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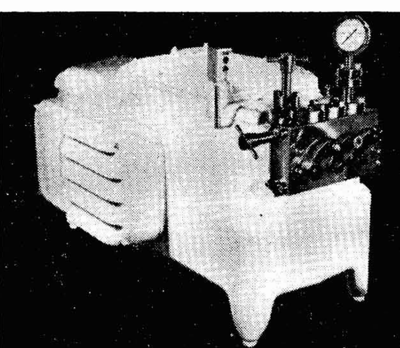

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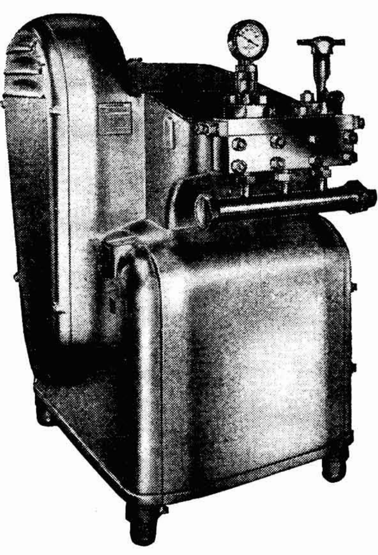

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THE PROTEIN AND NON-PROTEIN NITROGEN FRACTIONS IN MILK.[1] III. THE EFFECT OF HEAT TREATMENTS AND HOMOGENIZATION

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The effect of pasteurization on chemical and physical properties of milk has been studied by many workers. Many competitive claims have been made by proponents of raw and pasteurized milk, as to the desirability of pasteurized milk for human consumption and for manufacture of other dairy products. With these ideas in mind, the following experiments were designed to study the nitrogen distribution as affected by pasteurization and homogenization. The changes have both theoretical and practical values.

Almost all enzymes present in milk are proteinaceous and contain some nitrogen. Most of the enzymes are either partially inactivated or destroyed by different heat treatments; this may alter the form of nitrogen in which they occur. Differences in rennet coagulation time of pasteurized milk and heat stability of evaporated milk are other examples where physical and chemical changes are involved due to different heat treatments.

EXPERIMENTAL

Effect of pasteurization on nitrogen distribution in milk. The effect of holder-type pasteurization on N distribution in milk was studied in detail on eight milk samples. Raw milk samples were obtained from the milk supply as commercially received by the Department of Dairy Industry. After thorough mixing, each sample was divided into two lots. One lot was analyzed raw and the other lot was pasteurized, using a thermostatically controlled laboratory pasteurizer. It consisted of four separate water baths, each equipped with a separate steam inlet, electric heating element and thermo-regulator. The steam was used for heating and exact holding of the temperature was achieved by means of the electric heating element and thermo-regulator. Each bath was fitted to accommodate two aluminum vessels with covers through which aluminum, revolving, agitating paddles were applied. All the samples were pasteurized at 143° ± 1° F. and after holding for 30 min. they were cooled immediately.

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TABLE 1

Effect of pasteurization on nitrogen distribution in milk

Sample no.	Treatment	Total N	Casein N	Albumin N	Globulin N	Proteoses-pep. N	N.P. N	Ammonia N	Urea N	Creatinine[a]	Creatine[a]	Uric acid[a]	α Amino N	Unaccounted N
						(*Mg. per 100 ml. milk*)								
1.	Raw	481.1	380.3	32.5	26.0	19.7	22.6	0.62	7.64	0.19	4.06	2.40	3.85	8.35
	Past.	479.7	379.5	33.6	25.5	18.4	22.8	0.94	8.95	0.19	4.36	2.53	3.85	6.72
2.	Raw	486.5	383.5	38.0	24.4	13.6	27.0	0.51	7.21	0.20	3.90	2.53	6.05	11.10
	Past.	483.8	384.1	26.8	21.4	24.1	27.4	0.57	9.22	0.20	4.20	2.20	6.32	9.11
3.	Raw	463.0	368.7	35.7	17.9	18.2	22.4	1.08	8.95	0.55	3.55	2.43	4.54	5.63
	Past.	462.9	370.1	33.3	17.9	19.0	22.5	1.38	9.32	0.60	3.62	2.36	4.67	4.95
4.	Raw	442.3	343.3	31.4	28.5	15.6	23.5	1.19	7.21	0.55	3.67	2.53	2.89	10.00
	Past.	438.0	343.3	24.7	29.5	16.6	23.9	0.89	7.78	0.58	4.23	2.39	3.44	9.40
5.	Raw	486.9	377.7	39.6	27.5	20.5	21.5	0.84	8.58	0.55	3.85	1.55	3.02	7.10
	Past.	486.9	379.2	38.6	26.1	20.8	22.2	0.94	10.17	0.60	3.80	1.55	3.23	5.91
6.	Raw	448.2	353.1	35.3	24.2	17.6	18.1	0.70	6.54	0.50	3.85	1.67	2.20	6.68
	Past.	442.4	350.7	31.9	22.2	19.0	18.7	0.82	6.65	0.55	3.70	1.76	2.27	6.98
7.	Raw	454.0	356.0	32.8	24.2	15.9	25.2	0.62	10.85	0.47	4.13	2.30	3.51	7.93
	Past.	448.2	350.7	34.5	21.0	15.4	26.6	0.90	10.69	0.47	4.18	2.30	3.44	9.31
8.	Raw	471.4	365.6	43.9	22.3	10.9	28.7	0.79	10.80	0.50	3.90	2.19	3.92	11.05
	Past.	469.5	368.0	38.7	21.3	12.0	29.5	1.01	10.70	0.52	3.98	2.15	4.12	11.50
Av.	Raw	466.7	366.0	36.2	24.4	16.5	23.6	0.79	8.47	0.44	3.86	2.20	3.75	8.48
	Past.	463.9	365.7	32.8	23.1	18.2	24.2	0.93	9.19	0.46	4.01	2.16	3.92	7.98

[a] Reported as such. Unaccounted nitrogen calculated on the basis of nitrogen only.

All the milk samples were tempered to room temperature and analyzed together with the raw series. The data are presented in table 1. Previously, this type of work was done only by Kieferle and Gloetzl (3), who studied the changes involved in the protein and non-protein nitrogen fraction on different heat treatments. Menefee *et al.* (5) have studied the effect of processing on nitrogen distribution but their study was limited to the protein fractions only.

Effect of pasteurization and homogenization on nitrogen distribution in milk. Very little information was available about the distribution of nitrogen as affected by the commercially practiced pasteurization and homogenization. The present study was conducted to develop the subject along these lines.

In order to assure samples that were truly representative of homogenization by machines of commercial size, the milk samples were taken from the commercial pasteurizing and homogenizing operation of the Department of Dairy Industry. A holding type pasteurizer vat of 1,600-lb. capacity was filled with raw milk. After mixing thoroughly by means of agitators for 5 min., a representative sample of raw milk (I) was taken. The milk in the vat then was pasteurized at 155° F. for 30 min. (All the operations were kept normal, as usually conducted by the plant for commercial purposes.) In the meantime a two-stage Manton Gaulin-type homogenizer (capacity of 400 gal. per hour) was rinsed and tempered with clean hot water at 180° F. After pasteurization (the milk was being agitated constantly) a sample of the milk (II) was withdrawn and immediately cooled to room temperature. The milk from the pasteurizing vat was then pumped directly to the homogenizer and homogenized at 2,000 lb. pressure per in.^{2}. To avoid any dilution of milk with traces of water in the homogenizer, the first few gallons of milk coming through the homogenizer were discarded. A homogenized milk sample (III) then was taken directly from the outlet of the homogenizer. Since there should be a temperature rise due to heat of friction in the homogenizer, the temperature was observed at the point of taking the sample. The temperature of the outcoming milk always ranged from 148 to 152° F., which indicates that heat losses from pipe lines and the surface of the homogenizer more than offset the heat of friction. The homogenized milk samples were cooled immediately to room temperature, for immediate procedure with the analysis.

All the three samples, raw (I), pasteurized (II) and homogenized (III), were thoroughly agitated and analyzed for all the different protein and non-protein nitrogen fractions, using the methods described in the previous paper (6). The data are presented in table 2.

EXPERIMENTAL RESULTS

Pasteurization (table 1) and pasteurization and homogenization (table 2) produced no significant changes in the total and casein nitrogen. A very small increase was observed in the proteoses and peptones and non-protein nitrogen content. This was expected on the basis of the findings of the previous workers. Also, no appreciable change occurred in any non-protein nitrogen fraction, except that a slight increase was observed in the NH_3, urea and α-amino nitrogen in both instances.

TABLE 2

Effect of pasteurization and homogenization on the nitrogen distribution in milk

	Sample 1			Sample 2			Sample 3			Average		
	Raw	Past.	Homo.	Raw	Past.	Homo.	Raw	Past.	Homo.	Raw	Past.	Homo.
					(Mg. per 100 ml. milk)							
Total N	453.2	453.2	455.5	469.2	466.9	464.6	477.2	473.3	471.4	466.5	464.5	463.8
Casein N	352.5	349.6	354.8	393.7	398.2	397.1	387.9	386.9	385.0	378.0	378.3	379.0
Albumin N	41.2	43.4	42.6	22.6	19.2	19.7	27.6	26.2	26.2	30.5	29.6	29.5
Globulin N	19.5	17.9	14.7	13.5	9.4	8.3	20.6	15.7	14.3	17.8	14.3	12.4
Proteoses-Pep. N	19.7	21.3	22.1	19.3	19.6	19.2	18.7	20.1	20.7	19.3	20.3	20.7
N.P. N	20.3	21.0	21.4	20.2	20.5	20.3	22.3	24.4	25.2	20.9	22.0	22.3
Ammonia N	0.17	0.63	0.92	0.63	0.74	1.14	0.39	0.63	0.72	0.40	0.67	0.93
Urea N	6.70	6.81	6.23	6.67	6.70	6.16	7.94	9.63	11.36	7.10	7.71	7.92
Creatinine[a]	0.56	0.58	0.67	0.50	0.57	0.61	0.55	0.61	0.61	0.54	0.59	0.63
Creatine[a]	4.51	4.66	4.83	4.21	4.46	4.92	3.87	4.01	4.13	4.20	4.38	4.63
Uric acid[a]	2.32	2.30	2.25	2.20	2.16	2.14	1.91	1.65	1.82	2.14	2.04	2.07
α-Amino N	4.41	5.81	6.27	3.20	3.87	4.27	5.18	7.42	6.35	4.26	5.70	5.63
Unaccounted N	6.60	5.30	5.43	7.40	6.98	6.22	6.75	4.64	4.58	6.92	5.59	5.42

[a] See table 1.

Pasteurization of milk at 143° F. for 30 min. (table 1) did not cause any appreciable changes except in the case of albumin, globulin, NH_3 and urea content. About 9 per cent coagulation was observed in the case of albumin and 5 per cent coagulation for globulin. A slight increase was observed in NH_3 and urea content.

On pasteurization of milk at 155° F. for 30 min. and homogenization at 2,000 lb. (table 2) the albumin and globulin nitrogen decreased, whereas the proteoses-peptones, non-protein nitrogen, NH_3 and α-amino nitrogen increased. Creatinine and creatine also showed a slight increase. The globulin nitrogen decreased by about 20 per cent on pasteurization, with a further decrease of 10 per cent on homogenization. Decrease in the globulin nitrogen was in harmony with reports in the literature. The NH_3 nitrogen increased from 0.40 mg. per cent to 0.67 mg. per cent on pasteurization and to 0.93 mg. per cent on homogenization. The most probable source of this NH_3 is the cleavage of amide nitrogen from the proteins. The increase caused by homogenization probably occurs by virtue of the heat of friction in the process, although the violent agitation and impacts in the homogenization valve might conceivably be factors.

Sommer (8) has reported that coagulation of albumin and globulin is apparently about to commence at the heat treatment employed in the pasteurization of market milk. As expected, the albumin and globulin nitrogen decreased on pasteurization at 143° F. for 30 min. and at 155° F. for 30 min. Since the time of pasteurization was the same in both cases, one would expect a greater decrease at higher temperatures. In the present study, a greater decrease was observed in the total albumin plus globulin nitrogen at the higher temperature than at the lower, but strangely enough, at the lower temperature (143° F.) the albumin nitrogen decreased more than the globulin nitrogen, whereas at the higher temperature the decrease was mostly (20 per cent) in the globulin nitrogen.

These observations, which seem rather unusual, may involve the following speculative possibilities:

(1) In pasteurization at 143° F., a laboratory pasteurizer was used. A limited quantity of about 1.5 qt. of milk was pasteurized in the aluminum vessel provided for the purpose. Though the conditions of temperature and time were under perfect control, a greater area of heating surface was exposed per unit of milk as compared to a smaller heating surface exposed per unit of milk in the commercial pasteurizer. Not only the ratio of metal area to milk volume, but also the kind of metal may be important. In the present comparison, there is aluminum and a high ratio of metal area to milk volume *versus* stainless steel and a low ratio of metal area to milk volume. Any slight difference due to the metal might then be greatly magnified because of difference in relative area. That this factor may be important is indicated by the work of Springer (9) in relation to rennet coagulation of milk. Springer showed that besides temperature, the vessel used has a decided influence on the coagulation time of milk with rennet.

(b) With knowledge about albumin and globulin still very incomplete and with some indications that interconversion may take place, the possibility exists

that the latter factor may complicate the separate determination of the amounts of albumin and globulin coagulated by heat. Snyder (7) found that when albumins were present along with phospholipids in a fat emulsion, an interchange took place whereby the albumin nitrogen decreased and the globulin nitrogen increased.

In summation, no appreciable change occurred in the nitrogen distribution during pasteurization at 143° F. for 30 min. or pasteurization at 155° F. for 30 min. and homogenization, except in the cases of albumin, globulin, NH_3 and urea nitrogen.

Effects of storage on non-protein nitrogen content in evaporated milk. Anderson and Pierce (1) found that the non-protein nitrogen, amino acids and NH_3 content increased and that protein precipitation took place during storage of both raw and sterilized skimmilk at −10° F. and −14° F.

Hunziker (2) reported that the viscosity of evaporated milk decreases with age, and the rate of decrease is accelerated by higher storage temperatures.

Non-protein nitrogen determinations were made on four evaporated milk samples during different stages of its manufacture and storage at 100° F., taking advantage of the availability of such experimental samples from another research project (4).

The increase in non-protein nitrogen content was not very marked during forewarming and condensing, but during sterilization a maximum increase was observed. On storage, the rate of increase was highest during the initial stage, but the rate of increase became much less after 3 days. On the same samples, Maxcy (4) observed a decrease of viscosity during storage. The viscosity decreased at a higher rate during the first 10 hr. than during the latter period. As both problems, the increase and decrease of viscosity during processing and storage, respectively, and the increase in the non-protein nitrogen during all the stages, are related in the protein system in milk, it will be interesting to investigate this subject further.

SUMMARY

Influence of pasteurization on the nitrogen distribution was studied by pasteurizing milk at 143° F. for 30 min. in a laboratory pasteurizer. No appreciable change occurred on pasteurization, except in the case of albumin and globulin. About 9 per cent coagulation was observed in the case of albumin and 5 per cent coagulation for globulin.

Pasteurization at 155° F. for 30 min. and homogenization at 2,000 lb. pressure per in.^{2} produced no significant changes in the nitrogen distribution, except that globulin nitrogen decreased, whereas the non-protein nitrogen and α-amino nitrogen increased.

On storage of evaporated milk at 100° F., the non-protein nitrogen increased with a decrease in viscosity.

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AMMONIATED CANE MOLASSES AND SIMILAR PRODUCTS IN THE RATIONS OF DAIRY CALVES[1, 2]

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It has been demonstrated that urea (1, 2, 3, 4, 5, 8, 9, 11, 12, 13, 14, 15, 16, 17, 20), ammonium bicarbonate (5), ammoniated beet pulp (10) and ammoniated molasses and similar products (7) may serve as sources of nitrogen for growth and production by cattle. There are available considerable tonnages of industrial by-products which, if ammoniated, might prove to be valuable sources of feed for ruminant animals.

The purpose of the experiments presented in this report was to determine the value of some of these products in the rations of growing dairy calves.

EXPERIMENTAL

Preliminary observations.

Trial 1. A number of industrial by-products (ammoniated by Commercial Solvents Corp., Terre Haute, Ind.) were fed to 16-wk.-old Holstein bull calves to determine their effects upon palatability and also to obtain some indication of their effects upon growth. The materials used in these studies were as follows and had the following protein equivalent ($N \times 6.25$): Ammoniated cane molasses, 19.4%; ammoniated cane molasses neutralized with HCl, 16.8%; neutralized with H_3PO_4, 13.8%; neutralized with H_2SO_4, 13.8%; ammoniated inverted cane molasses, 26.2%; neutralized with HCl, 27.5%; neutralized with H_2SO_4, 30.0%; neutralized with H_3PO_4, 29.4%; ammoniated hydrol, 26.8%; ammoniated condensed distillers' molasses solubles, 12.5%; ammoniated citrus solubles, 15.7%; ammoniated citrus pulp, 18.77%; and ammoniated Masonex (Masonite Corp., Laurel, Miss.), 21.62%.

