose appeared to be highly digestible for the young calf, whereas corn starch and banana meal were less available.

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ELECTROPHORESIS OF MILK PROTEINS II. SOME EFFECTS OF METHODS OF PREPARATION ON THE ELECTROPHORETIC PATTERNS OF WHEY PROTEINS[1]

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Paper I of this series (11) reported several small differences in the electrophoretic patterns of whey proteins prepared by two methods. These methods were first, a salt-acid procedure devised by Harland and Ashworth (6) and second, a new salt-lyophylized procedure. Both of these patterns were approximately similar to patterns reported by Smith (10) and Deutsch (3) using whey proteins separated from casein with acid or with rennet.

Nitschmann and Lehmann (8) found that α-casein, normally a single component, when treated with rennet produced two electrophoretic peaks. Crystalline β-lactoglobulin also has been reported to be electrophoretically homogeneous (1) at some pH values, but to contain three peaks at others (7).

A more extensive electrophoretic comparison of whey proteins prepared by each of the above four methods seemed justified. A pH of 6.8 is near the natural pH value of fresh milk. Values of 7.8, 6.8, 5.8 and 3.8, therefore, were chosen for this study. Areas, mobilities and isoelectric points all seemed useful bases for comparison.

PROCEDURE

To insure that any observed differences would be due to methods of preparation and not to differences in the starting milk, the four procedures were used simultaneously on portions of the same samples of milk. Pooled raw whole milk, cooled as soon as milked, was obtained from the Kansas State College Dairy within 3 to 4 hr. after milking. The cream was separated in a Sharples supercentrifuge. The preparations of whey proteins by the salt-acid method and the salt-lyophilized method were exactly as previously described (11). The acid whey and the rennet whey also were dialyzed, lyophilized and redialyzed in a manner similar to that used for the salt-lyophilized whey.

For preparation of the acid whey, a procedure modified from Rowland (9) was used. Five hundred ml. of skimmilk were heated to 40° C. and placed in a rectangular fruit jar containing a motor stirrer. Fifty ml. of 10 per cent acetic acid were added dropwise, followed after 10-min. standing by 50 ml. of 1.0 *N* sodium acetate. The mixture then was cooled to about 5° C. and filtered after again standing for 2 hr.

Eckles *et al.* (4) described a commercial method for rennet cheese which was

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2 Contains portions of a thesis to be presented by W. G. Stanley as partial fulfillment of the requirements for the degree Doctor of Philosophy in Chemistry at Kansas State College.

adapted here to a laboratory production of rennet whey. Five hundred ml. skimmilk at 30° C. were placed in a rectangular fruit jar with stirrer and 2.5 ml. of a 1 to 20 dilution of commercial rennet extract were added dropwise. Stirring continued 5 to 10 min. until the first tendency to clot was observed. The curd became firm on standing 30 min. It then was cut into 0.5-in. cubes, warmed to 37° C., and again allowed to stand 30 to 40 min. before filtering. Best filtering was obtained with a pad of filter paper that had been pulped in a Waring blendor.

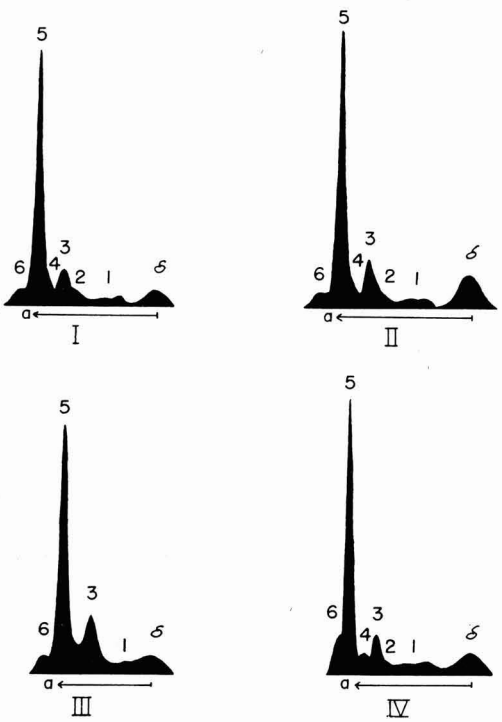

FIG. 1. Electrophoretic patterns of ascending boundaries of four whey protein preparations at pH 7.8; I, salt-acid; II, salt-lyophilized; III, acid-lyophilized; IV, rennet-lyophilized.

Three samples of "non-protein" filtrates from the salt-acid whey protein preparations also were dialyzed and lyophilized to 1/50th, 1/60th and 1/75th, respectively, of the volume of the initial milk. They were dissolved in buffer and dialyzed in a manner similar to that used for the whey proteins.

The buffers used for dialysis and as solvents for migrations were sodium veronal-citrate-chloride solutions. Twenty l. of buffer were made by dissolving 113.4 g. sodium barbital (NaV), 47.64 g. sodium citrate ($Na_3C_6H_5O_7 \cdot 5.5\ H_2O$) and 37.99 g. NaCl in distilled water. Concentrated HCl then was added to produce the desired pH.

Electrophoresis was carried out at 0.5° C. using a Tiselius apparatus equipped for schlieren scanning, as manufactured by Klett Manufacturing Co., New York City. A constant current of 20 ma. and a potential gradient of 6 to 7 volts cm.$^{-2}$ were used. Conductivity of a portion of each protein solution was measured in the electrophoresis bath. Pattern areas due to each component were measured with a planimeter from traced enlargements, using the Tiselius and Kabat (12) method of peak division. Mobilities were calculated for both ascending and descending boundaries. The distances migrated by a peak were measured from the δ and ϵ peaks and from a fixed reference line. A microprojector with a mechanical stage was used in making these measurements. The precision of measurement of actual distance moved was about 0.02 mm.

RESULTS AND DISCUSSION

At pH 7.8 the four preparations of whey proteins produced at least six peaks of approximately similar size and position (figure 1). Similar peaks previously had been found (3, 10, 11) with somewhat similar buffers. At pH values which were lower and nearer the isoelectric points, the small slow-moving peaks 1, 2 and 4 were less clearly resolved (figure 2). The most obvious change in

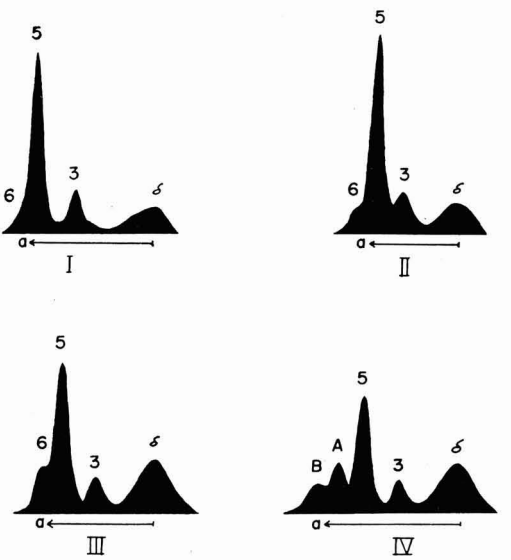

FIG. 2. Electrophoretic patterns of ascending boundaries of four whey preparations at pH 5.8; I, salt-acid; II, salt-lyophilized; III, acid-lyophilized; IV, rennet-lyophilized.

electrophoretic patterns was the appearance, at pH 5.8 in the preparation from rennet whey, of two large fractions labeled A and B. The two fractions A and B comprised 18.0 and 13.4 per cent, respectively, of the total pattern area. These areas seemed to be largely at the expense of the β-lactoglobulin, peak 5. The respective mobilities of peaks A and B (from ϵ peak) were 4.33 and 5.89

$\times 10^{-5}$ cm^2 volts^{-1} sec.$^{-1}$. These fractions also were present but less pronounced at pH 6.8 in the rennet whey preparation. Additional study is being given to several possible interpretations of this effect of rennet. Peak 6 of the acid whey preparation was larger than the corresponding peak of other preparations. At pH 5.8 its area was 10.5 per cent of the total pattern. However, its mobility was distinctly different from that of either A or B. Peak 4 at pH 5.8 appeared in all preparations only as an asymmetry in peak 5, while at pH 7.8 a small but distinct maximum was present in the preparation from rennet whey.

The mobilities at various pH values of component 5 for the four methods of preparation are shown in figure 3. Although the differences in the four curves are rather small, at least two features of figure 3 are typical of all avail-

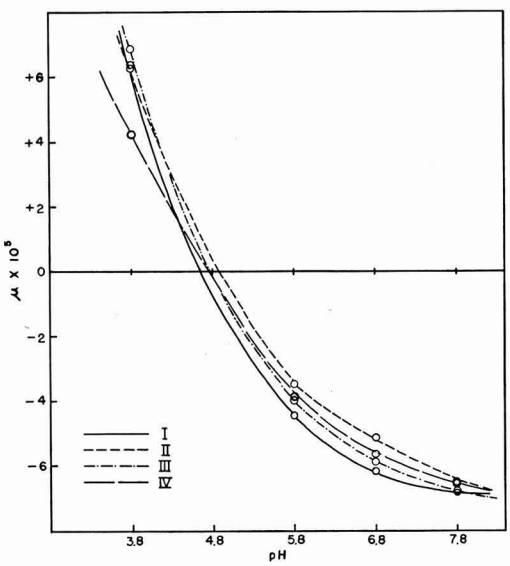

FIG. 3. pH-mobility curve of β-lactoglobulin (peak 5) from whey proteins prepared by four methods: I, salt-acid; II, salt-lyophilized; III, acid-lyophilized; IV, rennet-lyophilized.

able components. First, the pH-mobility curves differed considerably from a straight line. A parabola fitted by least squares did very well within the range studied. If all the methods of preparation of whey proteins produced exactly the same protein components, then the curves would be coincident; because these curves deviated somewhat from each other, some changes due to method of preparation must have taken place in protein structure. The magnitude of these changes is roughly indicated by the horizontal deviation from coincidence. Second, in the region of zero mobility the spread of pH values was less than in other mobility ranges.

From three preparations of the "non-protein" filtrate a total of 11 mobilities were determined at pH 7.8 (table 1). Only one value (13.1) was common to

TABLE 1

Mobilities at pH 7.8 of components of non-protein filtrate compared with components of salt-acid whey proteins

Protein component no.		1	2	3		5	6				
Mobility x 10^5		2.4	3.9	5.4		6.8	8.0				
Non-protein component											
Mobility x 10^5	1.5	2.9	3.4	5.1	6.0	7.4	8.4	10.3	12.0	13.1	15.7

TABLE 2

Areas of electrophoretic peaks from whey proteins prepared by four methods

Peak nos.	Methods of isolation															
	I Salt-acid				II Salt-lyophilized				III Acid-lyophilized				IV Rennet-lyophilized			
	pH															
	3.8	5.8	6.8	7.8	3.8	5.8	6.8	7.8	3.8	5.8	6.8	7.8	3.8	5.8	6.8	7.8
	Peak areas as percent of total area															
−1					1.9	....	....	....	4.4	....	....	....	....	....	....	....
1	8.4	....	5.9	7.0	5.0	....	6.1	4.5	17.9	....	10.8	....	....	....	....	9.0
2	8.8	....	8.5	8.2	11.3	....	....	7.8	10.1	....	5.3	....	....	....	....	4.4
1+2	17.2	6.1	14.4	15.2	16.3	....	....	12.3	28.0	....	16.1	11.1	28.3	....	....	13.4
3	22.4	17.4	17.3	16.9	41.8	21.7	18.3[a]	28.9	20.8	20.2	14.7	20.4	23.6	16.7	20.7	14.2
4	4.7	....	....	....	....	....	....	....	....	8.0	....	....	9.0	....	....	7.8
5[b]	55.6	70.7	63.0	60.9	40.0	69.3	69.8	53.6	46.9	61.3	62.3	59.4	39.1	51.9	63.0	56.3
6	....	5.9	5.3	5.4	....	9.0	5.8	5.3	....	10.5	5.5	6.1	....	....	....	5.6
7	....	....	....	1.6	....	....	....	....	....	....	1.4	3.0	....	....	....	2.7
A	....	....	....	....	....	....	....	....	....	....	....	....	....	18.0	....	....
B	....	....	....	....	....	....	....	....	....	....	....	....	....	13.4	16.3	....

[a] Peaks 2 + 3. [b] Includes peaks 4 and 6 when these could not be measured separately.

TABLE 3

Mobilities of electrophoretic peaks from whey proteins prepared by four methods

Peak nos.	Methods of isolation															
	I Salt-acid				II Salt-lyophilized				III Acid-lyophilized				IV Rennet-lyophilized			
	pH															
	3.8	5.8	6.8	7.8	3.8	5.8	6.8	7.8	3.8	5.8	6.8	7.8	3.8	5.8	6.8	7.8
	Mobilities cm^2, $volt^{-1}$, sec^{-1}, $\times 10^5$															
	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−
1a[a]	2.70	....	1.06	2.39	1.76	....	1.12	1.28	1.82	....	1.53	....	....	....	....	2.65
d	....	....	.71	2.15	....	....	1.07	....	....	....	1.31	1.44	....	....	....	1.62
2a	3.78	....	2.53	3.88	3.24	....	3.00	3.21	3.58	....	3.83	....	....	....	....	4.67
d	....	....	1.46	3.70	....	....	2.70	....	....	....	3.00	2.91	....	....	....	3.54
3a	4.56	3.26	4.49	5.41	4.14	2.16	3.80	5.62	4.42	2.57	4.58	5.30	....	2.32	4.07	5.40
d	3.55	2.06	2.47	4.69	1.75	2.07	3.63	....	3.68	2.07	4.34	4.47	3.79	1.74	3.17	4.66
4a	5.96	3.90	....	....	....	....	....	....	6.34	3.24	5.66	....	3.00	....	....	6.24
5a	6.39	4.58	5.90	6.84	6.28	3.50	5.14	6.50	6.70	4.01	5.86	6.79	....	3.88	5.63	7.11
d	6.22	3.40	3.52	6.32	5.34	3.40	5.16	....	6.44	3.29	5.64	6.21	6.26	2.95	4.65	6.20
6a	....	5.49	6.83	7.99	....	4.44	5.87	....	....	5.03	6.79	7.88	....	....	....	8.24
d	....	4.87	4.50	8.10	....	4.80	6.35	....	9.07	4.94	7.18	7.73	....	....	....	7.86
Aa	....	....	....	....	....	....	....	....	....	....	....	....	....	4.85	....	....
d	....	....	....	....	....	....	....	....	....	....	....	....	....	4.33	....	....
Ba	....	....	....	....	....	....	....	....	....	....	....	....	....	5.90	6.70	....
d	....	....	....	....	....	....	....	....	....	....	....	....	....	5.89	6.37	....

[a] a = Ascending boundary. d = Descending boundary.

all three preparations, and only one other (5.1) was common to two preparations. Quite likely all the six components from the corresponding salt acid whey proteins (also shown in table 1) were represented by the so-called "non-protein" filtrate components of nearest mobility. The differences in mobilities may be due to denaturation of the residues of protein components, to change of solvent, or to both these factors. The extra unmatched components of the "non-protein" filtrate either may be protein components too dilute to be detected without concentration, or denaturation products of recognized protein components. The areas of all measurable peaks are shown in table 2, and all determinable mobilities in table 3.

No one of the four methods of preparation yielded components which always moved fastest or slowest at all pH values. However, components from salt-lyophilized whey migrated either slowest or next to slowest in 21 of 26 comparisons, while components from salt-acid and acid-lyophilized whey migrated either fastest or next to fastest in 18 of 28 and 20 of 30 respective comparisons. These comparisons may indicate, as Briggs and Hull (2) have suggested for β-lactoglobulin, that the procedures producing greater mobilities have caused more denaturation of the components involved. On this basis, the salt-lyophilized procedure caused least change and the two acid procedures caused most change.

Another method of measuring differences in the four methods of preparation was to calculate for each pH the standard deviations of the mobilities. For components 3 and 5, using δ and ϵ boundaries, the averages of such deviations at pH 3.8, 5.8, 6.8 and 7.8 were, respectively, 5.0, 10.7, 10.7 and 2.5 per cent of the average values. Thus, the difference in mobilities was least at pH 7.8 and was greatest at pH 5.8 or 6.8.

TABLE 4

Isoelectric points of electrophoretic components of whey proteins prepared by four methods

Peak Nos.	Methods of isolation			
	I Salt-acid	II Salt-lyophilized	III Acid-lyophilized	IV Rennet-lyophilized
	pH of isoelectric points			
1	5.65	5.32	5.02	....
2	5.20	4.98	4.90	....
3	4.73	4.95	4.79	4.91
4	4.70	....	4.75	....
5	4.67	4.86	4.75	4.76
6	4.05	4.42	4.69	....

No constant relation was found between isoelectric points of components and method of preparation (table 4). For components 1 and 2 the salt-acid whey produced highest isoelectric points and acid-lyophilized whey produced lowest.

For components 3 and 5 the salt-lyophilized whey produced highest isoelectric points and the salt-acid whey lowest. For component 6 the acid-lyophilized whey produced highest and the salt-acid whey lowest isoelectric points. For components 3 and 5 the isoelectric points were definitely lower than the 5.2 (1) found electrophoretically for purified components, but were still higher than the 4.5 (5) estimated from the viscosity of milk.

CONCLUSIONS

The composition of the protein components evidently was modified by at least three of the four methods used in preparing whey proteins for electrophoresis. The difference in peak areas at pH 5.8 is one evidence of such modification. Differences in the mobilities and isoelectric points of corresponding components are other evidences. The increased number and mobility of peaks in the "non-protein" filtrate also indicate modification of the original components.

Isoelectric points for the albumin and β-lactoglobulin fractions from all four preparations (pH 4.8) were lower than literature values (pH 5.2) for electrophoresis of purified components. However, they were all higher than the value (pH 4.5) from viscosity studies of milk. Probably, then, the nature of all components reflected changes induced by all four methods used in their preparation.

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A RAPID METHOD OF TESTING FOR QUATERNARY AMMONIUM COMPOUNDS IN MILK[1]

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Various test methods have been recommended to determine concentration of quaternary ammonium compounds (QAC) in water solution and in certain foods. Most of these methods are dependent upon the alteration of the color of a dye by the cations of the QAC (1, 2, 3, 10, 11, 12, 13, 15). Other recommended methods have included a precipitation test using horse serum (8), a precipitation test using Tamol-N (7), argentimetric titration (5), triiodide formation (4, 9) and a ferricyanide method (19).

DuBois and Dibblee (6) have reported that the Hartley-Runnicles method (13) can be used to estimate concentrations ranging from 1:1000 (1,000 ppm.) to 1:20,000 (50 ppm.) of QAC in milk. Levels of QAC in milk detectable organoleptically vary considerably with type of compound (14, 18), and this form of detection therefore is neither sufficiently accurate nor practical. Some difficulties that many be encountered due to presence of QAC in milk have been reported in a previous paper (17). A test dependent upon the reaction of the quaternary nitrogen in weakly alkaline solution with bromophenol blue to form a product soluble in ethylene chloride has been recommended by Wilson (19, 20) for use on various foods including milk. On the basis of data obtained by cooperating investigators, Wilson (20) has suggested, however, that further studies be carried out before recommending this procedure as an official method for milk.

Miller and Elliker (16) have reported preliminary observations on a modification of the eosin-indicator method (10) for determining QAC concentration. The original method has proven satisfactory for water solutions of QAC, but not for QAC in milk. The modified eosin-indicator method will detect as little as 5 ppm. QAC in milk. This paper presents further improvements in the method and experimental data not previously published.

REAGENTS

Indicator solution: (a) Dissolve eosin yellowish dye (dye concentration of about 90 per cent, Biological Commission Color Index 768) in acetone (analytical reagent) at the rate of 0.5 mg. of dye to 1 ml. of acetone. (b) Add acetone-eosin solution to tetrachloroethane (technical grade) at the rate of 1 ml. of acetone-eosin to 9 ml. of tetrachloroethane. (c) Remove the reddish color from the solution by adding citric acid crystals (monohydrate, analytical reagent) at the rate of 10 mg. of crystals to each ml. of dye solution. (d) Shake for 1 min. or until the red color disappears. (e) Filter through filter paper.

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Buffer: Prepare a solution of citric acid (monohydrate, analytical reagent) at the rate of 25 g. to 100 ml. distilled water and adjust to pH 3.5 with 50 per cent NaOH (analytical reagent). Approximately 12 ml. of NaOH to 100 ml. of citric acid solution usually are required.

Anionic solution: Prepare a 0.01 per cent solution of active anionic compound from 10 per cent Fisher Laboratory Aerosol (10 per cent di-octyl sodium sulfosuccinate). This represents approximately a 1:1000 dilution of the 10 per cent Aerosol. Other anionic surface active agents may prove suitable for this solution.

PREPARATION OF STANDARDS

Prepare standards for comparison by adding known concentrations of the desired QAC to unhomogenized milk samples to provide final QAC concentrations covering the range suspected in the test samples. This method of standardization is necessary because different QAC preparations yield different titration values, and interpretation of the titration end point varies with different individuals.

PROCEDURE

(a) Place 1 ml. of the milk to be analyzed in a test tube. Add 5 ml. of distilled water, 1 ml. of indicator solution and 0.2 ml. of buffer solution. Shake vigorously for 10 sec.

(b) Centrifuge to separate solvent fraction from milk solids and water. This may be accomplished by centrifuging for 5 min. at 3200 RPM in a 10-in. centrifuge. A Babcock centrifuge can be used but may require as long as 25 min. for the same operation. Three distinct layers should be apparent following the centrifuging. The top layer should be a liquid; the middle layer should consist chiefly of precipitated protein; and the lower layer should consist of the solvent containing most of the QAC in the sample. The procedure up to this point may be employed as a qualitative test for detection of QAC in milk. Presence of a QAC is indicated by development of a red color in the solvent layer. A pink to red color usually indicates at least 5 ppm. QAC. The subsequent steps in the procedure outlined below are necessary for an approximate determination of QAC present.

(c) Remove the top layer. Pour out the solvent layer into a second tube. The precipitated protein should adhere to the first tube when the solvent layer is transferred. Flush remaining solvent fraction from this tube with 5 ml. of distilled water. Transfer this water to the solvent in the second tube and agitate for about 5 sec. to thoroughly mix contents. Allow the solvent to separate from the water layer. This can be accelerated by centrifuging for about 20 sec. Remove the top layer. Wash the solvent layer in the tube twice more. Each of these washings should consist of addition of 5 ml. of water to the solvent in the tube, agitation of contents, centrifuging for 20 sec. and removal of the water layer.

(d) Add 1 ml. of distilled water and 0.1 ml. of buffer to the solvent layer after the last washing. Titrate this mixture with standard anionic solution.

Add slowly and shake until the red color of the lower layer is removed. The quantity of QAC is indicated by the milliliters of anionic solution required to remove the red color. A microburette facilitates this titration. In some samples pigments such as carotene may slightly mask the red color formed by QAC and eosin. A few experimental trials, however, should enable accurate interpretation of the end point.