The products were included in the experimental rations so as to supply 22.4 per cent of the total nitrogen from the ammoniated products. The following basal ration was used: 20 lb. wheat bran, 60 lb. crushed oats, 84 lb. cracked corn, 0.2 lb. irradiated yeast (9F), 2 lb. salt, 2 lb. $CaHPO_4$, 2 lb. bone meal and enough cane molasses, ammoniated material and soybean oil meal (solvent process) to bring the protein level to 11 per cent. The percentage of nitrogen was kept low in order to secure as much of the total nitrogen from the ammoniated products as possible and still stay below 10 per cent of the total mix. While the same amount of nitrogen came from each ammoniated by-product source, this necessitated a variation of 3.5 to 10 per cent of the total weight of the final mixture. All of the resulting rations were eaten readily and all produced

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normal growth during the 15 days of observation. The feces appeared normal and no ill effects of any sort were observed.

Trial 2. Following trial 1 four of the materials studied previously were fed at a 10 per cent level in similar mixture at a rate of 6 lb. per day so as to study palatability. The products having the following protein equivalent were used: Ammoniated inverted cane molasses, 26.2% ; ammoniated inverted cane molasses neutralized with H_3PO_4, 29.4% ; and ammoniated cane molasses, 19.4%. All of the rations were found to be palatable, with calves eating this amount very rapidly. Similarly, growth in terms of gains in body weight and height of the withers was normal during the 15-day feeding period.

TABLE 1
Concentrate rations used[a]
(Trial 3)

Ingredient	Groups			
	Control	A	B	C
Cane Molasses	10	....	....	....
Ammoniated inverted Cane Molasses I (28.3% Protein equivalent)	....	10	....	....
Ammoniated Cane Molasses II (15.0% Protein equivalent)	....	....	10	....
Ammoniated CDMS[b] (13.4% Protein equivalent)	....	....	....	10
Soybean oil meal	16.5	7	11.5	12
Crushed oats	21.5	31	26.5	26
Yellow corn meal	26.3	26.3	26.3	26.3
Wheat bran	10.0	10.0	10.0	10.0
Linseed oil meal	2	2	2	2
Alfalfa meal	2	2	2	2
Dried skimmilk	5	5	5	5
Distillers' dried solubles	5	5	5	5
Vitamin A & D feed[c]	0.2	0.2	0.2	0.2
Dicalcium phosphate	0.5	0.5	0.5	0.5
Ground limestone	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5

[a] Data presented in lb.
[b] Condensed distillers' molasses solubles.
[c] 4,000 I. U. A. and 500 U. S. P. units D_2/g.

Trial 3. Four groups of six Holstein bull calves, each of which were similar in body weight, height at the withers and chest circumference, were used in a trial to determine the value of the following materials with their respective protein equivalents for growth of the calves to 16 wk. of age: Ammoniated inverted cane molasses, 28.3% ; ammoniated cane molasses, 15.0% ; and ammoniated condensed distillers' solubles, 13.4%. The calf starter formulae used in trial 3 are presented in table 1. The ammoniated products were included at the 10 per cent level because this level approached the maximum for mixing in the feed industry. On this basis 16.0, 8.4 and 7.5 per cent of the nitrogen in the mix came from ammoniated products in the starters fed to groups A, B and C, respectively. The calves were fed these rations *ad libitum* until they consumed a maximum of 6 lb. per day, after which they were continued at that level of

feeding. They also were fed all the good quality alfalfa hay they would consume throughout the trial. The calves were kept in individual pens with salt block and water bowl with water available at all times.

The calves were fed the following milk replacement formula which is now being produced by several manufacturers and has produced growth under experimental and farm conditions comparable to that of milk fed calves (18, 19): 50 lb. dried skimmilk, 10 lb. dried whey, 15 lb. distillers' dried solubles, 5 lb. oat flour, 10 lb. soluble blood flour, 7 lb. dextrose, 0.5 lb. vitamin A and D feed (500 U. S. P. units D_2 and 4000 I. U. units A per gram), 0.5 lb. mineral mixture (32% Ca, 2.6% Mg, 1% Mn, 0.13% Cu and 0.002% Co) and 2.0 lb. $CaHPO_4$. This replacement was fed as follows:

Age	Milk	Water	Replacement
(d.)	*(lb.)*	*(lb.)*	*(lb.)*
1– 4	4[a]	0	0.0[a]
5– 7	2	2	0.2[b]
8–10	1	3	0.4[b]
11–21	0	5	0.5[b]
22–35	0	6	0.6[b]
36–49	0	6	0.7[b]
50–56	0	7	0.7[c]

[a] Dam's milk.
[b] Twice daily.
[c] Once daily.

The replacement was mixed with warm water and fed at 100° F. in open pails. All rations proved to be palatable and were consumed readily.

The growth of the calves are presented in table 2. There was no significant difference in growth rates in terms of height at the withers or chest circumference between the control group and the group receiving the ration containing ammoniated inverted cane molasses (group A) during the first 12 wk. of the trial. Significantly ($P > 0.01$) lower rates of growth in these respects were obtained on those groups receiving the ammoniated cane molasses (group B)

TABLE 2

Rates of growth of calves fed ammoniated products

Group[a]	Withers Height	Chest Circumference	Body Weight
	(cm.)	*(in.)*	*(lb.)*
	Birth to 12 wk.		
Control	9.7	7.2	97.5
A	9.6	6.7	86.1
B	6.4	4.7	53.3
C	7.6	5.1	69.8
	12 to 16 wk.		
Control	4.5	3.2	57.7
A	4.7	3.3	59.6
B	5.2	3.8	59.4
C	5.6	3.2	55.1

[a] Groups correspond to those of table 1.

and ammoniated condensed distillers' molasses solubles (group C). Rates of growth in terms of gains of body weight were significantly ($P > 0.01$) poorer in all experimental groups receiving ammoniated products as compared to the controls during the first 12 wk. of the trial.

It should be noted in table 2 that the rates of growth in terms of height at the withers, chest circumference and body weight in the ammoniated products-fed groups were equal to those of the control group after the calves reached 12 wk. of age. No statistically significant differences could be found in rates of growth of any of the groups in gains made between 12 and 16 wk. of age.

During the last 10 days of trial 3, three calves of each group were fed 1.5 g. of chromium oxide according to the procedure of Kane *et al.* (16). The sampling and methods of analysis of the rations fed and feces also were those of the above authors. On the basis of this method the digestibilities of the nitrogen in the various rations fed were as follows: Control, 67.14%; Group A, 69.23%; Group B, 70.15%; and group C, 68.84%. This not only takes into consideration the starter fed but also the hay fed. On the basis of the data obtained it would appear that the nitrogen of the ammoniated products studied was as digestible as equivalent amounts of nitrogen from soybean oil meal and crushed oats in trials with calves between 15 and 16 wk. of age.

DISCUSSION

On the basis of the preliminary trials conducted with 16-wk.-old calves, it appears that ammoniated cane molasses and ammoniated condensed distillers' molasses solubles are palatable in dairy calf rations at the levels studied. It does not appear, however, that normal growth can be obtained in calves under 12 wk. of age where these ammoniated products are used under the conditions of these experiments. The growth of calves between 12 and 16 wk. indicates that once the rumen begins to develop and function that they can use the nitrogen from the ammoniated products. There are many other products which, upon ammoniation, may prove to be valuable sources of nitrogen and energy for dairy cattle. Considerable tonnages of ammonia can and are being produced economically and it is hoped that it can be developed as an economical source of nitrogen for dairy cattle.

SUMMARY

Ammoniated cane molasses, ammoniated inverted cane molasses and ammoniated condensed distillers' molasses solubles were fed to growing Holstein bull calves. The nitrogen from ammonia was fed as a replacement for the nitrogen of soybean oil meal and oats. It does not appear that nitrogen from ammonia can be used under the conditions studied in calves until they are nearly 12 wk. of age. In calves 12 to 16 wk. of age, gains of approximately 2.0 lb. per day were obtained with rations containing these products. Similarly, it was found that the nitrogen of ammoniated products was as digestible as that of the nitrogen of soybean oil meal and oats in calves at 15 to 16 wk. of age. It appears that ammonia nitrogen may serve as a valuable source of nitrogen for mature dairy cattle.

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FURTHER STUDIES CONCERNING SEX-LIMITED GENETIC INFERTILITY IN CATTLE[1, 2]

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INTRODUCTION

Gregory *et al.* (3) presented evidence indicating that a recessive autosomal gene conditioned infertility in certain Jersey and Holstein heifers of the California Agricultural Expriment Station herd. In an effort to exclude cases of sterility of environmental origin, the sterility considered was limited to heifers unable to produce calves when mated to known fertile bulls. Thus, any cow that had produced one calf was considered fertile even though she might be unable to reproduce thereafter. The same definition for fertility and sterility used for females in the earlier report (3) is used in this paper. The objectives contrast sharply with that of Tanabe and Casida (8), whose experiment was designed to study the nature of sterility of environmental or developmental origin. They purposely excluded from consideration all heifers that had never produced a calf, using only cows that had given birth to one or more calves prior to becoming infertile. In the 104 cows observed, they found that 10.6 per cent had abnormalities of the genital tract. This included 8.7 per cent in which the anomaly constituted a barrier to fertilization. Of the cows possessing normal genitals, 66.1 per cent had normal fertilized ova 3 days after mating, while the percentage of normal embryos had dropped to 23.1 per cent 34 days after mating. Reproductive performance at 34 days after mating was as follows: (a) failure of fertilization, 39.7 per cent; (b) embryonic abnormalities before 34 days post coitus, 39.2 per cent; and (c) normal embryos, 21.1 per cent.

Although no actual mating tests were made, Gregory *et al.* (3) and Mead *et al.* (5) presented evidence indicating that different genes probably were involved in conditioning sterility in the Jerseys and Holsteins studied. The estrous cycle of the Jerseys tended to approach normal and the genital tracts were for the most part normal, although upon autopsy an occasional sterile heifer manifested what the attending veterinarian termed a juvenile condition of the genital tract. This was not a consistent feature of sterile heifers, nor could any gross anomaly of the tract be correlated with the sterility. On the other hand, the sterile heifers of the Holstein breed manifested irregular estrus. Few ever were observed to be in heat and one exhibited nymphomania. Another had a malformed reproductive tract, but the veterinarian's report does not state the nature of the

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[3] This study is cooperative between California Agricultural Experiment Station Projects 716 and 1451. Project 1451 is in cooperation with the Bureau of Animal Industry, U.S.D.A. and the state experiment stations under Western Regional Project W-1 concerning Beef Cattle Breeding Research.

deformity. It was these differences which suggested the probability that different genes might be involved in conditioning sterility in the Jerseys and Holsteins, respectively.

Fincher and Williams (2) reported a Holstein bull that produced sterile daughters from a mating of sire to daughter. The ovaries, vulva and fallopian tubes of the sterile females were normal, but anomalies of the vagina, cervix and cornu were of such magnitude as to account for the sterility. They suspected that this sterility was of hereditary origin. A genetic analysis of their data by Gregory *et al.* (3) provided evidence that an autosomal recessive gene, sex-limited in nature, conditioned the sterility and that the sire involved was homozygous. It is an open question whether the female sterility of Holsteins studied by Fincher and Williams and by Gregory *et al.* is conditioned by identical or different genes.

Erikson (1) studied a form of sterility controlled by a single recessive autosomal gene with high but incomplete penetrance. This condition affects both males and females, hence the nature of the gene action differs from sex-limited types of sterility.

The purpose of this report is twofold: (a) to present additional genetic data on the "Jersey" type of female sterility; and (b) to present evidence of hereditary male infertility.

I. FEMALE STERILITY

Environment, management and stocks. All the animals involved in this study were owned by the University of California and were maintained by the Division of Animal Husbandry at Davis. With the exception of a few females which were purchased from commercial breeders cooperating with the breeding experiment, the dams of all the females tested were born and raised at Davis. The purchased females were substantially the same stock as that of the College, since the cooperators from whom they were purchased had been using University sires for 20 yr. or more. The whole Experiment Station herd is under essentially the same regimen of feeding, management and general environmental conditions. All the tests for fertility and sterility were made at Davis under conditions described in earlier reports (3, 5, 6, 7). The herd is free from brucellosis and trichomoniasis. *Vibrio fetus* has never been diagnosed or suspected. If there are other specific infectious agents in the herd that reduce fertility, none has been identified.

The animals considered in this study are of two different origins. Sire 753A is an inbred son of 370A, the chief herd sire for 6 yr. (See fig. 1.) Bull 753A and all the cows to which he was mated represent the Jersey stock that has formed the basis for all of the earlier reports. In 1944, plans were made to establish a second line of Jerseys. Since earlier studies had demonstrated that cattle carry a large number of deleterious recessive genes, the attempt was made to minimize the disastrous effects of recessives by establishing the new line in such a manner as to avoid, as far as possible, the introduction of unknown, undesirable recessives from foundation females. This was to be accomplished by

using foundation females from commercial dairymen who had for 20 yr. or more been using herd sires from the California Station Dairy Breeding Project. The cooperators supplying the foundation females for the second line were progeny testing herd sires produced in the California Experiment Station herd. Thus, the herds of cooperating dairymen provided almost as much knowledge concerning the genotype of individual animals as did the Station herd. The new line was to be established by using the foundation females in a backcross to a succession of inbred related sires unrelated to the foundation females. The possibility of new deleterious genes is almost completely eliminated from the foundation females. The known undesirables from the females can be easily controlled, and thus any unknown undesirable genes introduced from the sires

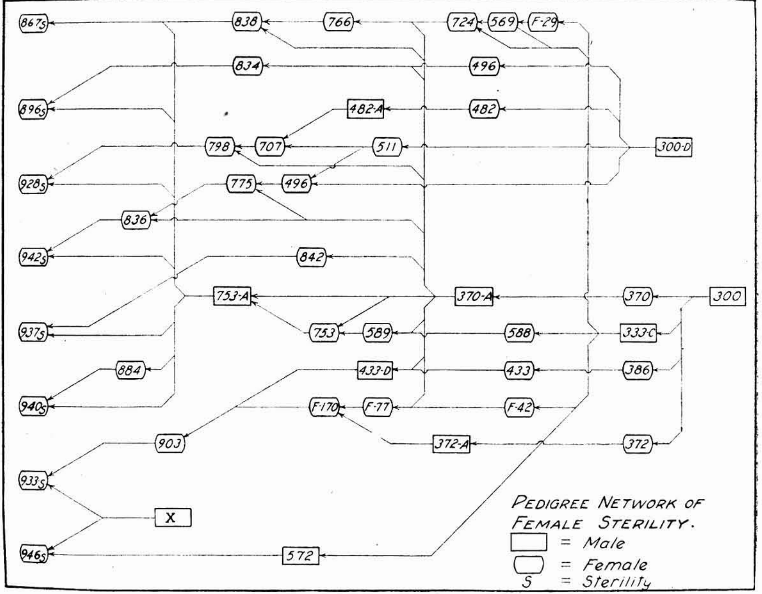

FIG. 1. A pedigree network showing the relationship of 8 cows that were infertile.

will not present such a serious problem. Cows 903 and 572 (fig. 1) are typical examples of the foundation females used to establish the second line.

Analysis of data. All the females included in this analysis are descendants of 300D, 333C and 370A and were born since 1943. The pedigree network shown in figure 1 is that of the eight sterile heifers. The relation of these animals to those used in the earlier study may be obtained from figure 1 of reference 3. To date, bull 753A has a total of 44 daughters that have been tested for fertility, out of dams sired by bulls that breeding tests indicated were heterozygous for female infertility. Thirty-eight have proved fertile and six have proved infertile. This ratio is in good agreement with the expected percentage of sterility, which is 12.5 per cent. This strongly supports the hypothesis advanced in an earlier report (3) that an autosomal gene conditions the female infertility.

Sterile heifers 933 and 946 were produced by mating an unrelated sire, X, to descendants of 370A and 333C. Both of these bulls produced sterile females as did their progenitor sire, 300. A logical conclusion is that bull X carries the same gene for female sterility that was present in the original line of Jerseys. This also is in agreement with the various lines of evidence presented by Mead *et al.* (5) that in the breeds of dairy cattle the gene for female sterility has a relatively high frequency.

The specific cause for reproductive failure in these cases of genetic sterility is of importance to physiologists and those interested in the relation of gene to character and also is of considerable economic importance. Since reproduction is an involved process, mutant gene action may interfere with or block a specific reaction at a definite period in the reproductive cycle either before or after fertilization. Thus, any number of mutant genes may interfere with the process. Some observations were made that throw light on the specific nature of reproductive failure. The estrous cycles of five sterile heifers were followed over a long period. While there were a few irregularities, these five heifers generally exhibit rather regular cycles when compared with the norms of Hammond (4).

TABLE 1

Autopsy data concerning the five sterile heifers that were sacrificed

Cow	Age of slaughter	Total no. of matings	Hours killed post-coitus	Status of ovum	Remarks
	(yr.)				
928	2.5	10	66	1 cell	Presumably fertilization was prevented by an ampullar structure anterior to the cervical canal which acted as a barrier to fertilization.
933	2.5	15	98	16 cells	Reproductive tract appeared normal.
937	2.25	8	65	8 cells	Reproductive tract appeared normal.
940	2.33	11	not bred		Cow in anestrus 3.5 mo. before slaughter—apparently because of a retained corpus luteum from a follicle that was accidentally ruptured during palpation.
942	2	6	89	Failed to recover	Ovulated at last estrus. Gross appearance of tract normal; faulty technique responsible for failure to recover ovum.