Test samples. In trials to evaluate the accuracy of the new method for determination of QAC in milk, each QAC to be tested was made into a distilled water solution containing 500 ppm. according to the concentration specified by the manufacturer. The concentration of this solution was checked by the test method of Harper *et al.* (10). The necessary quantity of the respective stock solution then was added to various milk samples so that the final QAC content would be 0, 5, 10, 25, 50 and 100 ppm. respectively. The recommended test procedure outlined above then was used to determine the concentration of QAC in each of the milk samples. Samples were prepared in such a manner that their QAC content was not known to the operator conducting the titrations.

RESULTS

The quantity of anionic solution required to titrate 0 to 100 ppm. of different types of QAC in milk by the new method is shown in table 1. Results indicate the method to be reasonably consistent for the types of QAC tested. Expected differences in titration values occurred between different types of QAC. Results similar to those shown have been obtained on 11 different types of QAC and three detergent sanitizers added to milk. Some difficulty has been experienced in determining concentrations greater than 100 ppm. The most consistent results have been obtained when concentrations of 50 to 100 ppm. QAC were present in milk.

The actual percentage recovery of the original QAC added to the milk samples was not determined in these studies. A number of trials indicate that more than half the original QAC added to the milk was recovered in the final, washed solvent fraction titrated in step *d* of the procedure. Results in table 1 and in a large number of other trials indicated the percentage recovery in this fraction to be fairly consistent. If desired, the recovery of QAC can be increased by subjecting wash water and milk solids fractions removed in step *c* of the procedure to further extractions by the indicator solution and washing until all QAC has been recovered. The recovered QAC can be titrated as in step *d*. However, under normal conditions, where suitable standards for comparison are included, the additional extractions are unnecessary for determination of QAC.

There was a difference in the sharpness of the end point when different types of QAC were tested. However, little difficulty was experienced in establishing the true end point. The intensity of red color in the solvent layer decreased progressively as the concentration of QAC decreased from 100 to 5 ppm. Certain compounds (for example alkyl dimethyl benzyl ammonium chloride) produced a red color in the solvent layer at concentrations below 5 ppm., and levels

TABLE 1

Quantity of anionic solution required to titrate various concentrations of added QAC in milk

QAC	ml. of anionic solution to titrate the following concentrations of QAC in milk:					
	0 ppm.	5 ppm.	10 ppm.	25 ppm.	50 ppm.	100 ppm.
	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)
Alkyl dimethyl benzyl ammonium chloride	0.00[a]	0.05	0.14	0.35	0.65	1.20
	0.00	0.05	0.12	0.37	0.67	1.21
	0.00	0.06	0.14	0.33	0.60	1.18
	0.00[a]	0.05	0.13	0.32	0.65	1.14
	0.00	0.04	0.13	0.33	0.62	1.19
Para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride	0.00[a]	0.05	0.14	0.29	0.61	1.19
	0.00	0.04	0.15	0.29	0.64	1.21
	0.00	0.05	0.13	0.36	0.61	1.17
	0.00[a]	0.03	0.12	0.32	0.62	1.20
	0.00	0.03	0.13	0.29	0.57	1.14
Methyl dodecyl benzyl trimethyl ammonium chloride	0.00[a]	0.03	0.04	0.21	0.30	0.70
	0.00	0.04	0.08	0.22	0.35	0.64
	0.00	0.03	0.07	0.22	0.34	0.68
	0.00[a]	0.03	0.07	0.23	0.34	0.66
	0.00	0.03	0.09	0.19	0.34	0.65

[a] The first three values for each QAC represent triplicate determinations on one set of unknowns; the last two values represent duplicate determinations on another set of unknown samples prepared the following day.

of 2 to 3 ppm. of this compound in milk could be estimated with use of a microburette.

Thus far, no false positive tests with the method have been encountered during several hundred determinations on various types of raw and pasteurized mixed herd milks, as well as on individual cow or herd milks. Results obtained on sour milk containing QAC were similar to those obtained on fresh milk. However, difficulty was encountered in determining concentrations of QAC added to homogenized milk.

A modification of the method for QAC determination in milk also may be applied to QAC determination of water solutions of detergent sanitizers (16). Detergent compounds in the preparation must be removed by successive washings by the same procedure used in the above method for determining QAC in milk.

DISCUSSION

The simplicity and consistent results obtained with the eosin-indicator titration method for quaternaries in milk should enable its application for detection of gross QAC contamination of milk. The first two steps of the procedure may be employed as a presumptive or qualitative test. Samples can be eliminated if no red color is present in the solvent layer at this stage. If the solvent fraction exhibits a red color, the subsequent washing steps and titration should provide an indication of total quantity of QAC present. The type of QAC in the test

samples must be known to prepare the appropriate standards for an accurate determination.

The most accurate range for QAC determinations for either milk or detergent sanitizers appears to be about 5 to 100 ppm. If concentrations higher than 100 ppm. are expected, the milk may be diluted to bring the QAC content of the sample in this range. Further studies are under way to improve the accuracy of the method, especially for determining concentration of QAC in milk at levels of 5 ppm. or less. The reason for differences in sensitivity with different types of QAC has not been investigated. The titration values, particularly with low concentrations of QAC, do not represent a linear function; the results obtained with this method indicate, however, that it should be sensitive enough to detect and estimate levels of QAC that might be inhibitory to lactic acid bacteria in milk.[2]

Further investigation also is necessary to fully explain the reactions between QAC and eosin indicator. Apparently, the successive washings remove certain compounds that interfere with the reversal of this reaction by the anion in the titration solution. The same principle (removal of interfering factors) also is important for QAC determination on detergent sanitizer and buffered QAC solutions. Unless this washing is sufficiently thorough and complete, the final accurate titration of QAC present in the solvent fraction is difficult or impossible.

Preliminary observations and a consideration of the general procedure involved suggest that this method or modifications of it may be employed for detection of QAC in some foods other than milk.

SUMMARY

A method has been developed for determination of QAC in milk. It is based on extraction and precipitation of QAC in a tetrachloroethane-acetone-eosin indicator solution. Interfering factors in the solvent-indicator fraction are removed by successive washings with distilled water and QAC then is titrated with a standard solution of anionic surface active agent.

The first two steps of the procedure may serve as a presumptive or qualitative test for detection of QAC in milk. The subsequent steps are necessary for a determination of quantity of QAC present. The method has proven suitable for determining concentration in milk of QAC preparations commonly employed in dairy sanitation procedures. With suitable standards for comparison the method will determine quantities of QAC in the range of 5 to 100 ppm. in milk.

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[2] However, results of Lundstedt (Eric Lundstedt, personal communication) indicate that *Leuconostoc* species necessary for aroma production in some dairy products may be inhibited by concentrations of less than 5 ppm. QAC in milk.

aration of the manuscript, and to T. E. Furlong for technical assistance with laboratory determinations.

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EFFECT OF QUATERNARY AMMONIUM COMPOUNDS ON ACTIVITY OF LACTIC ACID STARTER BACTERIA IN MILK AND CHEESE[1]

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The increased application of quaternary ammonium compounds (QAC) for dairy sanitation purposes has stimulated interest in the effect of various concentrations of these compounds on the growth of lactic acid bacteria in milk. The problem is important from the standpoint of attempts to prevent souring of milk or to reduce bacterial counts prior to delivery to the dairy plant. Another important factor is the effect of QAC on lactic acid bacteria in starters, cheese or other cultured milk products for which such milk might be employed. This study deals with the effect of various concentrations of QAC in milk on representative lactic acid starter bacteria.

DuBois and Dibblee (2) concluded that the presence of added QAC lacked any influence on the bacterial counts of raw or pasteurized milk at concentrations ranging from 1:500 (2,000 ppm.) to 1:25,000 (40 ppm.). It was observed that higher concentrations (200 and 2,000 ppm.) inhibited growth of Gram positive acid-producing organisms but not that of Gram negative types. A *Streptococcus* sp. isolated from milk was inhibited by 200 and 2,000 ppm. but not by 40 ppm. added QAC.

Mull and Fouts (10) reported a reduction in bacterial count when QAC was added directly to milk, but concluded that such compounds would have to be present in low quality milk in concentrations of 200 to 250 ppm. to bring about significant decreases in bacterial count.

Johns and Pritchard (6) reported a slight, but definite, bacteriostatic action when QAC were added to milk in the highest concentration which might hope to escape detection organoleptically, but concluded their preservative effect to be decidedly less pronounced than that of formaldehyde.

Moore (9) found that 25 ppm. of QAC in milk caused partial inhibition and 50 to 75 ppm. effected complete inhibition of commercial lactic acid starter organisms. Barber *et al.* (1) reported some inhibition of *Streptococcus lactis* at 100° F. in reconstituted skimmilk containing as little as 10 ppm. added QAC. Progressive decreases were observed with 25 and 50 ppm. and complete inhibition occurred with 100 ppm. QAC in the milk.

Preliminary work on the effect of QAC in milk on lactic acid starter bacteria has been reported by Miller *et al.* (7). Slight inhibition of acid production was observed when the QAC was present in concentrations as low as 5 ppm.

METHODS

The organisms selected for this study included a single strain lactic culture, *Streptococcus cremoris* (R-6), a mixed strain commercial lactic culture widely

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used in the manufacture of cottage and Cheddar cheese, and two Swiss cheese starter organisms, *Lactobacillus lactis* (39a) and *Streptococcus thermophilus* (C-3). The QAC preparations employed were as follows: Alkyl dimethyl benzyl ammonium chloride, para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride, methyl dodecyl benzyl trimethyl ammonium chloride and a detergent sanitizer containing para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride. For purpose of comparison, a commercial sodium hypochlorite also was included in the trials. Concentration of QAC stock solution was established by the titration method of Harper *et al.* (4), and concentration of hypochlorite solution by titration with sodium thiosulphate.

QAC or hypochlorite from the stock solution was added to 50-ml. quantities of sterile reconstituted skimmilk to provide added concentrations in the milk of 0, 5, 10, 15, 20, 25, 30, 40, 50, 75 and 100 ppm. of the compound under study. A concentration of 200 ppm. available chlorine as hypochlorite also was used. It was recognized that the hypochlorite in the above concentrations in milk dissipates rapidly due to reaction with organic matter and thus, the above concentrations do not necessarily represent final content of available chlorine in the milk. The quaternary compounds are more stable in milk than the hypochlorites, and the quantities of QAC as added in this experiment can be detected by a method reported in a subsequent paper (8).

Milks containing the various added concentrations of germicide were inoculated with 1 per cent of the respective cultures and incubated as follows: Commercial lactic culture, 37.8° C.; *S. cremoris,* 37.8° C.; *S. thermophilus,* 48° C.; and *L. lactis,* 44° C. After 6 hr. incubation, the titratable acidities were determined with 0.1 *N* NaOH to phenolphthalein end point. Incubation at temperatures near the maximum for the organisms involved has been employed as an activity test in other studies to more closely simulate manufacturing conditions to which the lactic acid organisms are subjected (3, 5).

The experiment also was repeated using sterile whole milk instead of skimmilk to determine whether or not the presence of butterfat might affect the bacteriostatic action of the various germicides. Trials also were carried out using an incubation period of 16 hr. at the following temperatures for the respective organisms: Commercial lactic culture, 21.1° C.; *S. cremoris,* 21.1° C.; *S. thermophilus,* 37° C.; and *L. lactis,* 37° C.

Triplicate experimental lots of Cheddar cheese were made with milk containing 0, 5 and 10 ppm. alkyl dimethyl benzyl ammonium chloride. The commercial lactic culture was used for these trials. One lot of cheese was made with 20 ppm. in the cheese milk. Effect of the QAC was followed by acidity measurement at various stages of manufacture.