The autopsy data on these five heifers are assembled in table 1. Heifer 940 was in anestrus and ovulation was not expected. The other four heifers ovulated; it is evident that there was no failure in the mechanism associated with the production of ova. Heifers 933 and 937 had eggs in the 16- and 8-cell stages, respectively. This is significant because it indicates (a) that the meiotic activity probably is normal and (b) that the eggs of genetically sterile females are capable of fertilization and cleavage. Heifer 928 possessed an ampullar structure in the anterior region of the cervical canal which is assumed to have

acted as a barrier, at least temporarily, and thus prevented fertilization. There is some doubt concerning the genetic status of this cow. Since the estrous cycles of the other cows were more or less regular, it is evident that the zygotes are aborted early, perhaps in the late cleavage or early blastocyst stage; thus, the approximate time of reproductive failure is indicated. Throughout this study it should be emphasized that the sterile cows were mated many times to a proven fertile bull and in some cases to more than one bull. This in conjunction with the genetic analysis indicates that the deficiency causing reproductive failure is not in the developing zygote but in the dam herself. Thus, cows of the specific genotype in question appear to be incapable of providing the environment that is necessary to sustain the developing zygote.

II. MALE INFERTILITY

Attention first was directed to male infertility during 1941 and 1942, when a bull 14 mo. of age was the sole sire used in a registered Holstein herd of 25 to 30 cows. The herd producing the sire was noted for acceptable type and better-than-average production. The females upon which the bull was used had a good record of reproductive efficiency and were known to be free from brucellosis and trichomoniasis; there were no reasons for suspecting *Vibrio fetus*. Practices of feeding and management were above average. The herd had been without a sire for several months and many of the cows were open. Hand breeding was practiced. The young bull manifested abundant libido; copulation usually was prompt and vigorous, but the cows failed to become pregnant. During the 1 yr. he was used as the sole herd sire, the bull fathered only one calf. He then was placed in another well-managed herd of about the same number of cows. Here, after 1 yr. of service, he duplicated his performance in the first herd by fathering one calf. As a sire he had such a low degree of fertility that for practical purposes, breeders classed him as sterile. Semen examinations from time to time consistently revealed a low concentration of spermatozoa with a high percentage of aberrant forms.

During this time, several other young bulls of the same breed were reported to have impaired fertility. Whenever possible, the pedigree of such a bull was obtained together with any additional pertinent data available. Unfortunately, it was impossible to obtain information on all the bulls alleged to be suffering from low fertility. Thus, over a period of several years, five young Holstein bulls manifesting greatly impaired fertility were found. The five bulls were born in four different herds and their fertility was tested in four other herds. Better-than-average dairymen operate all the herds involved; nutrition, management and other environmental factors conducive to good health and reproduction are practiced in all the herds. All the bulls involved appeared normal in every characteristic except fertility. All five bulls proved to be related. A pedigree network showing the relationship is shown in figure 2. Bull 1 is the sire described.

Bull 12 was born in the same herd as 1 but was used in a different herd. From 14 to 16 mo. of age, he was hand bred to 17 heifers for a total of 35 matings

without a single conception. Later, he was placed on pasture with the heifers, and during one period of about 2 wk. there were seven conceptions. This was his only record of fertility. An examination of a semen sample immediately before he was placed on pasture with the heifers revealed a low concentration of spermatozoa, a high percentage of which were abnormal.

Bull 20 has a unique record. As six cows produced calves from his early services, his fertility was assumed. Later, he was mated to a group of heifers none of whom conceived. He then was used in 40 different matings on 14 cows and five heifers without a single conception. A microscopic examination during the last series of matings revealed that the semen was practically devoid of spermatozoa.

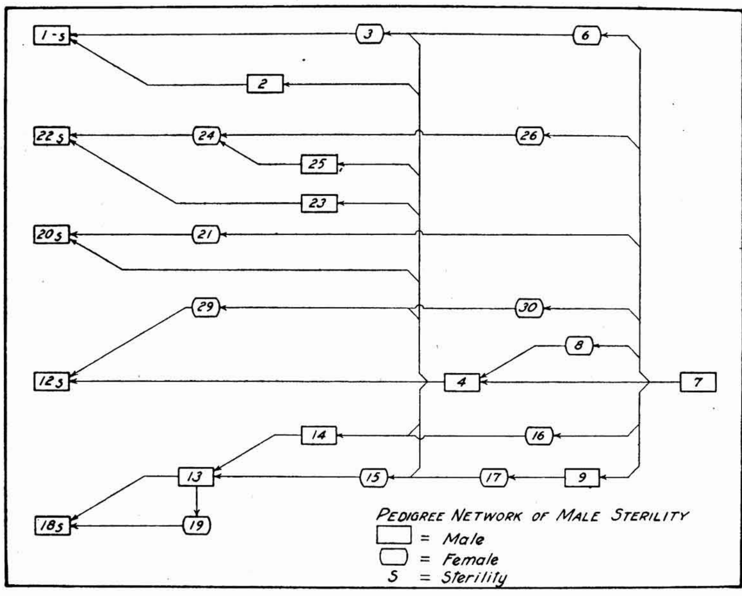

FIG. 2. A pedigree network showing the relationship of 5 bulls manifesting impaired fertility.

In October, 1947, when he was approximately 1 yr. of age, bull 22 was put in service by hand matings on a small herd of 15 to 20 cows, but there were no conceptions. A semen check revealed a low concentration of spermatozoa, with lower-than-average motility and a high percentage of tailless heads. Between June and September, 1948, he sired four or five calves. With the hope that abundant green feed might improve his fertility, he was placed on irrigated pasture for 3 mo. and later was given a grain ration with wheat germ oil. During this regime, he was mated to 12 cows without success. By August, 1949, his low fertility made it impractical to use him further.

When mating tests in the first herd proved that bull 1 had impaired fertility, he was replaced by bull 13 (fig. 2), who then was approximately 1 yr. of age.

All the cows were open when he became the herd sire, and within 3 mo. all had conceived. This bull has always been highly fertile. From a sire-daughter mating, sire 13 produced a son, bull 18, which proved to be sterile (fig. 2). Because of his superior type and good potentialities for high production, this bull was selected for use in an artificial insemination organization. His semen consistently manifested a low concentration of spermatozoa, a high percentage of which were abnormal. Semen collections were made systematically over a period of months but the quality never came up to the normal standard and none was ever distributed for insemination. Since the quality of the semen was so poor, he was used in very few mating tests.

Although a thorough study has not been made, the semen of these five bulls with impaired fertility has the common characteristics of a low concentration of spermatozoa with a high percentage of abnormal forms. The breeding histories of bulls 12, 20, 22 and perhaps of bull 1, indicate that the dysfunction causing infertility may be partially or completely overcome for short periods of time. Although the semen tests on bull 18 were more numerous than those of any of the other bulls, the actual mating tests were quite limited. With additional opportunities, however, he probably could have sired an occasional calf.

Genetic ratios concerning male fertility and infertility were not available from any of the breeders that produced or used infertile sires. On the other hand, the management in the Station herd was inadequate to provide critical data on progeny tests because most of the bull calves were either killed at birth or were not used for breeding.

All five infertile bulls are closely related and are descendants of bulls 4 and 7, two closely related sires.

Although they are not included in figure 2, two other bulls which are inbred descendants of bull 7, were found to have poor records of fertility. One had a conception rate of 25 per cent based upon 180 matings and the other had a conception rate of 33 per cent based upon 73 matings. The owner considers these conception rates too low to tolerate in a well-managed herd. Probably these sires have the same type of impaired fertility as the other five bulls but represent the upper limit of fertility.

The pedigree network shown in fig. 2 offers a clue to the inheritance of male infertility. The possibility of a sex-linked gene appears to be eliminated. As the fertility of females in all the herds involved was satisfactory, there is no basis for suspecting that this type of impaired male fertility is associated in any way with impaired female fertility. The working hypothesis is that the infertility observed affects males only and is conditioned by a single autosomal recessive gene.

DISCUSSION

Genetically impaired fertility in males and females of cattle is of general biological interest and of economic importance. Data from critical mating tests are limited and difficult to obtain. Therefore, several questions pertaining to heredity and physiology are yet to be answered; one concerns the number of

genes needed to account for the phenomena observed. The fact that no case of sterility or impaired fertility has occurred in male siblings of the female sterile heifers even though there is evidence of a relatively high frequency of the gene in the Jersey breed (3, 5) strongly supports the hypothesis that the condition is sex-limited. Additional evidence also comes from the fact that mating tests of one fully-fertile sire indicated that he probably was homozygous for the gene that conditions female sterility.

Even though the data are limited, no female sterility in any of the Holstein herds producing infertile bulls was recorded which could be associated with the gene conditioning male infertility. When all the evidence from both breeds is considered, the existence of two different autosomal genes which are sex-limited is a more tenable hypothesis than that of a single gene with expression in both sexes. Thus, there is evidence for three types of genetic infertility in cattle—female, male and a more inclusive type described by Erickson (1) that affects both sexes.

The specific part of the reproductive process that breaks down in either female or male infertility is of importance to the geneticist and physiologist. The fact that the genital tract appears normal in sterile females indicates that one must look further than gross morphology for the cause. Since zygotic abortion occurs during late cleavage or early blastocyst stages, some substance or reaction that is essential for implantation may be lacking or blocked. If implantation is a critical period, as it may well be, an endocrine dysfunction may be involved. Whatever the cause, the sterility may be temporarily overcome if the dysfunction is corrected for a short time, thus permitting the zygote to abridge a critical period. Even though limited in numbers, the ratios from mating tests indicate that the penetrance is high.

In addition to hereditary types of female sterility, there also are developmental types which occur subsequent to a successful parturition (8). These types constitute a heterogeneous group as to cause but include the so-called "shy" breeders that have escaped detectable injury during pregnancy and parturition but are inefficient reproducers. Sterility of this group of cows and that of the genetically sterile cows show a common characteristic in that the eggs of both groups are capable of fertilization and cleavage but fail to implant. There have been several Jersey heifers, classed as fertile, that are siblings to those classed as sterile. These have produced one calf but have been incapable of reproducing thereafter, even though the reproductive tract appeared normal. The possibility of a relationship between certain types of the so-called "shy" breeders as defined by Tanabe and Casida (8) and the type of female sterility defined by Gregory *et al.* (3) should be investigated.

Male infertility is associated with a low concentration of spermatozoa, many of which manifest abnormal morphology. Whether these deficiencies are caused from meiotic failure, inability of spermatids to metamorphose into normal spermatozoa, premature disintegration of spermatozoa, or from other causes, future investigations will have to determine. There is some evidence from the case

histories cited suggesting that at times favorable environmental conditions may tip the balance towards fertility. Investigations along the lines suggested should be fruitful and should contribute to a better understanding of the mechanism controlling the spermatozoa in the male reproductive tract.

SUMMARY

Data from additional mating tests support earlier evidence that the type of female sterility in the California Experiment Station Jersey Herd is conditioned by an autosomal recessive gene. Additional data also support an earlier conclusion that the gene conditioning the sterility is of relatively high frequency in certain lines in the Jersey breed. While more extensive studies are needed, a sample of the sterile females investigated manifest estrous cycles that, in general, appear to be in the normal range. The cause of failure in the reproductive mechanism was not determined. Some of the eggs are capable of being fertilized and undergo cleavage. Thus, normal oogenesis, meiosis and fertilization are indicated, as is zygote abortion in the late cleavage or early blastocyst stage.

A small group of related Holstein bulls manifesting abundant libido but with greatly impaired fertility was studied. The chief characteristic of the semen was a low concentration of spermatozoa, a high percentage of which were malformed. The conception rate per mating of five of the bulls varied from less than 1 to less than 10 per cent; other tests indicate that the upper limit of fertility may be as high as 30 per cent. A pedigree analysis suggests that the male infertility may be conditioned by a recessive autosomal gene. The data also suggests that different genes condition the male and female infertility.

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EFFECT OF GLYCEROL-MONO-STEARATE ON FAT ABSORPTION, GROWTH AND HEALTH OF CALVES[1, 2]

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The importance of emulsification in the digestion and absorption of fat has been demonstrated (8, 9). Additions of lecithin to cottonseed oil, either refined or hydrogenated, lowered the incidence of diarrhea in rats fed large amounts of these fats (2). Moreover, fats containing one-sixth or one-fifth crude lecithin were absorbed more rapidly than the same fats without added lecithin (2). Results reported by Esh *et al.* (7) indicated that soya lecithin increases the absorption and utilization of vitamin A in young dairy calves and reduces scouring during the first 7 days after birth. Jones *et al.* (11) found that in the human subject inclusion of an emulsifying agent, polyoxyethylene sorbitan monooleate, in the diet reduced fecal fat and increased body weight, indicating improved absorption of fat. Other workers (14), however, failed to affect absorption of corn oil by rats when either purified soybean phosphatide or Tween 80 was in the diet in excess of amounts needed to make excellent emulsions by mechanical means.

The present investigation was undertaken to gain additional information on the relationship of methods of dispersing the fat in the calf ration to various physiological responses in the calf.

PROCEDURE

The experiment consisted of a randomized block design involving three diets fed to 15 calves, of which five were assigned to each of the diets (table 1). Except for the addition of cobalt and the substitution of calcium lactate for $CaCO_3$ and ferric chloride for ferric citrate, the salt mixture used was similar to that of Phillips and Hart (12).

Preparation of diets. Mixing and processing of the diets were as follows: Casein was dissolved according to the method of Bird *et al.* (5). After the casein was added to the $NaHCO_3$ solution (while being stirred vigorously with a "Lightnin" mixer[4]), the temperature of the mixture was raised to 145 to 150° F. and maintained at this level for 30 min. Subsequently, glucose, salt mixture (dissolved in hot water), vitamins and melted fat were added in the order listed. When the emulsifying agent was included (diets B and C), it was added to the fat and melted with it to facilitate dispersion. After the ingredients were thoroughly

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[4] "Lightnin" mixer manufactured by the Mixing Equipment Co., Rochester, N. Y.

mixed, they were homogenized at a pressure of 2,500 lb. (with the exception of diet C), cooled and stored at 36° F. until used. All diets were fed within 7 days after processing.

Feeding and management of calves. These were Ayrshire, Holstein, Guernsey and Jersey males, which were assigned to the experimental groups without regard to breed. During the first 48 to 72 hr. after birth, the calves remained with their dams to receive colostrum. Subsequently, they were transferred to individual pens, which were bedded with dehydrated sugar-cane pulp, and were fed the experimental rations. Muzzles were employed to prevent consumption of bedding but were not entirely effective. The experimental feeds, given twice daily, were warmed to 35° C. and subsequently nipple-fed at the rate of 10 lb. daily per 100 lb. of body weight. Levels of feeding were adjusted weekly on

TABLE 1

Ingredients of the diets fed to young dairy calves

Constituents	Diet A[a]	Diet B[b]	Diet C[c]
	(%)	(%)	(%)
Casein[d]	4.0	4.0	4.0
Glucose	5.5	5.5	5.5
Salt mixture	1.25	1.25	1.25
Hydrogenated cottonseed oil	3.5	3.0	3.0
Glycerol-mono-stearate[e]	....	0.5	0.5
Water	85.75	85.75	85.75
Vitamins[f]	....	....	....

[a] Dispersed by homogenization at a pressure of 2500 lb./in.^{2}

[b] Dispersed by homogenization and emulsification with glycerol-mono-stearate.

[c] Dispersed by emulsification with glycercol-mono-stearate (no homogenization).

[d] "New Process" commercial casein purchased from Sheffield Farms Co., Inc., 524 West 57th St., New York, N. Y.

[e] Emulsifying agent.

[f] Mg. vitamins/lb. milk added to each diet:

Vitamin	Mg.
Thiamine hydrochloride	1.0
Pyridoxine	1.0
Calcium pantothenate	2.0
Para-aminobenzoic acid	1.5
Riboflavin	1.0
2-Methyl-naphthoquinone	0.5
Alpha tocopherol	1.0
Inositol	20.0
Choline chloride	125.0

the basis of changes in body weight, which was determined when the calves were placed on the experiment and weekly thereafter. All calves were given, *via* capsules, 30,000 I. U. of vitamin A and 1,000 I. U. of vitamin D on alternate days throughout the experiment. Calves that developed scours were treated with sulfathalidine, which usually effected a cure within 3 or 4 days.

Collection and preparation of tissue samples. When the calves were 2, 4 and 6 wk. of age, venous blood samples were collected within 15 min. after the morning feeding.

At 6 wk. of age, the calves were sacrificed and autopsied. The livers were removed, weighed and subsequently ground in a meat chopper. At this stage, a sample was taken for choline analysis and the remainder was dried in an oven

at 85° C. for the first 10 hr. and at 95° C. for an additional 12 hr. The dried livers were ground in a Wiley mill, sealed in air-tight containers and stored at a temperature of 4° C. until analyzed.

Methods of chemical analysis. Hemoglobin was estimated on the whole blood using the method of Shenk and Hall (13). Blood plasma fat was determined by Allen's procedure (1). In determining the choline content of blood the method of Engel (6) was modified so as to be suitable for liquid samples. Fifteen-ml. samples of plasma were mixed with 150 ml. of methyl alcohol, heated to boiling for 5 min. and filtered on Reeve Angel no. 273 filter paper. The precipitate was washed with methyl alcohol, allowed to drain completely and then extracted[5] for 4 hr. with methyl alcohol. The combined filtrate and extract then was analyzed by the procedure of Engel.

Lipide concentrations in the livers were determined, on a moisture-free basis, by ether extraction[5] for 4 hr. The choline content of the livers was determined both in samples in the fresh state and in samples dried to facilitate storage. This procedure was followed in order to detect losses that might result from storage. Analyses of both sets of samples (table 4) showed only small losses in the dried and stored samples. Engel's method (6) was used to determine the choline content of the dry livers, but the procedure was modified for the fresh liver, which was finely ground with a meat chopper. Two-g. samples were placed in 150 ml. of methyl alcohol and boiled for 5 min., ground with a mortar and pestle, filtered, washed and finally extracted for 18 hr. with methyl alcohol, using the Goldfisch extractor. The combined filtrate and extract then was analyzed according to Engel's method.

RESULTS AND DISCUSSION

Hemoglobin content of calf blood. The average hemoglobin values for the three dietary groups were essentially the same. The mean concentrations in the blood of all calves, irrespective of diet, were 13.5, 14.2 and 13.5 g. per 100 ml. of blood, respectively, at 2, 4 and 6 wk. of age. Since these values are higher than those observed by Wise *et al.* (17) in calves receiving a conventional ration of whole milk, concentrate mixture and hay, the diet apparently supplied adequate nutrients for normal hemoglobin formation.

Fat content of blood plasma. There was considerable variation among animals within the same dietary group, but the fat was significantly higher in plasma from calves receiving diet B (glycerol-monostearate and homogenized) than from those fed either diet A (homogenized only) or diet C (glycerol-monostearate only). Though the differences between A and B were not significant the second week, they were the fourth and sixth week, even at the 1 per cent level (table 2). The differences between B and C were significant only the fourth week. The plasma fat values for calves fed diet C were higher than the values for calves fed diet A, but the differences were not significant statistically.

[5] Goldfisch extractor manufactured by Laboratory Construction Co., Kansas City, Mo., was used.

Assuming that the level of the plasma fat in the blood reflects the degree of absorption, the combined action of homogenization and the emulsifying agent enhanced absorption of fats in the particular diets used. Although the data do not specifically indicate the independent roles of these two methods of dispersion, observations suggest that glycerol-monostearate was a greater factor than homogenization.

Since blood samples were drawn within 15 min. after the morning feeding, it was not known whether the differences found at this time in blood fat of calves of the various dietary groups would be maintained throughout the remainder of the day. A study of the fat of blood plasma collected periodically for 12 hr. following feeding indicated that the samples taken 15 min. following feeding were indicative of differences characteristic throughout the day.

Choline content of blood plasma. Calves receiving diet B had significantly higher choline values at 4 and 6 wk. of age than calves receiving diet A (table 3).

TABLE 2

Average blood plasma fat of dairy calves receiving diets in which the fat was dispersed by different means

Dietary group	Average plasma fat		
	2 wk.	4 wk.	6 wk.
	(*mg./100 ml.*)	(*mg./100 ml.*)	(*mg./100 ml.*)
A (Homogenized)	85.9	98.2	100.8
B (Homogenized & glycerol-monostearate)	109.7[a]	165.1	151.9
C (Glycerol-monostearate)	101.7	119.0	132.2
5% least significant difference	34.75	34.75	34.75
1% least significant difference	47.29	47.29	47.29

[a] Missing plot technic used to compute values for 1 calf in this group sacrificed at 12 d. of age.

Except for the second week, the plasma choline values were higher from diet C than A, but the differences were not significant. The differences between the choline values of calves fed diet B and C were not significant although they were higher for calves receiving diet B.

The values for plasma fat and for plasma choline indicate a possible direct relationship between these two components. For example, in two unthrifty calves that had low plasma fat throughout the experiment, plasma choline also was low. Likewise, calves that had the highest fat levels also had high plasma choline values. This observation is in accord with the work of Bender and Maynard (4), who found a parallelism between total blood lipids, phospholipides and cholesterol in lactating goats. Waugh *et al.* (16) also observed that the plasma choline and plasma fat of calves declined correspondingly during periods of mild fasting.

TABLE 3

Average concentration of choline in blood plasma of calves receiving diets in which the fat was dispersed by different means

Dietary group	Average plasma choline		
	2 wk.	4 wk.	6 wk.
	(mg./100 ml.)	*(mg./100 ml.)*	*(mg./100 ml.)*
A (Homogenized)	12.0	12.9	10.2
B (Homogenized & glycerol-monostearate)	15.1[a]	17.7	16.3
C (Glycerol-monostearate)	11.8	14.2	12.9
5% least significant difference	4.6	4.6	4.6
1% least significant difference	6.3	6.3	6.3

[a] Missing plot technic used to compute values for one calf in this group sacrificed at 12 d. of age.

Liver lipides and choline. The difference between the liver lipides of calves fed diets A and B were not significant, although slightly lower on diet A. Calves fed diet C had somewhat lower liver lipides than did calves fed either diets A or B. The differences were not significant statistically but approached significance in the latter comparison. The liver lipides of calves fed the respective diets were higher than found in calves fed whole milk (10, 15). This high fat content is in agreement with the findings of Jarvis and Waugh (10). The addition of the emulsifying agent apparently did not correct this fatty liver condition. The

TABLE 4

Average weight of liver and concentrations of lipides and choline in livers from calves that received dietary fats dispersed by different procedures

Dietary group	Fresh wt.	Lipides (dry basis)	Choline (dry basis)	
			Analysis of fresh liver	Analysis of dried liver
	(lb.)	*(%)*	*(mg./g.)*	*(mg./g.)*
A (Homogenized)	1.98	17.28	14.30	14.15
B (Homogenized & glycerol-mono-stearate)	1.92[a]	19.64	14.62	13.53
C (Glycerol-mono-stearate)	1.62	13.73	13.99	13.61
5% least significant difference	0.42	5.95	1.80	1.32

[a] Missing plot technic used to compute values for one calf in this group sacrificed at 12 d. of age.

lipotropic effect of choline is well known, but a deficiency of this compound hardly can be expected since the amounts in the diets were similar to those found in milk. There is a possibility, however, that the choline as supplied in the diets was not utilized so well as that normally occurring in milk.

During the course of the investigations with calves, a preliminary exploration of the effect of substituting corn starch for glucose in the diet was made. Two Toggenburg kids were placed on diet A (table 1) and three on a similar diet in which starch replaced the glucose. The average concentrations of lipides in livers of kids fed the latter diet was 7.23 per cent, while the average value for those fed diet A was 17.63 per cent. The difference was significant statistically at a 1 per cent level. From these limited observations it would appear that corn starch lowered the incidence of fatty livers.

Body weight gains and general health. The average daily gains in body weight for calves receiving diets A, B and C, respectively, were 0.6, 0.5 and 0.3 lb. Although the gains were higher for calves receiving diet A than for calves receiving either diets B or C, the difference was not significant statistically. There is the possibility that the reduced growth rate of calves fed diet C was due to the higher incidence of scours.

General appearance and health of the calves, especially those fed diet A, was good. Autopsies of the calves revealed a high percentage of arrested cases of pneumonia, which occurred in calves fed all diets but appeared to be more severe in calves that received diet C.

All calves receiving diet C developed dull hair coats and became denuded around the anus, tail and inside of thighs. This alopecia is in agreement with observations by Bate *et al.* (3). Losses of hair did not always follow scouring and could not be attributed to soiling of the hair-coat with feces. None of the calves fed either diet A or B manifested this condition.

SUMMARY

Three groups of five calves each were fed from the colostral stage to 6 wk. of age diets in which fat was dispersed by different means: Diet A consisted of the basal ingredients plus 3.5 per cent hydrogenated cottonseed oil dispersed by homogenization; diet B, the basal plus 3.0 per cent hydrogenated cottonseed oil dispersed by 0.5 per cent emulsifying agent, glycerol-monostearate and homogenization; and diet C was identical to diet B except that it was unhomogenized.

Blood samples were collected from the calves at 2, 4 and 6 wk. of age for analyses.

No significant differences in hemoglobin were noted.

At 4 and 6 wk., the plasma fat of calves receiving diet B were significantly higher statistically at the 1 per cent level than that of calves receiving diet A, whereas the plasma fat levels from diet C were intermediate, indicating that glycerol-monostearate aided in absorption of lipides.

Plasma choline values of calves fed diet B were significantly higher statisti-

cally at a 5 per cent level than those of calves fed diet A, and the levels of those receiving diet C were intermediate.

Post-mortem observations revealed fatty livers in all calves. Analyses of the livers showed no significant differences in either lipide or choline content, but the differences between the per cent lipides in livers of calves fed diets B and C approached significance.

Although the growth of calves fed diet A was somewhat better than that of calves fed either diet B or C, the differences in body weight gains were not significant statistically.

All calves fed the unhomogenized diet (C) lost hair around the anus, tail and inside of thighs.

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A PROCEDURE FOR APPLICATION OF THE THIOBARBITURIC ACID TEST TO MILK[1]

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A reaction of an unknown metabolite with arylamines and thiobarbituric acid was described by Kohn (6) and Kohn and Liversedge (7). Bernheim *et al.* (2, 3) concluded that the metabolite was a product of the oxidation of unsaturated fatty acids. These and other reports (1, 8, 10) indicated the possible applicability of the thiobarbituric acid (TBA) test in studies of oxidized flavor in dairy products. Results reported previously (5) demonstrated that the TBA test correlates closely with numerical flavor scores of milk samples having oxidized flavor of varied intensity. The test is proving helpful in research on oxidized flavor and the mechanism of its development. A procedure for applying the TBA test to milk and a study of some factors influencing its reliability are described in this paper.

Thiobarbituric acid combines with aldehydes by a simple condensation reaction (4) but the reaction involving oxidized lipids has not been elucidated. Results obtained by Bernheim *et al.* (2) indicated that a group containing three carbon atoms was removed from the oxidized fatty acid and combined with the thiobarbituric acid. The compound responsible for the red color in the reaction of 2-thiobarbituric acid with certain pyrimidines (9) has absorption characteristics similar to those of the compound(s) formed from oxidized lipids but the nature of the reaction product(s) has not been established.

METHOD

TBA test procedure. The TBA test is based upon a reaction involving the formation of a red color when oxidized milk is acidified and heated with 2-thiobarbituric acid. The procedure used in applying the test to lipids and tissue suspensions (7, 10) did not give satisfactory results with milk because the colored compound(s) adsorbed to the milk proteins. Greater reproducibility in estimating the intensity of red color was obtained by using a pyridine-isoamyl alcohol mixture to elute and extract the colored compound(s). After detailed study of a number of factors which influence results of the test, the standardized procedure outlined below was adopted for application of the TBA test to milk.

Reagents. TBA reagent: 0.025 *M* 2-thiobarbituric acid in *M* phosphoric acid, prepared by mixing equal volumes of 0.05 *M* 2-thiobarbituric acid and 2 *M* phosphoric acid. Discard when precipitate forms. Extraction mixture: 2 : 1 mixture of isoamyl alcohol and pyridine.

Method. Ten ml. of milk are pipetted into a 40-ml. centrifuge tube (without pourout), 5 ml. of TBA reagent added and mixed well. The tube is placed in a boiling water bath for 10 min. and cooled in cold water. Delay in completion

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of the test is permissible at this point if the samples are refrigerated. For color extraction 15 ml. of extraction mixture are added, the tube stoppered, shaken vigorously for at least 30 sec. and centrifuged 5 min. at a minimum of 3,000 rpm. in a centrifuge with 16 in. peripheral diameter. Part of the clear solvent layer is removed and its optical density at 535 mμ determined with a Beckman Model DU spectrophotometer.

EXPERIMENTAL RESULTS AND DISCUSSION

Acidification for color development and extraction. The amount of color developed and extracted was influenced both by the kind of acid used for acidification and by the pH of the reaction mixture. Phosphoric acid was chosen as the acidulant because it dispersed the precipitated protein more completely than

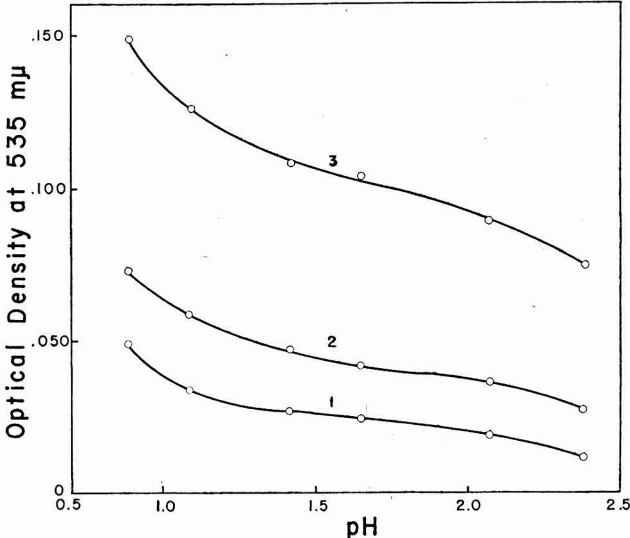

FIG. 1. Influence of pH of reaction mixture on color developed and extracted in the TBA test. 1—No oxidized flavor, 2—Slight oxidized flavor, 3—Pronounced oxidized flavor.

the other acids used, although citric acid also was satisfactory in this respect. Figure 1 illustrates the influence of pH (adjusted by varying the amount of phosphoric acid added) on the amount of colored compound(s) produced and extracted. The TBA reagent used in the standardized procedure buffered the reaction mixture at a pH of approximately 1.5 to 1.6 for most milk samples.

Influence of heating time on optical density. Figure 2 illustrates the influence of the time for which the reaction mixture is heated in the boiling water bath on the optical density of the extract. During the first 10 min. of heating, the rate of color development is greater in oxidized than in non-oxidized milk. After the first 10 min. the amount of red color continues to increase at a relatively constant rate for at least 90 min. in both non-oxidized and oxidized milk. These results indicate the importance of rigid standardization of the heating

period. A 10-min. heating period was arbitrarily adopted for use in the standardized TBA test.

Color extraction. The colored compound(s) adsorbs on the proteins and is not readily extracted by isoamyl alcohol alone. Pyridine aids in elution of the colored compound(s) and facilitates its extraction.

Color measurement. Typical absorption curves for isoamyl alcohol-pyridine extracts of TBA tests are presented graphically in figure 3. In curves no. 1, 2 and 3, two maxima are evident, one for the red compound(s) at 535 mμ wavelength and one for a yellow compound(s) at 450 mμ. These curves are similar to those published for other oxidized lipids (2, 10).

The amount of red color correlated with intensity of oxidized flavor, but the formation of the yellow compound(s) was variable and did not show a con-

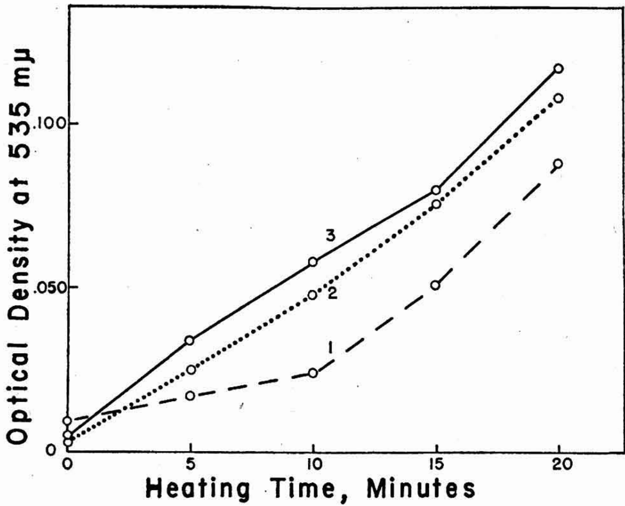

FIG. 2. Influence of time for which the samples were heated in boiling water on results of the TBA test. 1—No oxidized flavor, 2—Slight oxidized flavor, 3—Distinct oxidized flavor.

sistent relationship to red color production. The necessity for use of an instrument which provides sharp segregation of a narrow waveband at the maximum for the red compound(s) in order to prevent interference from the yellow compound(s) is illustrated by comparison of curves no. 3 and 4. These curves represent the same isoamyl alcohol-pyridine extract, but no. 3 was obtained with a Beckman Model DU spectrophotometer and no. 4 with a Coleman Model 11 spectrophotometer. Series of tests were ranked in different order of red color when results obtained with the Beckman Model DU spectrophotometer were compared with those of a Coleman Model 11 spectrophotometer and a Klett-Summerson Model 800-3 colorimeter with filter no. 54. For determination of optical density of TBA tests, the Beckman Model DU spectrophotometer gives satisfactory results.

Measurements at 535 mμ wavelength with the Beckman spectrophotometer conformed with Beer's law for optical densities at least as high as 1.2.

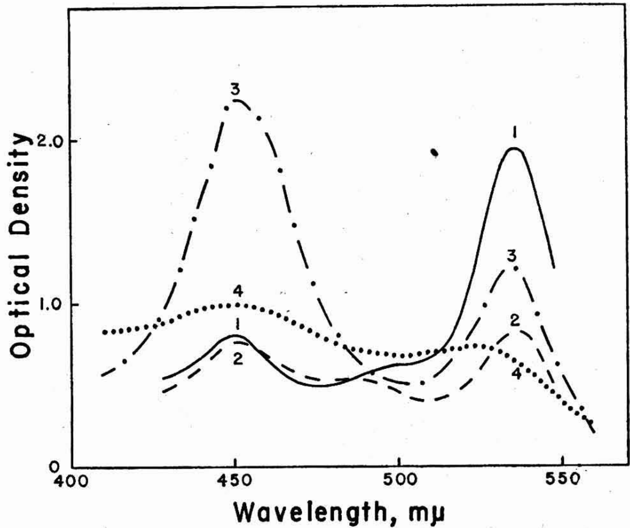

FIG. 3. Absorption curves for isoamyl alcohol-pyridine extracts of TBA tests. Nos. 1, 2 and 3 determined with Beckman Model DU spectrophotometer, No. 4 with Coleman Model 11 spectrophotometer. No. 1—Tallowy milk, oxidation catalyzed by sunlight; No. 2—Milk with oxidized flavor catalyzed by 0.5 ppm. copper; Nos. 3 & 4—Oxidized milkfat.