RESULTS

Results of the trials are shown in tables 1 and 2. Each value in the table represents the average of three experiments run on separate days. The values obtained in the three different experiments were practically identical throughout. Titratable acidities of inoculated milks were slightly lower than the uninoculated

TABLE 1

Effect of various quaternary ammonium compounds and a sodium hypochlorite on acid development of different lactic acid starter bacteria during a 6-hr. incubation period at temperatures near their maximum

Culture	Incub. temp.	Germicide[a]	Titratable acidity developed in sterile skimmilk containing the following ppm. concentration of germicide:[b]											
			0	5	10	15	20	25	30	40	50	75	100	200
	(°C.)		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Commercial Lactic culture	37.8	QAC 1	0.40	0.29	0.24	0.22	0.19	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 2	0.40	0.32	0.24	0.22	0.20	0.19	0.18	0.18	0.17	0.16	0.16	
		QAC 3	0.39	0.32	0.25	0.21	0.19	0.18	0.18	0.18	0.17	0.17	0.16	
		DS	0.40	0.31	0.24	0.22	0.19	0.18	0.18	0.17	0.17	0.16	0.16	
		NaOCl	0.38	0.37	0.37	0.37	0.36	0.36	0.35	0.31	0.30	0.24	0.21	0.16
S. cremoris (R-6)	37.8	QAC 1	0.34	0.32	0.30	0.26	0.23	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 2	0.35	0.33	0.28	0.25	0.22	0.19	0.18	0.17	0.17	0.16	0.16	
		QAC 3	0.36	0.33	0.30	0.27	0.25	0.21	0.19	0.17	0.17	0.16	0.16	
		DS	0.34	0.33	0.29	0.25	0.21	0.19	0.17	0.17	0.17	0.16	0.15	
		NaOCl	0.34	0.34	0.33	0.33	0.33	0.32	0.32	0.30	0.28	0.26	0.19	0.17
S. thermophilus (C-3)	48.0	QAC 1	0.34	0.30	0.29	0.27	0.25	0.24	0.22	0.19	0.17	0.15	0.14	
		QAC 2	0.31	0.29	0.27	0.27	0.24	0.24	0.21	0.19	0.16	0.15	0.14	
		QAC 3	0.32	0.28	0.27	0.26	0.24	0.23	0.23	0.19	0.17	0.15	0.14	
		DS	0.32	0.30	0.27	0.25	0.23	0.23	0.22	0.18	0.16	0.15	0.14	
		NaOCl	0.31	0.31	0.31	0.30	0.30	0.29	0.28	0.27	0.25	0.24	0.23	0.15
L. lactis (39a)	44.0	QAC 1	0.30	0.29	0.26	0.23	0.20	0.20	0.20	0.18	0.18	0.18	0.16	
		QAC 2	0.31	0.28	0.28	0.26	0.23	0.21	0.19	0.19	0.18	0.17	0.16	
		QAC 3	0.32	0.30	0.27	0.24	0.21	0.21	0.19	0.19	0.18	0.17	0.17	
		DS	0.30	0.29	0.26	0.23	0.20	0.19	0.19	0.18	0.18	0.17	0.16	
		NaOCl	0.29	0.30	0.30	0.30	0.29	0.29	0.28	0.28	0.26	0.25	0.23	0.18

[a] QAC 1 = para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride; QAC 2 = methyl dodecyl benzyl trimethyl ammonium chloride; QAC 3 = alkyl dimethyl benzyl ammonium chloride; DS = detergent sanitizer (para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride); NaOCl = commercial sodium hypochlorite.

[b] Titratable acidity of uninoculated control = 0.18 per cent.

TABLE 2

Effect of various quaternary ammonium compounds and a sodium hypochlorite on acid development of different lactic acid starter bacteria during a 16-hr. incubation period at normal temperatures

Culture	Incub. temp.	Germicide[a]	Per cent titratable acidity developed in sterile skimmilk containing the following ppm. concentration of germicide:[b]											
			0	5	10	15	20	25	30	40	50	75	100	200
	(°C.)		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Commercial lactic culture	21.1	QAC 1	0.79	0.77	0.70	0.63	0.48	0.37	0.32	0.23	0.21	0.18	0.16	
		QAC 2	0.82	0.78	0.74	0.68	0.61	0.43	0.34	0.24	0.21	0.18	0.16	
		QAC 3	0.79	0.72	0.67	0.57	0.48	0.39	0.32	0.23	0.20	0.17	0.17	
		DS	0.79	0.75	0.72	0.63	0.46	0.34	0.30	0.23	0.19	0.16	0.15	
		NaOCl	0.79	0.80	0.80	0.80	0.79	0.79	0.78	0.78	0.78	0.71	0.72	0.63
S. cremoris (R-6)	21.1	QAC 1	0.71	0.68	0.65	0.59	0.53	0.30	0.26	0.21	0.19	0.17	0.16	
		QAC 2	0.72	0.69	0.67	0.64	0.59	0.48	0.35	0.27	0.22	0.17	0.16	
		QAC 3	0.71	0.68	0.63	0.58	0.48	0.32	0.26	0.20	0.18	0.17	0.16	
		DS	0.69	0.67	0.63	0.58	0.43	0.30	0.25	0.21	0.18	0.16	0.15	
		NaOCl	0.71	0.71	0.72	0.72	0.71	0.70	0.71	0.69	0.70	0.68	0.67	0.59
S. thermophilus (C-3)	37.0	QAC 1	0.74	0.65	0.61	0.59	0.59	0.52	0.49	0.44	0.28	0.18	0.14	
		QAC 2	0.72	0.71	0.68	0.65	0.65	0.62	0.54	0.42	0.27	0.20	0.14	
		QAC 3	0.69	0.62	0.61	0.56	0.56	0.52	0.48	0.39	0.27	0.16	0.15	
		DS	0.73	0.71	0.64	0.60	0.53	0.48	0.42	0.36	0.25	0.16	0.14	
		NaOCl	0.72	0.72	0.71	0.71	0.71	0.70	0.68	0.67	0.65	0.59	0.55	0.40
L. lactis (39a)	37.0	QAC 1	0.78	0.77	0.69	0.67	0.60	0.52	0.49	0.42	0.28	0.15	0.14	
		QAC 2	0.77	0.76	0.72	0.68	0.63	0.59	0.54	0.46	0.31	0.24	0.14	
		QAC 3	0.75	0.67	0.64	0.61	0.58	0.54	0.50	0.44	0.32	0.16	0.14	
		DS	0.77	0.74	0.70	0.63	0.57	0.52	0.48	0.46	0.31	0.15	0.12	
		NaOCl	0.75	0.75	0.75	0.75	0.76	0.75	0.75	0.74	0.72	0.65	0.63	0.46

[a] QAC 1 = para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride; QAC 2 = methyl dodecyl benzyl trimethyl ammonium chloride; QAC 3 = alkyl dimethyl benzyl ammonium chloride; DS = detergent sanitizer (para di-isobutyl phenoxy ethoxy dimethyl benzyl ammonium chloride); NaOCl = commercial sodium hypochlorite.

[b] Titratable acidity of uninoculated control = 0.18 per cent.

control. This may be explained by the neutralizing effect of the cation of the respective germicide compounds added to the milk.

Table 1 shows the effect of the various germicides on growth and acid production of the starter organisms at temperatures near their maximum. Slight inhibition of acid production by 5 ppm. of the respective QAC or the detergent sanitizer was evident in the case of each culture. The degree of inhibition increased progressively with an increase in the concentration of the compound. Inhibition was almost complete with 25 to 30 ppm. of QAC in the milk. In general, the degree of inhibition of the organisms used was similar. The results suggest that *S. thermophilus* showed slightly greater resistance to bacteriostatic effect of the QAC than the other organisms. All titratable acidities, including the control, were somewhat low in the case of *L. lactis* because the culture was not fully activated following removal from the stock culture; however, the results are representative of the comparative inhibition of different concentrations and compounds against this type of organism. The effect of the QAC and detergent sanitizer on acid production in general was about the same. Complete inhibition apparently occurred with the sodium hypochlorite at a concentration between 100 and 200 ppm.

The presence of the milk fat did not greatly affect the bacteriostatic action of the QAC. When the experiment, as shown in table 1, was repeated with whole milk instead of skimmilk, identical results were obtained and therefore the data have not been included in this report.

Other studies have shown that the inhibitory effect of QAC in milk is not materially reduced by pasteurization of the milk after QAC addition, and in some trials inhibitory effect by QAC in milk has been apparent after more severe heat treatment, such as sterilization at 121.1° C. for 15 min.

Table 2 shows the inhibition by the various compounds at incubation temperatures normally employed for the different cultures. The relative effect of all types of germicides was less pronounced at the lower incubation temperatures than at near-maximum temperatures. With one exception, complete inhibition by the QAC present was not effected until the concentration reached 50 to 75 ppm. As much as 200 ppm. sodium hypochlorite did not completely inhibit growth at the normal incubation temperature of the respective cultures. The *S. cremoris* and commercial lactic culture appeared to be more susceptible to bacteriostatic effect of the QAC than *S. thermophilus* or *L. lactis*. The methyl dodecyl benzyl trimethyl ammonium chloride appeared less bacteriostatic on a ppm. basis against the lactic acid bacteria studied than the other QAC preparations used.

The effect of alkyl dimethyl benzyl ammonium chloride on acid production during manufacture of Cheddar cheese is shown in table 3. Since only two experimental vats were available for a single trial, it was necessary to include a 0 ppm. control vat for each concentration of QAC studied. Thus on 1 day, one vat contained 0 and the second, 5 ppm., and on the next day, one contained 0 and the second, 10 ppm. QAC. The entire experiment including 0, 5 and 10

TABLE 3

Effect of added quaternary ammonium compound in cheese milk on acid development during manufacture of Cheddar cheese

Operation	Time	Change in per cent titratable acidity with following concentrations of QAC:			
		Control 0 ppm.	QAC 5 ppm.	Control 0 ppm.	QAC 10 ppm.
	(*hr.: min.*)	(%)	(%)	(%)	(%)
Received milk	0:00	0.17	0.17	0.17	0.17
Added starter	0:15	0.18	0.18	0.17	0.17
Added rennet	1:15	0.19	0.19	0.18	0.18
Cut curd	1:45	0.12	0.12	0.13	0.13
Began cooking	2:00	0.12	0.12	0.13	0.13
Steam off	2:30	0.14	0.14	0.14	0.14
Drained whey	3:30	0.16	0.16	0.17	0.16
Packed curd	3:45	0.21	0.21	0.23	0.18
Cheddared	4:00	0.27	0.26	0.30	0.23
	4:15	0.34	0.31	0.40	0.27
	4:30	0.39	0.37	0.45	0.30
	4:45	0.44	0.42	0.52[a]	0.33
	5:00	0.52[a]	0.48		0.36
	5:15		0.52[a]		0.40
	5:30				0.48
	5:45				0.54[a]

[a] Milling time.

ppm. was run three times on successive days. The results were highly consistent through all three experiments and table 3 represents a typical trial. A concentration of 5 ppm. QAC in the cheese milk delayed milling time about 15 min. over the control with no QAC. A delay in milling time of 45 to 60 min. occurred with 10 ppm. QAC in the cheese milk. In an additional trial with 20 ppm. alkyl dimethyl benzyl ammonium chloride added to the cheese milk, the acidity of the whey reached only 0.20 per cent during a 7.5-hr. manufacturing period.