Copper interference. Copper causes an increase in optical density in the TBA test. This is illustrated by figure 4, which presents results of an experiment in which copper was added to milk in different concentrations immediately before making the TBA tests. This figure may be used for determination of cor-

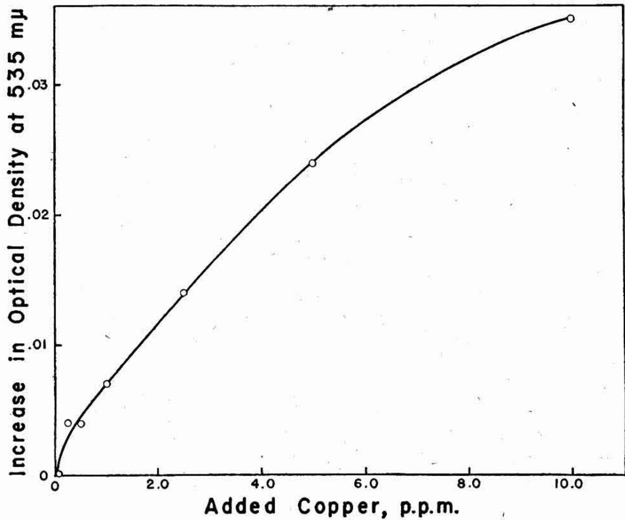

FIG. 4. Increase in TBA test caused by copper added to fresh milk immediately before the determination.

rection factors which should be subtracted from TBA tests of milk contaminated with more than 0.5 ppm. of copper.

Comparison with earlier TBA test. In the test used previously (5), the reaction mixture was adjusted to a lower pH and a 12-min. heating period was used. Consequently, the results were higher than those obtained by the procedure described herein. TBA tests were made by both procedures on a series of milk samples with oxidized flavor of varied intensity, and the relation between the tests calculated. For purposes of comparison, a test by the earlier procedure can be converted to its equivalent optical density by the procedure described above by deducting 0.010 and multiplying the remainder by 0.9.

Representative results. To illustrate the reproducibility and applicability of the method, TBA tests were run in duplicate on a series of samples which had been subjected to various treatments. The results are presented in table 1.

TABLE 1

Representative results illustrating reproducibility of duplicates and applicability of the TBA test

No.	Milk sample	Optical density at 535 mμ		
		1	2	Av.
1	Fresh raw	0.024	0.022	0.023
2	Homogenized	0.028	0.029	0.028
3	Slightly oxidized, catalyzed by 0.1 ppm. copper	0.042	0.041	0.042
4	Oxidized, catalyzed by 0.1 ppm. copper	0.097	0.096	0.096
5[a]	Oxidized, catalyzed by 0.5 ppm. copper	0.112	0.111	0.112
6	Exposed to sunlight for 30 min. (50 ml. in 150 mm. covered petri dish)	0.078	0.072	0.075
7	Exposed to two 15-watt germicidal lamps for 30 min. (50 ml. in 150 mm. open petri dish, 8 in. from lamps)	0.242	0.238	0.240

[a] 0.002 optical density deducted as correction for copper.

SUMMARY

A procedure for application of the thiobarbituric acid (TBA) test to milk is presented. Data reported illustrate the reproducibility and applicability of the test and the influence on the results of factors such as the pH of the reaction mixture, heating time, method of measuring color and copper contamination.

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INFLUENCE OF THE RATION ON THE EXCRETION OF CERTAIN VITAMINS BY RUMINANTS[1]

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In connection with a study of the influence of ration on rumen or intestinal synthesis of vitamins, the effect of method of preservation and storage of hay and the comparative effects of including cane and wood molasses in the ration, have recently been reported (1). Due to the high riboflavin content of the rations fed in that study, the importance of rumen synthesis of riboflavin, if it occurs, could not be determined. In a continuation of this general study such information was obtained and is reported herein along with additional data relating vitamin excretions to the following dietary items: corn silage, grass silage, mixed corn and grass silages, grass silage with cane molasses (80 lb. per ton) and a relatively low quality, late-cut native hay.

EXPERIMENTAL

The general experimental procedure and the analytical methods used were as previously reported (1). The experimental animals included one Jersey (no. 7), one Ayrshire (no. 9) and two Guernseys (no. 8 and 10). The vitamins studied were nicotinic acid, riboflavin and thiamine. The grass silage was prepared from the first cutting of a stand of mixed timothy hay, ladino, alsike and red clover.

RESULTS AND DISCUSSION

The average daily vitamin intakes, based upon analysis of the various rations, are presented in table 1. The low values seen for the late-cut native hay reflect

TABLE 1

Average vitamin intakes

Ration fed	Nicotinic Acid	Riboflavin	Thiamine
		(*mg./day*)	
Field-cured hay	162.0	43.2	4.5
Mow-cured hay	162.0	48.6	3.8
Low-quality native hay	54.0	21.6	1.3
Grass silage	154.8	20.7	5.0
Grass silage + molasses	177.6	24.6	5.6
Corn silage	162.8	46.3	3.8
Corn silage + grass silage	156.0	37.4	4.1

the very low quality of this hay with respect to its nicotinic acid, riboflavin and thiamine content. The only other instances of low vitamin intake involve riboflavin when grass silage was fed and are due to a considerably reduced riboflavin content of the grass when preserved as silage.

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TABLE 2

Vitamin excretions in feces

Cow no.	Field-cured	Mow-cured	Native hay	Grass silage	Grass silage + molasses	Corn silage	Corn silage + grass silage
			Nicotinic acid *(mg./day)*				
7	32.1	32.1	27.9	35.8	37.7	40.7	
8	31.9	36.5	26.7		38.2	41.8	36.3
9	36.4	35.4	33.4	37.9	40.4	39.9	
10	30.3	34.0	30.6		39.2	41.4	34.4
Av.	32.7	34.5	29.6	36.8	38.9	41.0	35.4
			Riboflavin *(mg./day)*				
7	9.1	8.2	8.1	8.9	11.9	15.7	
8	7.0	7.5	5.8		8.8	12.4	9.5
9	7.1	9.4	9.7	8.3	12.6	11.9	
10	7.6	7.8	8.9		8.2	13.5	9.3
Av.	7.7	8.2	8.1	8.6	10.4	13.4	9.4
			Thiamine *(mg./day)*				
7	3.0	3.6	3.1	3.0	4.1	6.5	
8	2.2	2.6	2.2		2.4	4.7	2.3
9	2.6	3.4	3.5	2.2	5.0	2.8	
10	2.1	1.8	2.1		1.5	2.2	2.2
Av.	2.5	2.8	2.7	2.6	3.2	4.0	2.2

Tables 2 and 3 present the average vitamin excretion values obtained with the various rations. Excretion of nicotinic acid was unaltered by the various rations with the exceptions of corn silage and late-cut native hay. In the latter case, the considerably decreased excretion merely reflects the lessened intake due to the

TABLE 3

Vitamin excretions in urine

Cow no.	Field-cured	Mow-cured	Native hay	Grass silage	Grass silage + molasses	Corn silage	Corn silage + grass silage
			Nicotinic acid *(mg./day)*				
7	15.7	17.8	9.0	16.6	18.7	16.5	
8	15.2	16.3	9.7		22.1	20.0	18.6
9	26.7	24.9	15.9	21.8	20.6	19.7	
10	19.5	17.5	9.7		21.1	17.0	17.1
Av.	19.3	19.1	11.1	19.2	20.6	18.3	17.8
			Riboflavin *(mg./day)*				
7	18.0	15.6	11.3	17.9	16.8	17.3	
8	13.7	12.9	10.8		17.6	17.2	12.6
9	17.9	14.5	11.7	16.4	14.7	16.4	
10	12.2	14.5	9.7		12.8	15.8	18.7
Av.	15.4	14.4	10.9	17.2	15.5	16.7	15.6
			Thiamine *(mg./day)*				
7	4.5	7.1	6.6	5.6	7.3	9.7	
8	6.2	3.7	4.9		6.4	7.3	6.7
9	9.3	8.6	7.2	13.7	6.0	6.3	
10	6.9	5.5	5.0		3.4	5.2	3.9
Av.	6.7	6.2	5.9	9.6	5.8	7.1	5.3

poor quality of the hay. The consistently increased fecal excretion of this vitamin when corn silage was fed could indicate a slight, but probably unimportant (1), enhancing effect by this ration on intestinal or rumen synthesis of nicotinic acid. From the point of view of nicotinic acid nutrition, method of preservation and storage of roughage evidently is unimportant.

Riboflavin excretions were essentially the same when the ration consisted of either field-cured or mow-cured hay. The low quality of the late-cut native hay, however, was reflected by a decreased excretion when this ration was fed. This decrease was evidently mainly in the urine. Corn silage apparently produced an increased riboflavin excretion. This could be due either to the greater intake or to an enhancing effect by silage on riboflavin synthesis in the digestive tract. The latter probability appears more likely when one considers the data with respect to grass silage plus molasses. In these instances, the excretion values were nearly the same as for field-cured and mow-cured hay; but the average daily intake of riboflavin was approximately only half that on the other rations. Actually, the total riboflavin excretion exceeded intake. This indicates that silage, and possibly molasses, favor the synthesis of riboflavin in the digestive tract of ruminants. The fact that a similar conclusion was not reached in a previous study (1) was no doubt due to the greater riboflavin content of the silage then fed.

In all cases thiamine excretion exceeded daily intake, corroborating the earlier suggestion (1) that rumen synthesis of this vitamin is of considerable importance in ruminant nutrition. Although the data indicate considerable thiamine synthesis in connection with all of the rations studied, the greater total excretions resulting from the silages suggest that silage favors rumen synthesis of thiamine.

SUMMARY

The low quality, with respect to nicotinic acid and riboflavin, of late-cut hay, was reflected in the decreased excretion of these vitamins by cows fed the hay. For the other rations fed, nicotinic acid excretion was unaffected, while the excretion values for riboflavin indicated that silage, and possibly cane molasses, favor the rumen or intestinal synthesis of this vitamin. Rumen synthesis of thiamine probably is of considerable importance in ruminant nutrition, and the feeding of silage appears to favor this synthesis.

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INTRAVENOUS AND ORAL ADMINISTRATION OF AN AQUEOUS SUSPENSION OF CAROTENE TO CALVES DEPLETED OF THEIR VITAMIN A STORES[1]

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The nutritive value of carotene stored within the body of the calf has not been determined. The possible contribution of body reserves of carotene to the vitamin A metabolism of the calf, especially during periods of suboptimum dietary intakes of carotene, is of importance. The main site of conversion of carotene to vitamin A in ruminants has been demonstrated *in vivo* to be the intestine (6, 8, 13). The conversion is believed to occur during the passage of carotene through the intestinal wall into the lymphatic duct (5). *In vitro* studies (23) have indicated that the liver as well as the intestine can convert carotene to vitamin A. In addition, the intravenous injection into calves of cow's plasma containing appreciable amounts of carotene has been reported to increase vitamin A storage in the liver (6).

In other species, the chick, rat and pig, the site of conversion of carotene to vitamin A has been found to be the intestine (1, 3, 7, 14, 17, 18, 24, 25, 26, 29). In the rat, intramuscular, intraperitoneal or subcutaneous injections of various water suspensions of carotene were utilized for growth restoration and disappearance of ophthalmia (2, 15, 16, 27). Oil solutions of carotene have been found not to be utilized as efficiently as aqueous solutions when both were administered parenterally (15, 27) and one report (21) indicated no utilization of an oil solution of carotene.

The objectives of this experiment were to ascertain the effects of intravenous and oral administration of an aqueous suspension of carotene on the liveweight, feed consumption, blood plasma and liver levels of carotene and vitamin A and histopathology of calves previously depleted of their vitamin A stores.

EXPERIMENTAL

Animals. Six Guernsey and six Holstein calves, previously raised on a limited whole milk, dry-calf starter and hay regime, were placed on a vitamin A depletion ration on the 106th day of age. The vitamin A-depletion ration[2] was

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[1] This work was supported in part by the Chas. M. Cox Co., Boston, Mass. and the Yantic Grain and Products Co., Norwich, Conn.

[2] A mixture by weight of one-third of dried beet pulp and two-thirds of a grain mixture composed of the following: 419.5 lb. ground barley, 500 lb. crimped oats, 500 lb. wheat bran, 150 lb. linseed oil meal (expellar process), 150 lb. soybean oil meal (expellar process), 200 lb. cane molasses, 40 lb. 500-potency B-Y dried fermentation solubles, 20 lb. steamed bone meal, 20 lb. iodized salt, and 0.5 lb. irradiated yeast (Standard Brands type 9-F) per ton of mixture. This depletion ration contained < 0.25 mg. of carotene per pound.

fed at the rate of 2.67 lb. per 100 lb. of bodyweight adjusted at successive 7-day intervals. When the blood plasma level of vitamin A reached less than 4 γ per cent for two consecutive 7-day periods, the calves were placed randomly with restriction as to breed and sex in one of three treatment groups: controls, intravenous administration of carotene and oral administration of carotene. The experimental period of 36 days consisted of three sub-periods: (I) a 6-day standardizing period to establish preliminary levels of criteria measured for assessment of subsequent data observed during treatment; (II) a 5-day period of administration of the dispersion agent (9% "Tween 80") to those animals in the carotene groups followed by a 5-day observational period to determine possible effects of the dispersion agent when administered alone and (III) a 6-day period in which the carotene was administered followed by a 14-day observational period to test the effects of oral and intravenous administration of carotene.

During the treatment periods, all calves were fed the same depletion ration at the same rate, which was adjusted to the weight of the calf for the day immediately preceding the start of each period. The "Tween 80" was administered twice daily between 8 and 9 a.m. and 3 and 4 p.m. with or without β-carotene, either by injection into the jugular vein or orally in gelatin capsules. Each calf received 17.1 ml. of 9 per cent "Tween 80" per 100 lb. of liveweight daily, based upon calf weights determined the day preceding period II. At this level the calves received 120 γ of carotene per pound of bodyweight for the first 6 days of period III, or approximately three times the daily carotene requirement as proposed by Moore *et al.* (20).

Preparation of aqueous carotene suspension. A weighed amount of β-carotene[3] dissolved in warm acetone was added dropwise from a burette to a constantly agitated 9 per cent "Tween 80"[4] solution which was maintained at 70 to 80° C. Heating and agitating were continued until most of the acetone was driven off, after which the remainder of the acetone was removed under vacuum. Distilled water then was added to bring the volume back up to the original volume of the "Tween 80."

A measured sample of the resulting carotene-"Tween 80" suspension was extracted with petroleum ether, B. P. 30 to 60° C., to ascertain the concentration of β-carotene. The petroleum ether extract was measured in an Evelyn macrocolorimeter using filter 440 against a standard of crystalline β-carotene. The concentration of carotene was then standardized at 0.7 mg. per milliliter of suspension by the addition of 9 per cent "Tween 80." The amount necessary for each calf per day then was measured into an Erlenmeyer flask, the air replaced by nitrogen and stored in the dark at room temperature until used.[5]

Observations and analyses. Daily feed intake was weighed to the nearest

[3] Crystalline β-carotene, General Biochemicals, Inc., Chagrin Falls, O.

[4] Polyoxyalkylene derivative of sorbitan monoleate manufactured by Atlas Powder Co., Wilmington, Del.

[5] Under identical storage conditions as existed during the experiment a sample with an initial concentration of 0.776 mg. of carotene per ml. contained 0.623 mg. of carotene per ml. at the end of a 12-day storage period.

0.1 lb. Liveweight and venous blood samples were taken every second day at 2 p.m. during the experimental periods. The blood samples were immediately chilled, centrifuged and a measured portion of the plasma frozen for carotene and vitamin A analyses. On the 36th or 37th experimental day, spinal fluid pressures were taken. The calves then were slaughtered and tissue samples were taken for subsequent histopathological examination. Liver and lungs were removed for carotene and vitamin A analyses.

Blood plasma carotene and vitamin A were determined by the method of Kimble (12), liver and lung carotene and vitamin A by the method of Davies (4) and spinal fluid as described by Moore (19). Histopathological techniques were those previously cited (11) and the statistical procedures used are those set forth by Snedecor (22).

RESULTS

Liveweight and feed consumed. Those groups receiving the carotene suspension either intravenously or orally had greater weight increases and feed consumption than the control group (table 1). The differences between groups were not statistically significant; however, analyses between days within a particular treatment group demonstrated significantly greater liveweight (log of liveweight $P < 0.05$) and feed consumption ($P < 0.01$) following either intravenous or oral administration of carotene than that observed for the days in period II prior to administration of carotene. In contrast, no difference between days were found for the control group. The dispersion agent alone, 9 per cent "Tween 80", had no apparent effect on liveweight changes or feed consumption.

Carotene and vitamin A. Both intravenous and oral administration of the carotene suspension caused an increase in the plasma levels of both carotene and vitamin A (table 1). Statistical analyses demonstrated that only the increases following the intravenous administration of carotene were significant ($P < 0.01$ for plasma carotene and $P < 0.05$ for plasma vitamin A). Further inspection of the data by individual days revealed that for the 20th, 22nd and 24th days, the plasma vitamin A levels for those calves receiving carotene orally were significantly greater ($P < 0.05$) than those observed for the control calves.

The carotene concentration of both the liver and lungs (table 2) in those animals receiving carotene intravenously was greater ($P < 0.05$) than that for the other two groups of calves. There were no real differences in vitamin A concentration of either the liver or lungs.

Spinal fluid and clinical observations. Spinal fluid pressures determined on the 36th or 37th day (table 1) immediately prior to slaughter were, on the average, lower in those calves receiving the carotene than in the control calves. In the two Guernsey controls, it was impossible to obtain satisfactory spinal punctures because of extreme muscular incoordination.

All calves had diarrhea and exhibited muscular incoordination and convulsive attacks during the experiment. The Guernsey calves appeared to be more prone to exhibit these symptoms than the Holsteins. The administration of

TABLE 1

The effect of oral and intravenous administration of an aqueous suspension of carotene on liveweight, feed consumed, blood plasma carotene and vitamin A and spinal fluid pressure

Expt. no.	Breed	Age at start of expt.	Liveweight			Per cent feed consumed			Mean plasma carotene			Mean plasma vitamin A			Spinal fluid, 36th or 37th day
			Period I at 6 d.	Period II at 16 d.	Period III[b] at 34 d.	Period I	Period II	Period III[b]	Period I	Period II	Period III	Period I	Period II	Period III	
		(days)	*(lb.)*			*(per cent)*			*(γ per cent)*			*(γ per cent)*			*(mm. H_2O)*
Controls															
3	H[a]	148	331	344	338	100	95	72	3	2	1	2.6	1.1	0.7	> 505
4	H	169	320	337	352	82	84	92	6	5	4	2.5	2.8	2.9	> 505
23	G	162	317	312	336	95	74	88	10	9	6	2.9	1.5	2.4	....
28	G	155	314	310	290	95	75	49	4	4	2	1.6	1.4	1.3	....
Mean		158.5	320.5	325.8	329.0	93	82	75	5.8	5.0	3.3	2.40	1.70	1.83	
Intra-venous carotene															
2	H	195	494	500	526	100	81	79	5	4	62	2.9	1.9	6.0	310
6	H	197	435	454	479	79	85	89	3	3	63	2.7	2.2	5.4	385
24	G	169	318	310	320	97	88	69	12	9	11	2.0	2.0	3.1	> 505
27	G	165	298	294	298	89	72	73	6	2	27	1.8	1.2	4.7	240
Mean		181.5	386.3	389.5	405.8	92	82	79	6.5	4.5	40.8	2.35	1.83	4.80	
Oral carotene															
22	H	182	432	385	436	87	79	84	2	3	4	2.1	1.6	3.2	505
5	H	204	507	508	558	66	55	81	6	3	10	2.4	1.1	5.7	465
25	G	174	328	334	341	100	74	85	5	6	14	1.4	2.4	1.7	....
26	G	142	278	296	298	96	91	98	15	13	9	2.5	2.0	1.6	285
Mean		175.5	386.3	380.8	408.3	85	73	86	7.0	6.3	9.3	2.10	1.78	3.05	

[a] All animals males except 4, 5 and 6.
[b] Value for the 36th day not included, since some calves slaughtered on the morning of this day.

carotene either intravenously or orally resulted temporarily in a lowered incidence of these symptoms. However, at the termination of the experiment, the symptoms generally had returned, but not at the same intensity as those found in the control calves.

Histopathology. Microscopic examination of the adrenal, eye, heart, kidney, liver, lung, pituitary, spleen and thyroid failed to reveal consistent histopathological changes. In the male calves, no spermatogenesis was observed. Squamous metaplasia was observed in the parotid interlobular ducts as well as in the main duct and also in the interlobular ducts of the submaxillary gland of all calves. The extent of the salivary gland lesions apparently were of equal intensity in all groups of calves.

TABLE 2

The effect of oral and intravenous administration of an aqueous suspension of carotene on liver and lung concentration of carotene and vitamin A.

Expt. no.	Liver			Lungs		
	Total wt.	Carotene	vitamin A	Total wt.	Carotene	vitamin A
	(*g.*)	(γ/*g.*)		(*g.*)	(γ/*g.*)	
Controls						
3	2909	0.21	0.04	1673	0.08	0.42
4	2944	0.24	0.58	1516	0.14	0.14
23	1883	0.33	0.04	1869	0.04	0.00
28	....	0.25	0.23	....	0.14	0.00
Mean	2578.7	0.258	0.223	1686.0	0.100	0.140
Intravenous Carotene						
2	3825	1.84	0.22	1704	13.61	0.57
6	3570	1.00	0.18	1630	3.12	0.47
24	2784	0.49	0.06	2445	2.07	0.07
27	....	0.57	0.10	....	5.72	0.26
Mean	3393.0	0.975	0.140	1926.3	6.130	0.343
Oral Carotene						
22	3952	0.18	0.02	1984	0.05	0.03
5	3200	0.52	0.10	1740	0.05	0.09
25	2620	0.76	0.07	1940	0.18	0.06
26	2705	0.36	0.05	2148	0.11	0.02
Mean	3119.3	0.455	0.060	1953.0	0.098	0.050

DISCUSSION

Although limited in number and exhibiting variable response, these data indicate the possibility that body stores of carotene can be utilized by young dairy calves as evidenced by growth restoration, increases in plasma vitamin A levels and decreases in clinical symptoms of vitamin A deficiency after the intravenous administration of a suspension of carotene. Therefore, it is plausible to consider carotene stored within the liver and other depots of nutritive value to the calf.

The data are largely in agreement with similar experiments conducted with rats (2, 15, 16, 27), in that growth restoration was observed in those animals

which received parenteral administrations of carotene suspensions. In the experiment reported herein, as well as in the rat experiments, considerable storage of carotene was observed upon slaughter. Similarly, no apparent increase in vitamin A stores was observed. However, it should be pointed out that a 14-day period elapsed between administration of the carotene and slaughter in this experiment. This length of time may have permitted the complete utilization of the vitamin A converted from the carotene.

The increase in the blood plasma vitamin A levels after the intravenous injection of carotene is of interest. Although the Carr-Price reaction is not specific for vitamin A (9) and oxidation products of β-carotene affect the reaction more than does β-carotene (10), these data indicate that the carotene stored within the body possibly can be converted to vitamin A. A more specific measurement of vitamin A is needed to establish this point (17).

The presence of large amounts of carotene in the lungs and smaller amounts in the liver of those calves injected intravenously with carotene indicates a filtering out of the larger particles of carotene. Sexton *et al.* (21) have suggested the Kupfer cells of the liver as a site of this filtration. Of interest is their finding that carotene in cow's blood migrates in a Tiselius apparatus with the β globulin. This may not be duplicated when the carotene is injected as a suspension using "Tween 80" as the stabilizing agent.

In a somewhat similar study as that reported in this paper, Warner and Loosli (28) found similar responses to the intravenous administration of a colloidal suspension of carotene to vitamin A depleted calves. These data lend support to those reported herein.

SUMMARY

The effect of intravenous and oral administration of carotene suspended in 9 per cent "Tween 80" was studied in 12 calves which had been depleted of their vitamin A stores. Growth rate and feed consumption were somewhat higher in those calves which received the carotene, but the differences were not statistically significant. Intravenous administration of carotene resulted in higher blood plasma levels of carotene and vitamin A and liver and lung concentrations of carotene than were found in either the control calves or those which received carotene orally. Oral administration of carotene resulted in increases in blood plasma levels of carotene and vitamin A, but these differences were statistically significant for only those days during the carotene administration. Spinal fluid pressures as well as degree of diarrhea, muscular incoordination and convulsive seizures were decreased by the administration of carotene. The interlobular ducts and main duct of the parotid gland and the interlobular ducts of the submaxillary gland exhibited squamous metaplasia typical of hypovitaminosis A. The degree of intensity of these histopathologic changes was not influenced by treatment.

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THE FERTILITY OF BULL SEMEN EXTENDED WITH YOLK–CITRATE CONTAINING VARYING PROPORTIONS OF EGG YOLK[1]

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The use of egg yolk in diluters or extenders of bull semen was inaugurated by Phillips in 1939 (6). He recommended three parts of fresh egg yolk and two to three parts of a phosphate buffer. Salisbury *et al.* (8) later developed an extender composed of equal parts of egg yolk and sodium citrate buffer. Mayer and Lasley (5) and Bogart and Mayer (1) have shown that egg yolk contains a factor which increases the resistance of spermatozoa to drastic changes in temperature, osmotic conditions and pH. Since the development of these extenders, there has been a large increase in the number of cows being bred artificially. Salisbury *et al.* (7) and Willett (12, 13) have shown that bull semen may be extended to as much as one part of semen to 100 parts or more of extender without impairing fertility. With large quantities of extended semen being shipped by many bull studs, the cost and labor of obtaining sufficient egg yolk have been items of major expense. Swanson (10, 11) found that there was very little difference in the maintenance of progressive motility and the resistance to cold shock when samples were diluted with yolk-citrate in proportions of 1:1, 1:3 and 1:7. Some bull studs have decreased the proportion of egg yolk but they have not reported controlled studies concerning the effect on fertility. Stewart *et al.* (9) found no significant difference in the fertility of semen in a control diluent of equal parts of egg yolk and 3 per cent sodium citrate solution and that in a trial diluent composed of one part of egg yolk and three parts of citrate buffer. Dunn and Bratton (2, 3, 4) have reported that whole eggs may be used satisfactorily in making up semen extenders. Their method was estimated to reduce the cost of preparing extenders to only 25 per cent of that required for the usual yolk-citrate-sulfanilamide extender. The present study was undertaken in an effort to gain further information regarding the fertility of bull semen extended with yolk-citrate containing less than 50 per cent egg yolk.

EXPERIMENTAL PROCEDURE

During the months of June and July, 1950, there were 182 semen samples collected from 26 bulls. Each sample was split into two equal portions and extended with yolk-citrate. The extenders used in the month of June consisted of either equal parts of 2.9 per cent sodium citrate dihydrate and fresh egg yolk or

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one part of egg yolk to three parts of the citrate buffer. During the month of July, the proportion of yolk to citrate was either 1:3 or 1:5. The rate of extension averaged about one part of semen to 40 or 50 parts of extender. Penicillin and streptomycin were added to all of the extenders at the rate of 500 units of each per milliliter. The semen was shipped four times a week to 35 local cooperatives of the Kentucky Artificial Breeding Association. Shipments to each local were alternated so that approximately the same number of cows would be bred with each semen treatment throughout the experimental period.

RESULTS

During the month of June, 1,273 cows were bred with semen extended with yolk-citrate composed of equal parts. Of these cows 70.8 per cent did not return for second service within 120 to 150 days. There were 1,318 cows bred with semen extended with yolk-citrate composed of one part of egg yolk and three parts of citrate buffer and 69.2 per cent of these did not return for second service. The difference in fertility (1.6 per cent) was not statistically significant.

During July, 1,309 cows were bred with semen extended with yolk-citrate prepared in a ratio of 1:3. There were 74.0 per cent non-returns. Similarly, 1,404 cows were bred with semen extended in 1:5 yolk-citrate and 66.2 per cent did not return for second service within 120 to 150 days. The difference of 7.8 per cent, when tested by Chi-square, was statistically highly significant ($P = < 0.01$).

SUMMARY

During the months of June and July, 1950, 182 semen samples from 26 bulls were split into two equal portions and extended with yolk-citrate prepared to contain egg yolk and sodium citrate solution in proportions of 1:1, 1:3 or 1:5. Semen in each of the extenders was used to breed 1,200 to 1,400 cows. There was no significant difference between the 1:1 and the 1:3 series. However, the semen extended in 1:5 yolk-citrate had a fertility of 7.8 percentage units lower than that extended in 1:3 yolk-citrate. This difference, when tested by Chi-square, was highly significant.

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THE SOLUBILITY OF WHOLE MILK POWDER AS AFFECTED BY PROTEIN STABILIZERS AND BY EMULSIFIERS[1]

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The addition of salts for protein stabilization has been practiced extensively by the evaporated milk industry for about 25 yr. The work of Sommer and Binney (10) and Sommer and Hart (11) contributed a great deal toward solving the problem of heat stabilization of the proteins in evaporated milk. The use of sodium citrate in small amounts also has been suggested by Wilster *et al.* (12) as a means of improving the solubility of milk powder.

Josephson and Reeves (6) employed a mineral-ion-exchange treatment of milk to obtain the desired salt balance. A portion of the Ca was replaced with Na by means of a synthetic resin column to produce a heat stable evaporated milk.

Controlled pancreatic digestion in milk was introduced by Anderson (1) as a means of preventing oxidized flavor in market milk. Since the pancreatic enzymes break down the protein, it seemed desirable to study their effect on whole milk powder as a combination antioxidant and protein stabilizer.

The use of emulsifiers in dairy products has been limited mainly to the ice cream industry as whipping aids. However, other food industries have made extensive use of these agents. In this study, emulsifiers of different hydrophilic-lyophilic balance were used with the idea of reducing the surface tension between the milk powder and the water for improved reconstitution. Also, information as to their effect, if any, on keeping quality of the milk powder was desired.

EXPERIMENTAL PROCEDURE

The experimental work may be divided into four series, (a) a salt-balance study, (b) a mineral-ion-exchange study, (c) an enzymatic-treatment study and (d) an emulsifier study. Each series consisted of at least five replications made from separate bulk milk supplies and included control powders made from each supply of milk.

Fresh milk was obtained from the College herd, preheated to 170° F. for 30 min. in a 30-gal. pasteurizer and evaporated to approximately 40 per cent total solids in a Rogers experimental vacuum pan. The concentrated milk was divided into several lots for the various treatments.

In the series devoted to salt-balance studies, the individual salts were added to the concentrated milk in the following percentages by weight of the reconstituted milk: 0.01 per cent ascorbic acid, 0.2 per cent sodium citrate, 0.2 per cent sodium citrate plus 0.01 per cent ascorbic acid, 0.1 per cent sodium citrate plus 0.1 per

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cent Na_2HPO_4, 0.075 per cent sodium hexametaphosphate (Calgon), 0.2 per cent Na_2HPO_4 and 0.03 per cent Ca as $CaCl_2$.

In the ion-exchange studies, Amberlite 1R-100 in the Na cycles was used for the ion exchange material. Fresh skimmilk at 40° F. was passed down flow through a glass column 4 ft. long and 4 in. in diameter, at the rate of 8 bed volumes per hr. The back-washed and drained-bed volume was 4800 ml. The treated skimmilk was condensed and spray-dried in an experimental drier based on a design given by Coulter (5). This low Ca skimmilk powder was hermetically sealed in no. 2 cans and stored at 45° F. for future use in treating milk. The ion-exchange treatment of whole milk was accomplished by adding the necessary amount of low Ca skimmilk powder (reconstituted to 30 per cent total solids) to the concentrated whole milk to attain treatment at the level of 1, 3 and 5 per cent of the total solids.

Pancreatic enzyme digestion was carried on in fresh whole milk by adding 0.5 g. of pancreatic powder to 10 gal. of fresh whole milk at 100° F. After incubating for 10 min., the temperature was raised to 170° F. during the next 10 min. and held for 30 min. The milk was cooled to 125° F. and condensed to approximately 40 per cent total solids. Other lots of concentrated milk had emulsifying agents added at the rate of 0.05 per cent by weight on a reconstituted basis. The emulsifiers used were Span 62 (sorbitan monostearate), Tween 60 (polyoxyethylene sorbitan monostearate), Myverol 1800 (glycerol monostearate) and Myverol 1885 (mixed monoglycerides of cotton seed oil).

In all cases the concentrated whole milk, with or without additional materials added, was passed twice through a Manton-Gaulin, 25 gal. per hour, homogenizer at pressures of 2,000 lb. on the first stage and 500 lb. on the second stage. The concentrate was cooled to 80° F. and spray-dried at the rate of 1 l. every 15 min. The resulting powder was air-packed, hermetically sealed in no. 2 squat cans and stored at 45 and 85° F.

The total solids content was determined on both the evaporated and dried milk (vacuum oven). The fat content of the powder was determined by the HCl-ether extraction method given by Mohr and Hasing (8). The solubility index was determined by the method of Cone and Ashworth (4), but modified by adding an additional quantity of powder to compensate for the moisture in the powder. The efficiency of homogenization was determined by Ashworth's turbidity method (2). The apparent density of the powder was determined as described by Manus and Ashworth (7). The alcohol stability test as given by Ashworth and Hibbs (3) was modified in the final step by estimating the quantity of soluble nitrogen by a turbidometric method. A standard curve for this method was obtained by measuring the turbidity (Evelyn colorimeter 515 mμ filter) of a large number of samples which were made up to contain increasing increments of soluble nitrogen (from 0 to 40 mg. of soluble nitrogen) per gram of powder.

The flavor scores of the reconstituted powdered whole milk samples were determined by a panel of seven experienced judges. The milk powder was reconsti-

tuted on a basis of one part of powder to seven parts of distilled water and judged for flavor. The following arbitrary standards were used: 9–10, excellent; 7–9, good; 5–6, fair; 3–4, poor; 1–2, bad.