DISCUSSION

The minimum concentration of 5 ppm. QAC added to milk in these studies caused very slight inhibition of four different lactic acid starter cultures. If excessive quantities of QAC should enter the milk supply, through intentional adulteration or accident, definite inhibition of lactic acid starter bacteria may occur. The effect of higher concentrations of QAC (5 to 10 ppm.) would vary with the manufacturing conditions employed for a dairy product. For example, the degree of inhibition of the lactic acid bacteria might be more pronounced in manufacture of Cheddar cheese than in cottage cheese or buttermilk. Presumably, the near-maximum growth temperature employed in Cheddar cheese manufacture accentuates the bacteriostatic effect of the germicide on lactic starter organisms. The delay of 45 to 60 min. in milling time caused by 10 ppm. QAC is considered significant. Such an extension in time would be detrimental to economical operation of a cheese plant. A concentration of 5 ppm. QAC in the cheese milk might not cause a noticeable delay in acid development in such a product. Another problem suggested but not investigated in these

studies is the possible effect of QAC in the cheese milk on rate of ripening and final flavor of the cheese.

Results of these studies emphasize that a delay in souring of raw milk may be accomplished by 20 ppm. of QAC. Unless a milk grader is familiar with the bitter flavor contributed to milk by most quaternary compounds, he may fail to detect such a quantity in the milk. However, concentrations as low as 5 ppm. QAC in milk can be detected by a modified eosin indicator titration method developed in the course of these studies (8).

SUMMARY AND CONCLUSIONS

A study was carried out on the effect of three QAC products (quaternary ammonium compounds), a QAC containing detergent sanitizer and a sodium hypochlorite on acid development by four types of lactic acid starters in sterile skimmilk and whole milk.

A mixed-strain commercial lactic culture, *S. cremoris, L. lactis* and *S. thermophilis* was slightly inhibited by 5 ppm. of each of the QAC compounds added to the milk.

The inhibition of acid development was nearly complete in all cultures with 25 to 30 ppm. of QAC in the milk when the organisms were incubated at temperatures near their maximum. At incubation temperatures normally used for culturing the organisms, 50 ppm. QAC in the milk effected nearly complete inhibition.

Milling time was delayed 15 min. by presence of 5 ppm. and 45 to 60 min. by 10 ppm. QAC in the manufacture of experimental Cheddar cheese.

Results obtained suggest the necessity of employing farm and plant sanitizing procedures that will avoid contamination of milk with inhibitory concentrations of QAC.

ACKNOWLEDGMENT

This study was supported in part by a grant from Klenzade Products, Inc. Acknowledgment is due R. Streiff and J. A. Cornett for preliminary trials on this problem and assistance in manufacture of experimental cheese.

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ABSTRACTS OF LITERATURE

Prepared in cooperation with the
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BUTTER

O. F. HUNZIKER, SECTION EDITOR

99. Process of standardizing dairy product. I. J. LUNDAL and R. P. ROBICHAUX (assignors to Sugar Creek Creamery Co. and Cherry Burrell Corp.). U. S. Patent 2,536,297. 12 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 146. 1951.

A dairy product containing at least 70% milk fat in the continuous phase is agitated at temperatures above the melting point of the fat, while various standardizing agents, including non-fatty materials are added. The uniform dispersion then is cooled with agitation until stiff enough to maintain a desired shape. R. Whitaker

100. Cream sediment tester. W. R. WILBORN. U. S. Patent 2,536,406. 7 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 174. 1951.

A perforated disc in the bottom of a funnel-shaped receptacle supports a layer of filter material. The cream sample is drawn through the filter by vacuum supplied by an aspirator attached to the bottom outlet of the funnel.

R. Whitaker

Also see abs. no. 119.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

101. Cheese modifying enzyme product. M. G. FARNHAM. U. S. Patent 2,531,329. 17 claims. Nov. 21, 1950. Official Gaz. U. S. Pat. Office **640,** 3: 1018. 1950.

Desirable characteristics are imparted to cheese by hydrolyzing glycerides and proteins with lipolytic and proteolytic enzymes obtained from the head and neck tissues of mammals receiving milk in their diet. This product gives results such as are obtained by "rennet paste".

R. Whitaker

102. Problems in the production of cottage cheese. S. L. TUCKEY, Univ. of Ill., Urbana. Milk Plant Monthly, **40,** 1: 18–20, 22–24. Jan., 1951.

A discussion is given of the following factors relative to the manufacture of high quality cottage cheese: (a) quality of milk, (b) composition of milk, (c) pasteurization, (d) cultures, (e) manufacturing procedures, (f) coagulating agents, (g) cutting acidity, (h) cooking methods, (i) washing procedures, (j) standardization, (k) curd storage and (l) spoilage. J. A. Meiser, Jr.

103. Cheese cutter. P. N. NELSON. U. S. Patent 2,533,682. 4 claims. Dec. 12, 1950. Official Gaz. U. S. Pat Office, **641,** 2: 541. 1950.

A hand cheese slicer of the taut wire type is described. R. Whitaker

Also see abs. no. 130, 131, 166.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

104. Zur Untersuchung von Brot, Milch- und Magermilchbrot (Examination of bread, milk bread and skimmilk bread). E. HELBERG. Mitt. Leb. Hyg., 41, 3/4: 373–380. 1950.

The paper gives a way of differentiating the 3 types of bread. The titration numbers are 9–11, 10.2–11.2 and 4.3–6, the extraction numbers 12–21, 13–17 and 35–80, the alcohol extract values 390–465, 494–550 and 500–600, the reducing values 190–270, 310–350 and 320–370, the Ca contents 0.7–1.3, 3–5 and 3–7 and the P values 3.3–5.9, 8.3–9.4 and 6.2–9.4 for bread, skimmilk bread and milk bread, respectively. Alcoholic extract, reducing value and Ca and P contents show whether there is milk in a bread; titration and extraction numbers show if the milk used was skim. Titration no.: 5.00 g. bread is extracted with 50 cc. 96% ethanol and titrated with water until turbid; titration no. = cc. water used. Extraction no.: mg. substance extracted with 50 cc. 96% ethanol out of 5.00 g. bread. Alcoholic extract: mg. substance extracted with 50 cc. 48% ethanol out of 5.00 g. bread. Reducing value: Fehling solution is reduced, values are given as mg. $Cu_2O/5$ g. bread. Ca and P contents: values are given as mg. CaO or mg. $P_2O_5/5$ g. bread. A. Fasler

105. Semisolid food product and process for making same. W. P. M. GRELCK. U. S. Patent 2,536,438. 2 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 183. 1951.

Dried whey, nonfat milk solids, brewers' yeast and water are blended to form a smooth homogeneous paste, containing approx. 40% moisture and having the lactose in a noncrystallized form, with the whey as the principal solid ingredient.

R. Whitaker

106. Food product and method of production. W. P. M. GRELCK. U. S. Patent 2,536,439. 2 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 183. 1951.

Same as product described in abstract 105 with salt added. R. Whitaker

107. Frozen food product and method of making same. N. H. LUNDQUIST (assignor to Cresthaven Farms). U. S. Patent 2,536,137. 6 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 104. 1951.

The buttermilk obtained by churning sweet cream is condensed, sweetened and frozen in an ice cream freezer. R. Whitaker

Also see abs. no. 132, 133, 150.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

108. Improving staining procedures in the direct microscopic examination of milk. B. S. LEVINE, Milk and Food Sanitation Section, Environmental Health Center, Cincinnati, O. J. Milk & Food Technol., **13:** 321–328. Nov.–Dec., 1950.

The physico-chemical phenomenon of adsorption as it applies to differential staining of milk proteins and the bacterial cell with respect to ages, viability and virulence was studied. The stain used was a 0.6% certified methylene blue chloride in 95% ethanol. Five examples of staining procedures are given in the article.

H. H. Weiser

109. Don't let quats ruin that flavor. E. LUNDSTEDT, Sante Fe Foods, Inc., Goshen, N. Y. Food Inds., **22,** 12: 49–51. Dec., 1950.

Difficulties in the production of high quality cheese starters and cottage cheese often arise from the presence of quaternary ammonium compounds in milk. These compounds are more effective against the aroma-producing bacteria than against the lactic acid organisms. Selective action was demonstrated using a citrated whey medium prepared from starter culture and testing for acidity, CO_2, flavor and acetylmethyl carbinol or diacetyl. Two ppm. of quaternary ammonium compound prevented development of aroma but had little effect on acid development. With large inoculations of starter (6%) results were similar. Penicillin and streptomycin, in concentrations of less than 1 unit per ml. of milk, definitely inhibited acid production but showed little effect on aroma development. Special attention should be given to rinsing equipment before use, where quaternaries are employed in sanitizing.

T. J. Claydon

110. Modern methods of mastitis treatment cause trouble in the manufacture of fermented dairy products. H. C. HAUSEN, G. E. WIGGINS and J. C. BOYD, Univ. of Idaho, Moscow. J. Milk & Food Technol., **13:** 359–365. Nov.–Dec., 1950.

Inhibition of lactic acid bacteria grown in milk when penicillin, streptomycin, aureomycin, sulfanilamide and sulfamerazine were injected into infected mastitis udders was studied. Apparently, there is no diffusion of these compounds from the treated quarters to the untreated quarters. Cream separated from milk treated with penicillin and streptomycin showed a marked effect on the growth of lactic acid bacteria. whereas sulfanilamide, sulfamerazine and aureomycin were less effective against the organisms.

When penicillin and streptomycin were used, reconstituted milk and milk powder containing 1% milk from treated quarters inhibited the lactic acid organisms. When sulfanilamide, sulfamerazine and aureomycin were used in the quarters and the milk reconstituted, a definite stimulating effect on the test bacteria was observed. H. H. Weiser

111. Some observations on bacteria isolated from milk that grow within a psychrophilic temperature range. LURA KENNEDY and H. WEISER, Dept. of Bact., Ohio State Univ., Columbus. J. Milk & Food Technol., **13:** 353–357. Nov.–Dec., 1950.

Pasteurization of raw milk reduced the psychrophilic types of organisms, but did not completely destroy all of them. Storage of milk at 10° C. for several days tended to increase the number of psychrophilic bacteria. H. H. Weiser

112. The value of the coliform count in the routine examination of milk and dairy products. D. SACKETT and G. GRALAK, Clinical Lab., Elgin State Hosp., Elgin, Ill. J. Milk & Food Technol., **13:** 350–352. Nov.–Dec., 1950.

No correlation existed between the standard plate count and the coliform count in raw milk. Their study showed 98% of the samples of milk satisfactorily pasteurized had a coliform count of less than 5 organisms/ml., regardless of the plate count. Therefore, coliform count of 0–5/ml. should be used as a standard for grade A pasteurized milk, using desoxycholate agar as the plating medium. H. H. Weiser

113. Inactivation of bacteriophage of lactic acid streptococci at high and low pH levels. C. C. PROUTY, Dept. of Dairy Husbandry, Wash. Agr. Expt. Sta., Pullman. J. Milk & Food Technol., **13:** 329–331. Nov.–Dec., 1950.

The author investigated 3 strains of bacteriophage and their resistance to different pH levels, comparable to those used in various cleansing agents. The results seem to indicate that the ordinary acid and alkaline detergents now being used would not be effective in controlling an outbreak of bacteriophage infection.

H. H. Weiser

114. A propos des analyses bactériologiques quantitatives: Dans quelle proportion les germes de l'air peuvent-ils polluer les plaques lors de l'ensemencement et durant l'incubation? (Bacteriological analyses: How much can air germs pollute plates during inoculation and incubation?). E. NOVEL, Hyg. Inst., Genève, Switzerland. Mitt. Leb. Hyg., **40,** 3/4: 255–267. 1949.