RESULTS

The replicate averages for solubility index and flavor score of the control powders are plotted in figures 1 and 2, respectively. The controls for the milk

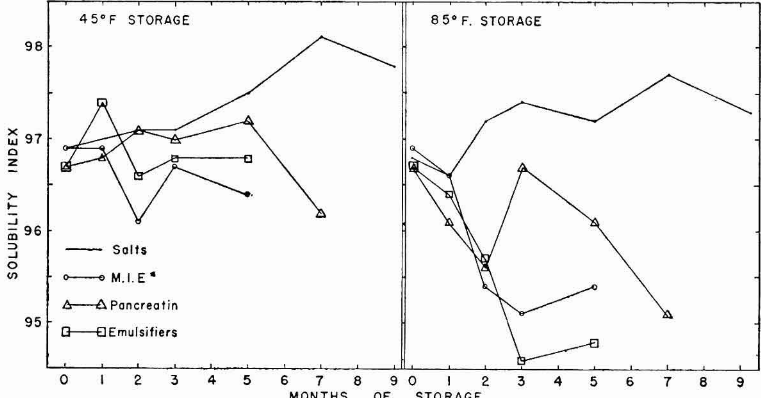

FIG. 1. Solubility index for control powders of each series as affected by storage at 2 temperatures. (* M.I.E. means mineral ion exchange.)

receiving salt treatment retained their solubility better than did the other controls. This may be attributed to the season of the year. The milk for the salt-addition studies was procured in the spring and early summer. As shown in figure 1, at 45° F. storage the solubility of the control powders tended to decrease with a progression of the seasons. Milk for the salt-addition study was obtained

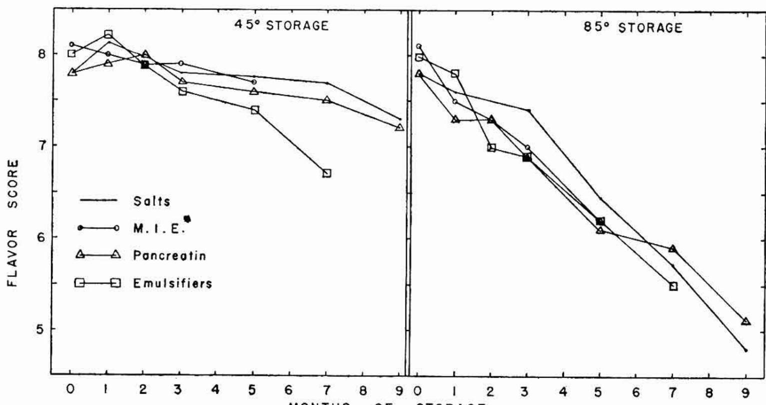

FIG. 2. Flavor score for control powders of each series as affected by storage at 2 temperatures. (* M.I.E. means mineral ion exchange.)

in the spring, that for the pancreatic study was summer milk, the emulsifier study made use of autumn milk and the mineral-ion-exchange study was carried out using winter milk. However, due to the small number of samples, these differences were not statistically significant.

The flavor scores of the control powders in figure 2 show no seasonal variation, but the influence of storage temperature is evident. The control powders stored at 45° F. were on the borderline between fair and good after 9 mo. of storage, whereas the control powders stored at 85° F. for 9 mo. were on the borderline between poor and fair.

Powders containing protein stabilizers. The values given in table 1 are the difference between the replicate averages of the solubility of the control powders and those of treated powders. At 85° F. storage, the salts as a whole tend to

TABLE 1

Differences in solubility between the treated powders and their respective controls

Treatment	Solubility differences after storage at:					
	45° F.			85° F.		
	2 mo.	5 mo.	7 mo.	2 mo.	5 mo.	7 mo.
Ascorbic acid				− 2.5	− 3.6	− 3.5
Na citrate & ascorbic acid				− 0.6	− 0.6	− 0.4
Na citrate				− 2.3	− 3.0	− 3.0
Na citrate & Na_2HPO_4	+ 0.1	+ 0.2	− 0.5	− 0.1	− 0.4	− 0.7
Na_2HPO_4	+ 1.0	+ 0.2	− 0.1	+ 0.5	− 0.6	− 1.7
Cal.	+ 0.2	− 0.4	− 0.5	− 0.3	− 0.6	− 0.3
$CaCl_2$	− 0.5	− 0.9	− 0.6	− 1.0	− 2.3	− 1.4
1% mineral-ion exchange	+ 0.9	+ 0.3		− 0.1	− 0.6	
3% mineral-ion exchange	+ 0.9	+ 0.7		− 0.15	+ 0.1	
5% mineral-ion exchange	+ 0.8	0		− 0.1	− 0.3	
Pancreatin	− 0.1	− 0.1	+ 0.2	− 0.1	+ 0.5	+ 0.4
Tween 60	− 11.7	− 10.9	− 10.9	− 5.8	− 6.3	− 6.3
Span 62	− 2.8	− 1.4	− 2.2	− 0.4	+ 0.2	− 0.4
Myverol 1800	− 2.3	− 3.1		− 0.2	+ 1.0	
Myverol 1885	− 2.4	− 2.0		− 0.4	+ 1.7	

decrease the solubility of the milk powder as compared with the controls. At 45° F. storage, there appears to be little difference between the controls and the salt-treated samples and only small changes in solubility during the storage period of any of the samples. At 45° F. storage, Na_2HPO_4 was most effective in preventing the decrease in solubility of the powder, whereas $CaCl_2$ caused the most rapid decrease in solubility of the treated milk powders. The powders containing the mixture of sodium citrate and ascorbic acid had solubilities similar to the controls, whereas sodium citrate used alone was detrimental to solubility.

With the exception of the detrimental effect of $CaCl_2$, there was no significant effect of any of the salts on the flavor of the reconstituted powder at 45° F. storage. The average flavor scores were all within ± 0.5 point difference from the control. At 85° F. storage nearly all treatments yielded powders having better keeping quality than the controls. Initially the samples had a slight flat flavor

and were not considered as palatable as the control; after the first month, this lack of flavor also was apparent in the controls. The combination of ascorbic acid and sodium citrate gave the greatest keeping quality improvement of all the salts added.

The effect of mineral-ion-exchange (M.I.E.) treatment on solubility is shown in table 1. The treated powders stored at 45° F. consistently were more soluble than the control powders after 1 mo. of storage. Treatment of 3 per cent of the non-fat milk on the ion exchange column gave better results than either the 1 per cent or the 5 per cent treatments. For the first month of storage, the milk powder containing 5 per cent treated milk was less soluble than the control powders. By the end of 2 mo., however, the reverse was true.

There was very little effect on palatability from M.I.E. treatment when the milk powder was stored at 45° F., other than a very slight salty taste at the 5

TABLE 2

Difference in flavor between treated powders and their respective controls

Treatment	Flavor differences after storage at:					
	45° F			85° F.		
	2 mo.	5 mo.	7 mo.	2 mo.	5 mo.	7 mo.
Ascorbic acid				+ 0.3	+ 0.2	+ 0.4
Na citrate & ascorbic acid				+ 0.4	+ 0.7	+ 0.6
Na citrate				+ 0.2	+ 0.3	+ 0.2
Na citrate & Na_2HPO_4	0.0	− 0.3	− 0.2	+ 0.2	+ 0.2	+ 0.5
Na_2HPO_4	− 0.1	− 0.2	− 0.1	+ 0.1	0.0	+ 0.4
Calgon	0.0	− 0.3	− 0.1	− 0.2	− 0.4	+ 0.8
$CaCl_2$	− 1.2	− 0.5	− 0.8	− 1.2	− 2.0	− 2.7
1% mineral-ion exchange	0.0	− 0.2	− 0.1	+ 0.1	+ 0.6	+ 0.7
3% mineral-ion exchange	+ 0.1	+ 0.1	+ 0.1	− 0.2	+ 0.5	+ 0.7
5% mineral-ion exchange	0.0	− 0.1	+ 0.1	− 0.1	+ 0.7	+ 0.6
Pancreatin	0.0	− 0.4	− 0.1	− 0.1	+ 0.1	0.0
Tween 60	+ 0.2	+ 0.9	+ 1.5	+ 1.2	+ 1.5	+ 2.2
Span 62	− 0.2	+ 0.7	+ 1.3	+ 0.5	+ 1.4	+ 0.8
Myverol 1800	+ 0.2	+ 0.4	+ 0.8	+ 0.3	+ 1.0	+ 2.1
Myverol 1885	+ 0.3	+ 0.3	+ 0.7	+ 0.1	+ 0.7	+ 2.1

per cent level. At 85° F. storage, the treated powders generally had better flavor than the control powders. As the storage period progressed, the decrease in flavor score of the control samples was much more rapid than that of the M.I.E.-treated milk. After 5 mo. of storage, the control samples were distinctly stale, whereas the M.I.E.-treated milks were only slightly stale.

Initially the pancreatin-treated milk powders were less soluble than their controls, but the control powders decreased in solubility at a more rapid rate than did the treated powders. At 45° F. storage very little difference existed between the controls and treated powders after the second month of storage. At 85° F. storage, the treated powders were more soluble than the controls after 5 mo. of storage.

The pancreatic digestion appears to be detrimental to the palatability of the resulting milk powder, the pancreatin-treated powders at 85° F. became stale

more rapidly than did the controls, particularly at 3 mo. of storage. However, by the fifth month of storage, the controls had degraded more than the treated powders. After 7 mo., no difference was found between the controls and the treated powders. The same trend was noticed in the powders which were stored at 45° F., but the changes in flavor were more gradual.

The solubility of whole milk powder containing emulsifying agents was difficult to measure accurately, especially with those powders containing polyoxyethylene sorbitan monostearate (Tween 60). There the butterfat churned out while reconstituting the samples. The samples stored at 85° F. churned surprisingly less after only 1 mo. in storage. After 3 mo. at 85° F., the powders containing Span 62, Myverol 1800 and Myverol 1885 were more soluble than their respective controls. Myverol 1885 appeared to best prevent a decrease in

TABLE 3

The influence of the various treatments on the alcohol stability of reconstituted whole milk powder

Treatment	No. of individual batches scored as:		
	Stable	Slightly unstable	Unstable
Controls	13	7	10
Ascorbic acid	1	2	1
Sodium citrate	5	0	0
Disodium phosphate	6	0	0
Calcium	0	0	8
Citrate plus ascorbic acid	5	0	0
Citrate plus phosphate	6	0	0
Mineral-ion exchange:			
1% Treated	4	1	0
3% Treated	5	0	0
5% Treated	4	1	0
Pancreatic digestion	2	1	3
Emulsifiers:			
Tween 60	2	3	0
Span 62	3	0	2
Myverol 1800	3	0	0
Myverol 1885	3	0	0

solubility with age when the powder was stored at 85° F. In all cases, the powders stored at 45° F. churned during reconstitution, thereby giving an apparently low solubility index. The powders containing emulsifiers were the only ones that were more soluble for the entire storage period, when stored at 85° F. than at 45° F.

The palatability of the whole milk powder was greatly improved by the use of emulsifiers. The initial flavor of the treated milk was "full," whereas the flavor of the controls was slightly "flat." The highly desirable flavor found in the treated powders was maintained throughout the storage period at 45° F. and for more than 5 mo. at 85° F. storage. The flavor of the controls became less desirable, developing a stale flavor in 3 mo. at 85° F. and 5 mo. at 45° F. storage.

The glycerol monostearate and the mixed mono-glycerides of cotton seed oil were more satisfactory, since they maintained both keeping quality and solubil-

ity of the milk powders which were stored at 85° F. The polyoxyethlene sorbitan monostearate had a desirable influence on keeping quality, but caused pronounced churning when the samples were reconstituted. It is not known whether the influence on keeping quality will be maintained when the amount of the emulsifying agent is reduced enough so that churning will not take place during reconstitution of the powder.

The stability of the reconstituted milk to precipitation by alcohol was affected only when the material added was of a nature capable of influencing the ionic charge on the protein molecule (table 3).

Using the methods of Snedecor (9), a highly significant correlation ($r = 0.75$, $n = 30$) was found between the solubility index and the efficiency of homogenization, which indicates that care must be taken to have properly homogenized milk when the fat layer formed during the solubility test is to be included as insoluble material, as is done in the Cone-Ashworth method (4).

No significant correlations were found between (a) apparent density of the powder and initial solubility index, (b) moisture content of the powder (1 to 3 per cent moisture) and initial solubility index, (c) fat content of the control powders and initial solubility index and (d) fat content of the control powders and homogenization efficiency index.

CONCLUSIONS

Na_2HPO_4 (0.2 per cent) appears to be the best salt to use in stabilizing milk for drying as far as solubility is concerned. However, none of the salts added seemed to improve the solubility to any great extent. The combination of sodium citrate (0.2 per cent) and ascorbic acid (0.01 per cent) was most influential of the salts in preventing the development of "off" flavors.

The addition of emulsifying agents at the rate of 0.05 per cent on a reconstituted basis caused churning of the butterfat when the powder was reconstituted. However, the use of emulsifier very materially improved the keeping quality of the milk powder during storage at both 45 and 85° F.

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METHODS OF TESTING FOR ABSORPTION VALUE OF NONFAT DRY MILK SOLIDS IN BREAD DOUGH[1]

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It has been known for a long time that it is necessary to heat-treat skimmilk in order to improve its baking quality. Greenbank *et al.* (1) showed that the ability of nonfat dry milk solids to bind water in the dough increased with heat treatment and pointed out the correlation of this water-binding ability and the viscosity of the condensed milk with increasing heat treatment. Skovholt and Bailey (7) measured the effect of heat treatment by means of the Farinograph. Stamberg and Bailey (8) used the Farinograph and a polarographic method to show the presence of sulfhydryl groups in low-heat nonfat dry milk solids samples. Larsen *et al.* (3) found a correlation between loaf volume and Farinograph and Extensograph measurements. Shipstead (6) suggested the use of the Mixograph for testing the weakening action of low-heat samples of nonfat dry milk solids and showed the correlation between high-temperature, short-time heat treatment, whey protein denaturation, viscosity, curd tension and absorption value with the Mixograph and Farinograph tests. It is evident from these data that the minimum heat treatment sufficient to denature the whey proteins, thereby eliminating the weakening effect on the bread dough as shown by the Mixograph and Farinograph tests, increases the absorption value only to 1.0. This indicates that the loaf volume test, which depends upon the gluten strength of the dough, can only correlate with absorption values up to 1.0. Neither the Mixograph nor the Farinograph test with 6 per cent nonfat dry milk solids showed any change in dough consistency with nonfat dry milk solids of absorption values ranging from 1.0 and up. The only test which seemed to show some correlation with absorption values over 1.0 was that of viscosity of reconstituted solution of the milk powder samples.

It has been a general rule for the baker to use 1 lb. of extra water for every pound of nonfat dry milk solids used in bread dough. Since it is evident that it is desirable to manufacture nonfat dry milk powder with high absorption values to reduce its actual cost to the baker, it becomes very important to the dry milk manufacturer to have a reliable method for testing absorption value. Such a method will help the production department to develop and standardize methods of heat treatment that will give uniformly high absorption values.

EXPERIMENTAL PROCEDURE

Source of samples: The experimental control samples used for the measurements of absorption value were prepared from skimmilk heated to various temperatures and held for definite periods of time, condensed to 40 per cent solids

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and dried in an experimental spray drier to a moisture content of around 3.5 per cent. The temperatures and holding times used are given in table 1.

Twelve commercial samples were obtained from different sections of the country. Information regarding the heat treatment of the samples was requested. Sample H was marked low-heat and sample E high-heat by the manufacturer and information regarding samples M and N was received, which indicated that the heat treatment was insufficient for good baking quality.

Determination of soluble whey proteins and non-protein nitrogen. The determinations of soluble whey proteins and non-protein nitrogen were done according to Rowland (4). The results were calculated on a moisture-free basis as per cent of dry solids. There seems to be a considerable variation in the values for soluble whey proteins in samples with high-heat treatment, which may be caused by variations in proteose and peptones present in the original milk. Reconstituted 9 per cent samples of 191 and 192 (which were made from the same batch of skimmilk), heated to 100° C. for 10 min. were tested for soluble whey

TABLE 1

Heat treatment of experimental control samples of nonfat dry milk solids

Sample	Heat treatment		Moisture content of milk powder	Absorption value by mixing bowl test
	Temperature	Holding time		
(*no.*)	(° *C.*)	(*min.*)	(%)	(*ratio of water to n.f.d.m.s.*)
191	63	30	3.24	0.58
152	99	0.5	3.68	0.92
144	110	0.5	3.24	1.08
192	88	15	3.46	1.33

proteins, and the results were 1.85 per cent for 191 and 1.93 per cent for 192. This indicates that no. 192 had been heated sufficiently before drying to denature all of the lactalbumen and lactoglobulin in the original milk. The slight increase for 192 could be due to protein degradation, which would increase the non-protein nitrogen value according to Rowland (5). Sample 191 showed a large drop in soluble whey protein from 6.25 to 1.85 per cent due to the extra heat treatment at 100° C. for 10 min. Similar tests made with samples P and K show a complete denaturation with practically no increase in non-protein nitrogen. This proves that the proteose and peptone content can vary in nonfat dry milk solids from 1.04 to 1.95 per cent (table 3). The high percentage of soluble whey proteins in samples J, K and T could possibly be explained by the high total protein content which naturally might tend to increase the soluble whey fraction.

Mixing bowl method. The mixing bowl method referred to by Shipstead (6) was developed by one of the major dairy companies. It is used in connection with the standard baking test for loaf volume with 6 per cent nonfat dry milk solids by the sponge dough method. The inside of the bowl is highly polished, and the test consists in observing at what water level the dough would clear from the bottom of the mixing bowl in a certain length of time. A standard sample of nonfat dry milk solids manufactured with minimum heat treatment sufficient

to overcome the weakening effect causing low loaf volume is used for comparison. This has been found by commercial baking tests to have an absorption value of six parts of extra water per six parts of nonfat dry milk solids per 100 parts of flour or an absorption ratio of 1.00. A modified mixing bowl method was developed using a smaller mixing bowl made by the National Manufacturing Co. It can be used without the subsequent baking test and with a straight dough instead of the sponge dough. Attempts were made to eliminate all other ingredients, but it was found necessary to use both yeast and salt in order to get results comparable to the original test with a complete sponge dough.