Pollution was found to average 0.49 colonies/plate of 9-cm. diameter (mean of 100 plates "inoculated" with sterile water and incubated at 20° C. for 15 d.). For plates of 12- and 14-cm. diameter the figures were 1.15 and 4.6 colonies/plate, respectively. Pollution usually does not falsify results, if operations are done properly.

A. Fasler

115. The utilization of formaldehyde by propionic acid bacteria. F. W. LEAVER, Western Re-

serve Univ., Cleveland, O. J. Am. Chem. Soc., **72,** 11: 5326–5327. Nov., 1950.

C^{14} formaldehyde (0.001 *M*) was utilized by *Propionibacterium arabinosum,* principally via conversion to CO_2. A comparison with glycerol, glucose, i-erythritol and pyruvate fermentations indicates that formaldehyde participates in the formation of propionic acid in a manner common to all the known fermentations with this organism. Formaldehyde may be an essential intermediate in the synthesis of propionic acid.

H. J. Peppler

116. Chemical nature and synthesis of the Lactobacillus bulgaricus factor. E. E. SNELL, G. M. BROWN, V. J. PETERS and J. A. CRAIG, Univ. Wis., Madison, and E. L. WITTLE, J. A. MOORE, V. M. MCGLOHON and O. D. BIRD, Parke, Davis & Co., Detroit, Mich. J. Am. Chem. Soc., **72,** 11: 5349–5350. Nov., 1950.

On papergrams the Rf value of the sulfur-containing fragment in hydrolysates of the *L. bulgaricus* factor (LBF) was found to be the same as that of β-mercaptoethylamine (0.43, pyridine-water solvent). A non-crystalline product prepared from the reaction of methyl pantothenate, β-mercaptoethylamine and acetamide is indistinguishable from natural LBF in Rf values and activity for *L. helveticus, L. arabinosus* and *Saccharomyces carlbergensis.* The most active fractions of the synthetic product contained as much as 30,000 LBF units/mg. solids. LBF may exist as N-(pantothenyl)-β-aminoethanethiol or as the corresponding disulfide. H. J. Peppler

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

117. C_{18} unsaturated acids of butterfat. F. B. SHORLAND. Nature, **166,** 4226: 745. 1950.

Appreciable amounts of linolenic as well as linoleic acid are present in milk fat from cows in New Zealand, which are fed almost entirely on pasture. Composition of the fat apparently varies with the composition of the dietary fat.

R. Whitaker

118. La chimie analytique des corps gras et l'emploi d'ether contenant du peroxyde (Analyses of fats and the use of ether containing peroxide). J. TERRIER, Cantonal Lab., Genève, Switzerland. Mitt. Leb. Hyg., **40,** 3/4: 245–247. 1949.

Ether containing peroxide not only is dangerous but gives wrong results when used in extracting fats. A fat was dissolved in ether with a positive peroxide reaction (it had been standing for 3 wk. in a white bottle on a table in the sun), the ether was distilled off and the fat dried and weighed. An increase of 1% of its weight was observed, while ether without peroxide increased it only 0.1% with the same treatment. The Lea no. was 60, compared to 1 for the same fat treated with peroxide-free ether. The author advises keeping the ether in a brown bottle, away from the light, with addition of 2 mg. diphenyl amine per 1.

A. Fasler

119. Le dosage de certains composants du beurre: matière grasse, lactose et mateières protéiques (Determination of certain butter components: fat, lactose and proteins). J. TERRIER, Cantonal Lab., Geneve, Switzerland. Mitt. Leb. Hyg., **40,** 3/4: 247–252. 1949.

Fat is extracted from dehydrated butter by mixing it with ether, centrifuging and decanting the ether solution, similar to the procedure in use for the determination of fat in cacao and chocolate. This procedure is more satisfying than extracting the fat with ether and taking an aliquot part of ether solution for the determination, as the latter often gives too high fat values due to evaporation of ether. The residue of the extraction can be used for determining lactose or N.

A. Fasler

120. Some effects of salt and moisture on rancidity in fats. IRENE CHANG and BETTY M. WATTS, College of Home Economics, Syracuse Univ. Food Research, **15,** 4: 313–321. July–Aug., 1950.

These studies, using pure lard as the test fat and peroxide values as criteria of rancidity (oxidative), indicate that in dilute solution (less than 5%) NaCl exercises a slight antioxidative effect. At higher concentrations (over 15%, and especially at the saturation point), it is strongly prooxidative. The activity of the salt was not found to be influenced by the presence of hemoglobin or muscle extract. $CaCl_2$ has a greater and KCl a lesser prooxidative effect than NaCl.

F. J. Doan

121. Fat determinations in milk and milk products. L. GERSHENFELD and B. UCKO. Dept. of Bact., Philadelphia College of Pharmacy, Phila., Pa. J. Milk & Food Technol., **13,** 342–343. Nov.–Dec., 1950.

The Schain method for determining the fat content in milk has been modified. The authors claim this technique gives clear-cut results. It is a rapid method and is applicable to most milk products. Solution A (containing 3.5 ml. of the nonionic detergent) is added to solution B or 10 ml. of the anionic detergent to make 1 solution. A boiling water bath is used instead of a bath controlled at 82° C. H. H. Weiser

122. Separation of γ-casein. N. J. HIPP, M. L. GROVES, J. H. CUSTER and T. L. MCMEEKIN, E. Reg. Research Lab., Philadelphia, Pa. J. Am. Chem. Soc., **72,** 11: 4928–4931. Nov., 1950.

Separation was accomplished by fractionation of washed casein with 50% ethyl alcohol and with water at pH 5.8. This preparation is electrophoretically homogeneous in solutions alkaline to the isoelectric point (pH 5.8–6.0) but inhomogeneous in acid solutions. The composition and properties of γ-casein are similar to the alcohol-soluble, low-phosphorus casein isolated by Osborne and Wakeman and characterized by Mellander. H. J. Peppler

123. Isolation of an electrophoretically homogeneous crystalline component of β-lactoblobulin. B. D. POLIS, H. W. SCHMUKLER, J. H. CUSTER and T. L. MCMEEKIN, E. Reg. Research Lab., Philadelphia, Pa. J. Am. Chem. Soc., **72,** 11: 4965–4968. Nov., 1950.

The slow-moving component of β-lactoglobulin, designated β_1-lactoglobulin, was isolated as a pure crystalline protein by a combination of the procedures of differential solubility at pH values acid and alkaline to the isoelectric point and subsequent fractionation with alcohol at low ionic strength. A comparison of the physico-chemical properties of β_1-lactoglobulin and of the normal complex reveals the most striking variations are in the solubility and electrophoretic data.

H. J. Peppler

124. Analyse d'un lait d'éléphant (Analysis of an elephant's milk). S. KRAUZE and B. LEGATOWA, Hygiene Inst., Warsaw, Poland. Mitt. Leb. Hyg., **40,** 5/6: 321–324. 1949.

Analysis 6 mo. after birth was: fat, 13.2%; SNF, 9.05; lactose, 3.85; proteins, 4.27; ash, 0.93; Cl+, 0.045; specific gravity, 1.0325; acidity only, 0.04%; reaction alkaline towards litmus.

The milk had a smell similar to coconut oil, though there had been no coconuts in the food of the elephant. The Reichert-Meissl no. of the fat was 4.7 and the Polenske no., 30.4. The authors think it probable that the fat contains caprylic, lauric and myristic acids which are characteristic for coconut oil. A. Fasler

125. The kinetics of trypsin digestion. D. FRASER and R. E. POWELL, Princeton Univ. Princeton, N. J., and Univ. of Calif., Berkeley. J. Biol. Chem., **187,** 2: 803–820. Dec., 1950.

By means of a simple graphical technique (called the slide-fit technique) data for the self-digestion of trypsin and the digestion of purified casein by crystalline trypsin are presented and analyzed. Enzymatic self-digestion is unimportant in the rate decline of casein-trypsin digestion. Casein behaves as a mixture. The proportions of components determined kinetically are close to those obtained from electrophoretic measurements. The minor component digests approx. 5 times as fast as the major component; the rate constant for the latter is 0.018. H. J. Peppler

126. Casein filaments treated with mercuric salt and formaldehyde solution. J. F. CORWIN, J. R. CALHOUN and T. M. BUZZO (assignors to Borden Co.). U. S. Patent 2,533,356. 8 claims. Dec. 12, 1950. Official Gaz. U. S. Pat. Office, **641,** 2: 454. 1950.

Casein filaments from the spinnerets are hardened at 150–190° F. at pH 2.0 and 6.0 in a solution of formaldehyde and a mercuric salt.

R. Whitaker

127. Decrease of reduced ascorbic acid in goat's milk during storage. A. D. HOLMES, Mass. Agr. Expt. Sta., Amherst, Mass. Food Research, **14,** 6: 468–471. Nov.–Dec., 1949.

Goat's milk, cooled immediately after milking and held at 10° C. in the dark, was found to suffer comparable losses of reduced ascorbic acid over a 4-d. period to those experienced by cow's milk. Average total losses in the study were 61.2% for goat's milk and 65.5% for cow's milk.

F. J. Doan

128. The DDT content of milk products. H. D. MANN and R. H. CARTER, Bureau of Entomology, and R. E. Ely, Bureau of Dairy Industry, U.S.D.A., Washington, D. C. J. Milk & Food Technol., **13:** 340–341. Nov.–Dec., 1950.

The effect of DDT in various dairy products made from raw milk containing this compound was studied. Cows were fed soybean-oil plus DDT, then the milk was processed and the different products analyzed for their DDT content by the Schechter-Haller colorimetric method. The DDT content is not appreciably reduced in any of the dairy products. Pasteurization of the milk had very little effect in reducing the amount of DDT. H. H. Weiser

129. Method for improving the strength of artificial insolubilized protein filaments. D. TRIALL and G. K. SIMPSON (assignors to Imperial Chemical Inds.) U. S. Patent 2,535,103. 8 claims. Dec. 26, 1950. Official Gaz. U. S. Pat. Office. **641,** 4: 1109. 1950.

Wet spun protein filaments are treated under tension with a water-soluble soln. of an ionizable mercuric salt to improve the strength.

R. Whitaker

Also see abs. no. 101, 104, 163.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

130. Cheese hoop holder for milk can washing machines. A. FARRAR (assignor to Kraft Food Co.). U. S. Patent 2,534,345. 7 claims. Dec. 19, 1950. Official Gaz. U. S. Pat. Office, **641,** 3: 811. 1950.

A frame having the same dimensions and shape of a milk can holds a cheese hoop in an inverted position. The frame and holder pass through a milk can washer, the mechanism being actuated by the can-shaped frame. R. Whitaker

131. Cheese press. P. MUELLER (assignor to Paul Mueller Co.). U. S. Patent 2,535,592. 2 claims. Dec. 26, 1950. Official Gaz. U. S. Pat. Office, **641,** 4: 1237. 1950.

The cheese press described consists of a U-shaped trough in which the hooped cheese is pressed by an hydraulic press against an end plate held by lugs evenly spaced along the side of the trough. R. Whitaker

132. Method of and apparatus for heat treating evaporated milk. P. C. WILBUR (assignor to Food Machinery and Chemical Corp.). U. S. Patent 2,536,115. 42 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 98. 1951.

Rapid heating in a continuous sterilizer is accomplished by subjecting the cans to high temperatures. To prevent "burn-on" of this product on the can walls, a non-condensable gas is mixed with the steam used for heating. R. Whitaker

133. Method and apparatus for heating foodstuffs in containers. P. C. WILBUR (assignor to Food Machinery and Chemical Corp.). U. S. Patent 2,536,116. 15 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 98. 1951.

Same heating medium as in abstract 132 is employed. A constant pressure is maintained in the heating chamber, but the temperature gradient increases in the direction the cans move.
R. Whitaker

134. Apparatus for and method of testing pasteurization time. R. N. PERKINS (assignor to Foxboro Co.). U. S. Patent 2,536,082. 11 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 89. 1951.

The time milk is held in a HTST pasteurizer holding tube is measured on a chart on a 2-pen temperature recorder. The bulbs are placed at each end of the holding tube in the milk stream.
R. Whitaker

135. Continuous freezing machine. C. R. STOELTING, O. E. STAMBERG and E. F. VILTER (assignors to Industrial Research Labs.). U. S. Patent 2,535,462. 6 claims. Dec. 26, 1950. Official Gaz. U. S. Pat. Office, **641,** 4: 1201. 1950.

A continuous ice cream freezer of the horizontal type is described, in which the mix is introduced at 1 end and the finished product drawn from the other. Cooling is accomplished by expanding compressed liquid refrigerant in a jacket.
R. Whitaker

136. Vented closure for milk tank manholes. J. H. DEFREES (assignor to Pa. Furnace and Iron Co.). U. S. Patent 2.533,771. 2 claims. Dec. 12, 1950. Official Gaz. U. S. Pat. Office, **641,** 2: 563. 1950.

A manhole cover for milk tanks equipped with a flutter valve to equalize the pressure between the tank and the outside atmosphere is described.
R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

137. How to ease the profit-squeeze. F. MERISH. Milk Plant Monthly, **39,** 11: 52–55. Nov., 1950.

Efficient business management in a war economy may be accentuated by: (a) improving accounting systems, (b) accurate checks on working capital, (c) preparation of monthly profit and loss statements, (d) prompt collections, (e) use of stock control records, (f) departmentizing of costs and (g) avoiding unproductive labor costs.
J. A. Meiser, Jr.

138. Get yourself a robot bookkeeper. F. MERISH. Milk Plant Monthly, **39,** 11: 67–70. Nov., 1950.

Bookkeeping machines greatly increase the efficiency of business management in milk plants since they reduce clerical work, simplify route settlements, simplify inventory control and assemble truck costs easily.
J. A. Meiser, Jr.

139. Efficiency in truck loading. Anonymous. Milk Plant Monthly, **40,** 1: 52–54. Jan., 1951.

Routemen make up load sheets 1 d. in advance. The employee who trucks bottled milk and other products into the cooler assigns definite spaces for each routemen's load as necessitated by the above sheet. The following morning routemen roll out their individually stacked orders on hand trucks to delivery vehicles. Not only does this system improve efficiency in preparing loads but simplifies record keeping as well.
J. A. Meiser, Jr.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

140. Cow-hay burners or grain burners. J. B. KITCHEN. The Jersey Bull., **69,** 24: 2044, 2081–2083. Dec., 1950.

At the N. J. Agr. Expt. Station cows were divided into 3 groups of 10 cows each. Group 1 was fed an all-roughage ration of U. S. no. 1 extra green leafy alfalfa hay and corn silage. Group 2 was fed the roughage and 1 lb. of grain for each 6 lb. of 4% milk produced. Group 3 received the roughage and 1 lb. of grain to each 3 lb. of 4% milk. More than 50 cows have completed 1 or more lactations in this experiment. Taking group 3 as 100%, the cows in group 2 produced 95% as much milk and those on roughage alone produced 81.6% as much. Tables show profit over feed cost at various milk prices with the 3 levels of grain feeding and with grain at $40, $55 and $75 per ton.
A. R. Porter

141. Metabolism of acetate and propionate in the ruminant. I. G. JARRETT and B. J. POTTER. Nature, **166,** 4221: 515. 1950.

Acetate (Ac) and propionate (Pr) are produced by bacterial fermentation in the rumen and are absorbed in significant amounts. Total ketone bodies in the blood are increased by Ac but the effect is counteracted when Pr also is absorbed. Both Ac and Pr increase the volatile acid in the blood. Pr increases the pyruvic acid in the blood but the effect is reduced when Ac also is absorbed. Lactic acid in the blood is increased by Pr and mixtures of Ac and Pr but not by Ac alone.
R. Whitaker

Also see abs. no. 128.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

142. Milk cooling device. J. A. KINGSTON (assignor to Gascoignes, Ltd.). U. S. Patent 2,536,752. 2 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 265. 1951.

A milk can is inserted in a cylindrical tank slightly larger in diameter than the can. A cover, placed on the tank, is so constructed that the milk is conducted from a supply tank mounted on top to a distributing trough which feeds the milk as a film down the inside of the can. A refrigerated liquid, pumped into the bottom of the cylinder, rises in the space between the can and cylinder through spiral channels to overflow from an outlet in the cover. As the can fills with milk the air is vented through a tube in the lid.
R. Whitaker

143. Combination dairy stanchion gate and feed box. W. F. THOMAS. U. S. Patent 2,536,236.

8 claims. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 129. 1951.

An overhead feed box supported on and operated by the stanchion gate is described.

R. Whitaker

144. Calf weaner. J. R. RHINEHART. U. S. Patent 2,535,158. 1 claim. Dec. 26, 1950. Official Gaz. U. S. Pat. Office, **641,** 4: 1123. 1950.

A nipple-coupling to be attached to a feed pail is described. R. Whitaker

145. Nipple attachment for feed pails. J. R. RHINEHART. U. S. Patent 2,535,159. 2 claims. Dec. 26, 1950. Official Gaz. U. S. Pat. Office, **641,** 4: 1124. 1950.

A nipple coupling equipped with a check valve for attaching to a feed pail is described.

R. Whitaker

146. Milking timer. L. J. SCHILLING (assignor to Babson Bros. Co.). U. S. Patent 2,534,927. 12 claims. Dec. 19, 1950. Official Gaz. U. S. Pat. Office, **641,** 3: 963. 1950.

A timer which may be set to stop a milking machine at a desired time interval is described.

R. Whitaker

147. Bull bridle. M. R. WARRICK. U. S. Patent 2,533,857. 7 claims. Dec. 12, 1950. Official Gaz. U. S. Pat. Office, **641,** 2: 587. 1950.

A halter for bulls consists of a frame which encircles the head of the animal and attaches to the ring in the nose. R. Whitaker

148. Barn cleaning apparatus. C. MATTHEWS and H. M. WENGER. U. S. Patent 2,533,676. 3 claims. Dec. 12, 1950. Official Gaz. U. S. Pat. Office, **641,** 2: 539. 1950.

An apparatus for mechanically cleaning the gutters of a cow barn is described.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

149. Calculating the solids content of ice cream mixes. J. A. MEISER, JR., Mich. State College, E. Lansing. Milk Plant Monthly, **40,** 1: 26, 28–29. Jan.. 1951.

A procedure for calculating the fat and total solids content of mix formulae using average compositions or analytical tests of the mix ingredients is given. J. A. Meiser, Jr.

150. Ice cream from buttermilk. Anonymous. Ice Cream Trade J., **46,** 12: 26–28. Dec., 1950.

Research workers in the Bureau of Dairying, U.S.D.A., have prepared sweetened condensed buttermilk following the procedure used in making sweetened condensed milk. Cane sugar (12.5–13.5 lb./100 lb. of buttermilk) was added to an equal weight of water, the solution boiled, filtered and drawn into the vacuum pan following the buttermilk. The mixture then was concentrated to between 72 and 74% total solids, cooled to 86° F., seeded with lactose and cooled with further agitation to 68° F. and then stored at 60° F. until used. Plain condensed buttermilk containing 27–30% total solids was made by concentrating fresh buttermilk. Sweetened condensed buttermilk was prepared from neutralized sour cream and from buttermilk in which different degrees of acidity were developed and neutralized before concentration. These products were used in ice cream as a source of serum solids. Solids from sweet cream buttermilk are an excellent source of solids-not-fat for ice cream and may be used interchangeably with skimmilk solids and may be blended with them to improve whipping properties of the mix. The buttermilk solids from neutralized sweet cream having a titratable acidity at the time of churning in excess of 0.25% is unsuitable for use in ice cream.

W. H. Martin

151. Emulsifiers in ice cream. W. S. ARBUCKLE, Univ. of Md., College Park. Ice Cream Trade J., **46,** 10: 106. Oct., 1950.

Emulsifiers decreased the rate of melting. The lecithinated products, glycerides and fatty acid esters reduce the rate of melting, but not to an objectional extent. The polyoxalkylene derivatives greatly reduce rate of melting and the finished ice cream may be criticized for not melting. Emulsifiers produce smaller ice crystals and somewhat smaller air cells and smoother ice cream. Excessive emulsifier may cause short body, slow melting and a curdy meltdown in the finished ice cream. W. H. Martin

152. Basic viscosity of ice cream mixes. J. TOBIAS and P. H. TRACY, Univ. of Ill., Urbana. Ice Cream Trade J., **46,** 10: 64, 183. Oct., 1950.

Basic viscosity is the constant viscosity value of the mix and results when the apparent viscosity of a mix, stabilized with gelatin, is reduced as far as possible by agitation. The latest and fastest method for arriving at basic viscosity takes only 90 sec. The mix is agitated at 3000 rpm. and the sample is raised and lowered during the agitation period. The viscosity then is measured with a Hoeppler viscosimeter. Duplicate determinations varied only from 0.3–1 centipoise.

The new method is very satisfactory in that large numbers of samples may be run in less time than required for older methods. The 90-sec. method is simple, easy to run and affords the best reproducible results yet obtained.

W. H. Martin

153. Chocolate ice cream. C. W. DECKER, State College of Washington, Pullman. Ice Cream Trade J., **46,** 9: 34, 93–94. Sept., 1950.

Cocoa (22% fat), chocolate liquor (50–55% fat) and chocolate flavoring blends (35–40% fat) are available for use in ice cream. The author recommends that ice cream be flavored with a combination of 3% cocoa and 1.5% chocolate liquor together with 18% sugar. Those wishing a variation in this formula may replace a portion of the chocolate liquor with cocoa at the rate of 0.25% cocoa for each 0.5% chocolate liquor until they consider the flavor desirable.

To prepare chocolate syrup heat water to boiling in a steam kettle or water bath, add chocolate liquor and add mixed cocoa and sugar with

vigorous stirring. Heat at 185° F. for 10–15 min. with vigorous agitation, preferably with something like a Lightning agitator. Add enough water to replace that lost by evaporation. Homogenization may be used, but is not essential. Skimmilk may be used in place of the water but should not be boiled. The syrup can be cooled and stored. Such a syrup will be quite viscous and will have to be warmed in hot water previous to adding to the freezer before it can be handled properly.

W. H. Martin

154. Concentrated fruit essences. C. C. FLORA, L. L. DAVIS and C. W. HOLDAWAY, Va. Agr. Expt. Sta. Ice Cream Trade J., **46,** 10: 112, 128–129. Oct., 1950.

Strawberry and peach essences were prepared from fruit collected in the season of 1947. Blakemore strawberries were washed, capped by machine and sorted on an inspection belt. The discards used in these studies were frozen in 50–lb. tins stored at 0° F. Elberta peaches were lye-peeled, washed, neutralized, inspected and sliced. Sugar with ascorbic acid was added to make a commercial 4:1 pack. The sliced peaches were frozen in 30-lb. tins and stored at 0° F.

The frozen fruit was thawed overnight without removal from the container and the juice pressed from the fruit in a small cider press. The essence was stripped from the juices, using a portable volatile flavor recovery unit originally designed for apple juice. The unit was operated to give a theoretical concentration of essence of strawberry of 108-fold and of peach of 100-fold. The essence was placed in tightly-stoppered bottles and stored in a refrigerator at 50° F. A standard ice cream mix containing 12% fat, 11% serum solids, 15% sugar and 0.3% stabilizer was used to determine whether or not the use of this method was feasible in manufacture of ice cream. The fruits and essence being added at the freezer, trials were conducted using both pureed and whole fruit with variations in amount of fruit and essence used. Flavor on the basis of official score card for ice cream judging by 4 experienced judges, desirable flavor intensity by experienced judges and consumer preference tests were used for evaluation. In strawberry ice cream canned strawberries plus 20 ml. of essence and frozen berries plus 20 ml. of essence were preferred. In peach ice cream canned peaches plus 20 ml. of essence and frozen peaches plus 10 ml. of essence were preferred by consumers.

W. H. Martin

155. Soft ice cream. H. F. SWENSON, Sweden Freezer Mfg. Co., Seattle, Wash. Ice Cream Trade J., **46,** 12: 44–46, 74–78. Dec,. 1950.

"Soft ice cream" is a term applied to a variety of products sold under different trade names such as Dairy Queen, Zesto and Sweden Freez. It usually is lower than ice cream in butterfat and overrun. It is served directly from the freezer. The volume of sales now is estimated at about 120,000,000 annually. This volume has resulted in an increase in mix business. W. H. Martin

156. Mass production of novelties at Huber's, Bridgeport, Conn. Anonymous. Ice Cream Trade J., 46, 9: 40–41. Sept., 1950.

With the increased volume of ice cream on-the-stick has come the demand for a method of mass production of these items which will save space and labor. Huber Ice Cream Co. accomplished this by organizing machines to do the job rapidly. Included in the production line are an Anderson filler, demolding and coating vats and a bagging machine. With this arrangement, the firm produces some 5,500 doz. novelties in an 8-hr. day, with a crew of 12 persons. The labor required was cut at least 7 persons and faster production rate obtained.

W. H. Martin

157. Method and apparatus for making frozen confections. M. B. RASMUSSON (assignor to Vitafreze Equipment, Inc.). U. S. Patent 2,535,-231. 21 claims. Dec. 26, 1950. Official Gaz. U. S. Pat. Office, **641,** 4: 1142. 1950.

Details are given of a continuous process for making coated-stick novelties. R. Whitaker

158. Apparatus for coating frozen confection and the like. A. FRIEDMAN (assignor to Joe Lowe Corp.). U. S. Patent 2,536,635. 1 claim. Jan. 2, 1951. Official Gaz. U. S. Pat. Office, **642,** 1: 235. 1951.

Frozen confections are coated by dipping them into a tank of melted chocolate or other coating. This tank is kept full by a pump which circulates the coating from an outer tank into which the first tank overflows. The outer tank is jacketed to maintain the proper coating temperature.

R. Whitaker

159. Current factors influencing consumer buying of ice cream. Ice Cream Trade J., **46,** 10: 80–81, 188–190. Oct., 1950.

The ice cream industry has been experiencing sales decline for the 4th consecutive yr. Pioneer Ice Cream Div. of the Borden Co. conducted an interview to discover factors influencing the ice cream sales. Of 472 persons interviewed 177 said they bought more ice cream than last yr.; 157 bought less and 81 bought about the same amount. The reasons why consumers bought more ice cream in 1950 than in 1949 were: increased appetite, 28.2%; larger family, 23.2%; entertaining more, 11.3%; warm weather, 10.2%; television parties, 8.5%; miscellaneous, 18.6%. The reasons given why consumers bought less ice cream were: smaller income, 27.4%; diet, 23.5%; installment buying, 22.3%; smaller family, 14.0%; cooler weather, 4.0%; miscellaneous, 8.8%

W. H. Martin

160. Formula for forecasting ice cream consumption. F. W. KIMBALL and D. NICKEL, Prod. Services Dept., Golden State Co., Ltd., San Francisco, Cal. Ice Cream Trade J., **46,** 10: 88, 180. Oct., 1950.

A mathematical equation has been developed for use in forecasting ice cream consumption. The formula is: Ice cream consumption in lb./person equals 0.037 times real disposable income

in dollars/person minus 9.3. When the per capita consumption is about 20 lb./person, the formula will have an error of less than 5%, 2/3 of the time. The error of the method is less than 1 lb./person 67% of the time and less than 2 lb., 95% of the time. Principal factors which determine ice cream consumption are price, weather and purchasing power of the consumer. Purchasing power is calculated on the basis of national disposable income (income after taxes), population and cost of living. W. H. Martin

161. Dispensing machine for ice cream and sherbets. D. C. MADDUX. U. S. Patent 2,534,782. 13 claims. Dec. 19, 1950. Official Gaz. U. S. Pat. Office, **641,** 3: 925. 1950.

Ice cream or sherbet is dispensed by an apparatus which strips off a ribbon of product from the open end of a cylindrical carton as it is rotated. R. Whitaker

Also see abs. no. 135.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

162. Milk flavors. J. A. NELSON, Montana State College, Bozeman. Milk Plant Monthly, **39,** 11: 38, 40. Nov., 1950.

Dividing the causes of abnormal flavors in milk into 4 categories, the author presents a discussion of the frequency of occurrence and methods of eliminating off-flavors in milk.

J. A. Meiser, Jr.

163. The curd tension of low fat milk. G. ANDERSON and K. G. WECKEL, Univ. of Wis., Madison. Milk Plant Monthly, **40,** 1: 37–38. Jan., 1951.

To determine the effect of heat treatment and homogenization on curd tension of low-fat milk, raw skim separated from fresh whole milk was standardized by the addition of cream to contain 0.5, 1.0 and 1.5% fat. Following standardization, individual lots were heated to 150, 160 and 170° F. and homogenized at 2,000 lb. pressure. Only a temperature of 170° F. for 30 min. produced a product that fell within the accepted meaning of low curd milk. Homogenization had no additional effect on curd tension of milks heated to 170° F. Homogenization of the 1.0 and 1.5% milks heated to 160° F. lowered the curd tension, but had little effect on 0.5% milk. Aging of low-fat milks for 3 d. at 40° F. following heat treatments and homogenization did not materially change the curd tension.

J. A. Meiser, Jr.

164. A comparative study of milk from a refrigerated bulk dispenser and similar milk delivered in sealed bottles. M. P. HORWOOD, M.I.T., Cambridge, Mass. J. Milk & Food Technol., **13:** 367–371. Nov.–Dec., 1950.

A refrigerated bulk milk dispenser was studied at an M.I.T. dormitory over an 8-mo. period. The milk should have a low bacterial count and be held around 40° F. Under these conditions the milk could be dispensed to consumer more economically without sacrificing public health protection provided by sale of milk in individual containers. H. H. Weiser

165. Uniforms for routeman. Anonymous. Milk Plant Monthly, **40,** 1: 44–46. Jan., 1951.

For 40 qt. of added business during the fall quarter, a cap, jacket and pair of trousers were awarded routemen. Since added business is hard to obtain during the summer months, a quota of 8 qt. for a 30-d. period enable the men to obtain a cap, summer jacket or pair of trousers. This incentive aids routemen who must buy their own uniforms and helps dairies uniform all their men alike. J. A. Meiser, Jr.

166. Year round promotion builds sales. F. FLAGG. Milk Plant Monthly, **39,** 12: 44–46. Dec., 1950.

To increase sales, a booklet entitled *Baby Post* was sent every month to mothers with babies. This series containing 12 booklets provided helpful advice on the care of infants. Delivered personally to consumer and non-consumer they provided excellent opportunities for routemen to meet prospective customers.

To promote the sale of cottage cheese, routemen received 1¢/pkg. for increases up to 25% of their previous month's sales; 2¢/ pkg. was paid for sales exceeding this percentage. Additional premiums of $20, $10 and $5 were offered.

J. A. Meiser, Jr.

Also see abs. no. 111, 112, 134, 139.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

167. Nature of the milk-ejection process. W. G. WHITTLESTON. Nature, **166,** 4232: 994. 1950.

The release of milk from the mammary gland is caused by a let-down hormone, which (a) contracts the myoepithelium around the alveoli, thus increasing the pressure within the latter and (b) opens the small ducts as the result of tightening of the longitudinal myoepithelial structures surrounding them. These actions produce a fall in duct impedance and the consequent rapid flow of milk into the large ducts and gland cistern.

R. Whitaker

168. Plasmacytosis of the bovine udder during colostrum secretion and experimental cessation of milking. B. CAMPBELL, R. M. PORTER and W. E. PETERSEN. Nature, **166,** 4230: 913. 1950.

Plasma cells, which are believed to be the origin of antibodies transmitted to the calf by the colostrum, were found to be present in the secretory tissue a few days prior to and after calving. At other times, no cell formation was noted. Experimental cessation of milking of 2 quarters of the udder of a normal lactating cow also produced plasma cells for a few days but these disappeared again shortly after milking was resumed.

R. Whitaker

169. Wall structure and closing mechanism of the bovine teat. W. D. POUNDEN, and J. D. GROSSMAN, Ohio Agr. Expt. Sta., Wooster. Am. J. Vet. Research, **11,** 41: 349–354. Oct., 1950.

Careful dissection of fresh and preserved teats showed the presence of distinct fibrous layers below the mucosa and between the skin and the intermediate layer. The fibrous layers showed considerable tension. It is important to consider each layer in surgical repair of the teat. Experiments to determine the important closing mechanisms of the teat were performed on teats before and after death of 5 cows. Only slightly less pressure was required to cause teat leaking postmortem than antemortem. This was interpreted to indicate the minor role of nerve stimuli upon the teat sphincter. Other structures shown to be important in maintaining teat closure were Furstenberg's rosette, circular elastic fibers at the lactiferous outlets and accumulation of desquamated epithelial cells. Removal of the latter was more complete by machine than by hand milking, and was followed by milk remaining between the folds of the teat opening. This could provide a pathway for bacterial growth into the teat. E. W. Swanson

170. Physiological mechanism of milk secretion. S. J. FOLLEY. Nature, **166,** 4229: 853. 1950.

This is a review of the papers presented at the international colloquium on the physiology of milk secretion, held Aug. 22–29, 1950, in Paris by the Centre National de Recherche Scientifique and the Rockefeller Foundation. R. Whitaker

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

171. A method for measuring the cleanliness of milk cans. J. JENSEN and J. WATERSON, Mich. Agr. Expt. Sta., E. Lansing. J. Milk & Food Technol., 13: 332–335. Nov.–Dec., 1950.

The method consists of hand-washing the milk can with a mixture of a detergent and wetting agent-condensed phosphate added to 1 qt. of water. A sediment test was made of the wash water using a Langsenkamp-Wheeler tester. The sediment pads were graded into 4 classes according to the amount of material on each disc.

H. H. Weiser

172. A study in the selective method of farm inspection. E. MANDT and M. S. HILBERT, Wayne County Health Dept., Eloise, Mich. Milk Plant Monthly, **39,** 11: 56–58, 60. Nov., 1950.

Shortage of personnel necessitated adoption of a method of screening producers in need of special assistance in improving production methods. Following direct microscopic counts on raw milk samples from individual producers, farm visits were made to those farms showing bacteria counts in excess of 200,000/ml. Immediate improvement in the raw milk supply followed, yet plate counts on retail milk remained high. Laboratory-pasteurization techniques then were incorporated to determine those raw milk sources responsible for high counts in the pasteurized milk. Producers' milk having counts in excess of 30,000 following laboratory pasteurization was regarded as excessive. Personal contact of these producers greatly improved the plate counts of the retail pasteurized milk. J. A. Meiser, Jr.

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