The speed of the mixer was reduced to 52 r.p.m., which gave a normal dough in 3 min. of mixing. Twelve g. of nonfat dry milk solids were mixed with 200 g. of flour and placed in the mixing bowl. A stock solution containing 40 g. of yeast cake and 40 g. of NaCl per 1,000 ml. was made up with distilled water, and 100 ml. of this solution containing about 97 g. of water were added per batch of dough. Additional amounts of distilled water varying from 45 to 55 ml. were added, depending upon the expected absorption value of the milk powder sample. If the bottom of the mixing bowl was covered with dough at the end of 2 min. of mixing, the test was repeated with decreasing amounts of water in increments of 1 ml. or more until the dough would clear the bottom in exactly 2 min. If, on the other hand, the first test had given a dough clearing the bottom in less than 2 min., then additional tests were made with increasing amounts of water until the dough would clear the bottom of the bowl in exactly 2 min. It was found important to regulate the room temperature and the temperature of the various ingredients so that the dough temperature at the end of 3 min. of mixing was about 27° C. (80° F.).

Water doughs with no addition of nonfat dry milk solids seem to behave differently from the milk doughs in the mixing bowl. Therefore, it was necessary to use a standard sample of nonfat dry milk solids of known absorption value for comparison. Commercial sample A, having an absorption value of 1.25, was used as a standard. This absorption value had been verified by tests made in a commercial bakery. The absorption values of all experimental and commercial samples tested by the modified mixing bowl method were calculated from the relative amounts of water found in comparison with the standard. For example: standard sample A required 151 ml. water to give a dough clearing the bottom of the mixing bowl in just about 2 min., whereas the experimental sample 191 required only 143 ml. water. Assuming that control sample A has an absorption value of 1.25, this means that 15 ml. of the water corresponds to 12 g. of sample A. Sample 191 takes 8 ml. less, or only 7 ml. of water which calculates to $\frac{7}{12} = 0.58$.

No. 191 is a typical low-heat sample of nonfat dry milk solids. It was heated to 63° C. for 30 min., which is normal pasteurizing requirements. Two other samples with low-heat treatment also were tested for absorption value by the mixing bowl method, and the absorption value of both samples was found to be 0.58. The soluble whey protein content and viscosity measurements of these samples compared with those of sample 191. This fact suggests the idea that a

low-heat sample of nonfat dry milk solids could be used as an additional control sample to establish the lowest point of the absorption value scale at 0.58, or three and one-half parts of extra water to six parts of nonfat dry milk solids with 100 parts of flour.

The mixing bowl method is an empirical test based on differences in adhesion to metal versus cohesion between parts of the dough at a certain stage of mixing. Control samples with known heat treatment and assumed absorption values based on commercial baking tests must be used to establish the basis for calculation of absorption values of the test samples. The reproducibility of the test depends upon maintaining a uniform temperature and the ability of the operator to judge the condition of the mixing bowl. Tests with the control samples should be made at definite intervals to check the results. The increments in changes of water addition have to be made in 1 ml. or multiples of same, in order to make sufficient difference in dough consistency to be noticed. This means that the method is not very sensitive, and absorption values are given in intervals of 0.08.

Fifty per cent Mixograph method. In the 6 per cent Mixograph test suggested by Shipstead (6), slight differences were found in the initial viscosity of the dough mixograms with various nonfat dry milk solids samples, but the maximum viscosity and time of development were not affected by absorption values over 1.0. However, when the addition of nonfat dry milk solids was increased to 18, 36 and 50 per cent on the flour basis, a marked effect was observed. A series of tests were made with three experimental samples of low, medium and high absorption values with varying levels of water additions that would give maximum viscosity values below and above 67 (chart units). Tests with a water dough showed that the flour used in these experiments would take 77.4 per cent water to give a maximum viscosity of 67. The maximum viscosity values of the various milk doughs were plotted against percentage water for each percentage nonfat dry milk solids and each sample, and from the curves obtained the per cent water, which corresponds to a maximum viscosity of 67, was estimated. By calculating the amount of water equivalent to 77.4 per cent of the flour used in each series and deducting this from the total water, it is possible to find the amount of extra water required for each sample and each percentage of milk powder to give a maximum dough viscosity of 67. The ratio of extra water to the amount of milk powder with increasing amounts of nonfat dry milk solids in the dough are then calculated. The results of these tests and calculations are given in table 2.

The absorption values of samples 191, 152 and 192 used in these tests were determined by the mixing bowl method and found to be 0.58, 0.92 and 1.33, respectively, as shown in table 3. It is evident that the calculated absorption ratios for the 50 per cent doughs made with the three samples of nonfat dry milk solids, as judged from the maximum viscosity of the doughs, are less than half of the absorption ratios with normal 6 per cent doughs, but the calculated absorption ratios of these 50 per cent doughs vary approximately in the same proportion as the absorption values with 6 per cent doughs.

It was observed that the mixograms of the 50 per cent dough test made with

TABLE 2

Calculated absorption ratios for low-, medium- and high-heat n.f.d.m.s. samples with increasing concentrations

Sample	Nonfat dry milk solids		Flour weight per test	Water to make maximum viscosity 67		Water equal to 77.4% of flour per test	Water available for milk solids per test (b)	Calculated ratio: water to milk solids $\left(\frac{b}{a}\right)$
	On flour basis	Weight per test (a)		On total flour & n.f.d.m.s. basis	Weight per test			
(no.)	(%)	*(g.)*	*(g.)*	(%)	*(g.)*	*(g.)*	*(g.)*	
191	18	6.4	35.6	72.3	30.37	27.55	2.82	0.44
152	18	6.4	35.6	75.3	31.63	27.55	4.08	0.64
192	18	6.4	35.6	77.3	32.47	27.55	4.92	0.77
191	36	11.15	30.85	63.3	26.59	23.88	2.71	0.24
152	36	11.15	30.85	68.5	28.77	23.88	4.89	0.44
192	36	11.15	30.85	72.2	30.32	23.88	6.44	0.58
191	50	14.0	28.0	59.3	24.91	21.67	3.24	0.23
152	50	14.0	28.0	63.9	26.84	21.67	5.17	0.37
192	50	14.0	28.0	69.0	28.98	21.67	7.31	0.52

a constant water addition of 75 per cent of total flour and dry milk mix and various samples of nonfat dry milk solids showed marked variations in the initial viscosity and time of development to maximum viscosity depending upon the absorption values. The total amount of flour and milk powder mixture was increased to 42 g. instead of 37.1 as used in the 6 per cent Mixograph test. This amounts to 28 g. of flour and 14 g. of nonfat dry milk solids sample, which are well mixed before adding the water. Figure 1 shows five mixograms with dry milk samples of absorption values varying from 0.58 to 1.50. Similar mixograms were made with the standard sample A and the other commercial samples. These data are tabulated in table 3, together with the results of various tests such as

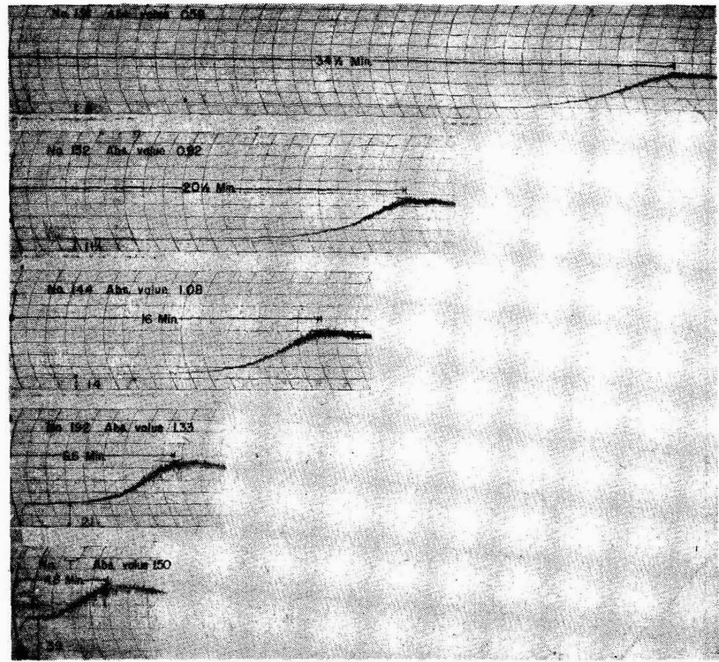

FIG. 1. Mixograms of doughs with 50% nonfat dry milk solids of varying absorption values.

total proteins, soluble whey proteins, non-protein nitrogen, the weakening angle of the 6 per cent mixograms and the viscosity of 25 per cent solutions of the nonfat dry milk solids (on a moisture-free basis). Figure 2 shows the correlation of initial viscosity, and figure 3 shows the correlation of time of development with the absorption value as determined by the 6 per cent mixing bowl test. Smooth curves have been drawn through the values for the four experimental control samples. The commercial samples fall reasonably close to this calibration curve, which indicates that the 50 per cent Mixograph test is fairly reliable for measuring the absorption value of nonfat dry milk solids. However, there seems to be less correlation at very high absorption values, probably due to consider-

able variations in viscosity of samples. In routine tests of a large number of samples, it might be advisable to stop the test after 3 or 4 min. if the initial viscosity is only 12 or less, since this indicates a relatively low absorption value and time of development would probably be about 18 min. (which means that the test would take about 22 min. to complete). Such samples could be marked unsatisfactory, and a more complete test made later on.

It is very important to maintain a fairly constant temperature not varying more than ± 1° C. from that at which the test was calibrated. If the room tem-

TABLE 3

Correlation of Mixograph tests with soluble whey protein, viscosity and absorption values of various n.f.d.m.s. samples in 6% bread dough

Samples		Total[a] protein	Soluble[a] whey protein	Non-protein[a] nitrogen	Absorption value by mixing bowl test	6% Mixograph test weakening angle	50% Mixograph test: Initial viscosity	50% Mixograph test: Time to maximum viscosity	Viscosity of 25%[b] solution at 23.9° C.
	(no.)	*(%)*	*(%)*	*(%)*	*Ratio: water to n.f.d.m.s.*	*(degrees)*	*(chart units)*	*(min.)*	*(centipoises)*
Standard	A	33.5	1.14	0.32	1.25	16.5	15	14	7.3
Experimental Control	191	33.2	6.25	0.40	0.58	30	8	34.5	5.0
	152	34.85	3.05	0.43	0.92	22	11.5	20.5	5.9
	144	34.3	1.92	0.36	1.08	17.5	14	16	6.4
	192	33.6	1.47	0.39	1.33	18.5	21	8.6	17.1
Commercial	H[c]	33.1	4.75	0.31	0.67	28	9	29.5	4.7
	M[d]	35.4	5.54	0.39	0.67	31	10	24.5	5.5
	N[d]	33.5	2.80	0.43	0.92	26.5	11.5	21	6.0
	L	35.0	1.68	0.42	1.08	18.5	14	14	6.1
	O	33.5	1.14	0.32	1.17	18.5	14	15	6.6
	E[e]	34.6	1.04	0.30	1.17	20.5	16	12	7.3
	P	34.3	1.51	0.39	1.25	15	20	6.7	20.5
	I	39.2	1.33	0.40	1.33	16	23.5	6.1	11.8
	J	39.0	1.95	0.30	1.33	19.5	29	5.3	53
	K	41.0	1.88	0.30	1.42	18	34	4.7	25
	T	41.3	1.86	0.27	1.50	18	39	4.8	20

[a] On moisture-free basis.
[b] 25% moisture-free solids.
[c] Low-heat treatment indicated by manufacturer.
[d] Insufficient heat treatment indicated by manufacturer.
[e] High-heat treatment indicated by manufacturer.

perature cannot be controlled satisfactorily during the hot season, it might be advisable to make new calibration tests with the standard control samples with low and high absorption values at a higher temperature. Table 4 gives the results of tests on two samples at three different temperatures, which show the effect of temperature variations on the initial viscosity and time of development.

The initial viscosity is measured about 2 to 3 min. after start. With samples of very low-heat treatment, which give mixograms with low initial viscosity, it is important to push the recording arm up to a reading of about 30 to 40 chart units and let it come down slowly to find its level from above. Otherwise, ex-

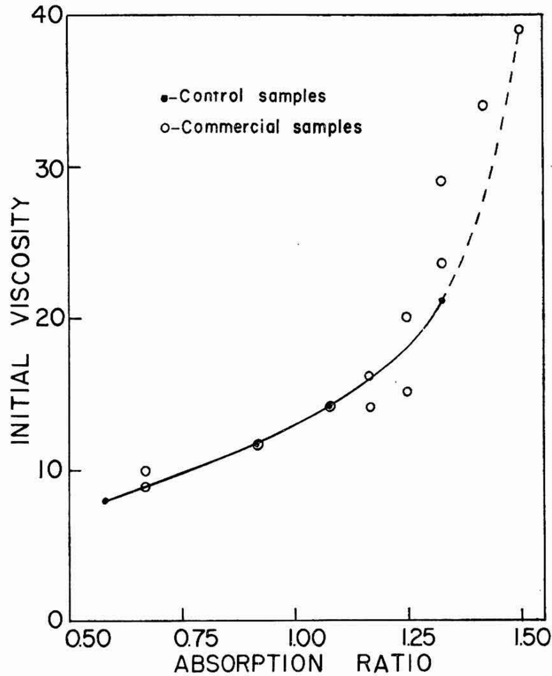

FIG. 2. Correlation of absorption values with initial viscosity of 50% Mixograph test.

tremely low values will be obtained due to a certain friction at the low readings holding back the upward movement of the arm.

The time of development to maximum viscosity is found by drawing lines through the middle of the ascending and descending parts of the mixogram, as shown in figure 1. Where these two lines intersect is the theoretical maximum point, according to Swanson and Johnson (9). The distance of this point from the starting line in chart units gives the time of developing in minutes and fractions thereof.

Viscosity of reconstituted milk. The viscosity measurements were made with solutions of reconstituted nonfat dry milk solids samples containing 25 per cent

TABLE 4

Effect of temperature on 50% Mixogram data

Temperature of room and ingredients		Sample #191			Sample #192		
		Initial viscosity	Time of development	Maximum viscosity	Initial viscosity	Time of development	Maximum viscosity
(° C.)	(° F.)	*(chart units)*	*(min.)*	*(chart units)*	*(chart units)*	*(min.)*	*(chart units)*
21	70	8	56.5	48.5	19	11.7	59
26.5	80	8	34.5	40.5	21	8.6	54
32	90	8	26	33	20.5	7.4	45

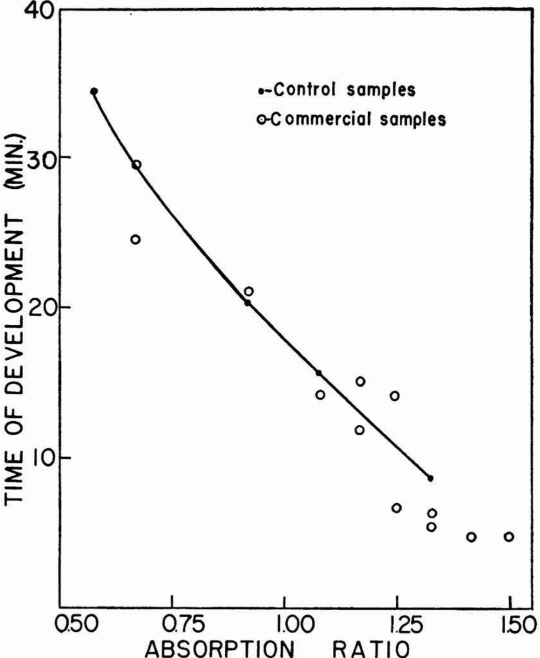

FIG. 3. Correlation of absorption values with time of development of 50% Mixograph test.

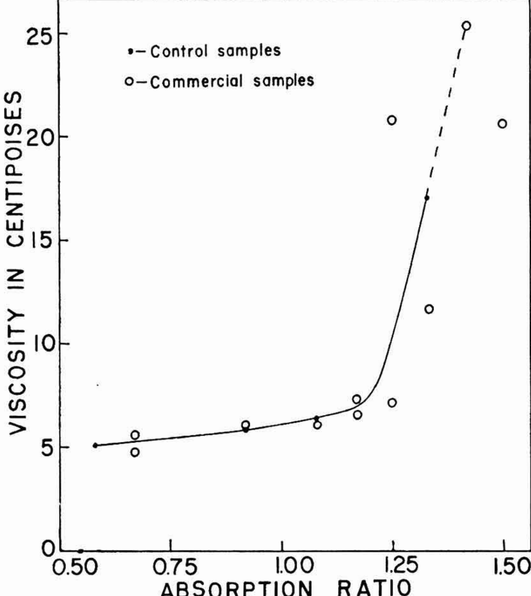

FIG. 4. Correlation of absorption values with viscosity of reconstituted 25% solutions of nonfat dry milk solids.

moisture-free solids. As a standard of viscosity, a 40 per cent sucrose solution was used, and all tests were made at 23.9° C. (75° F.). It was convenient to use an Ostwald-type viscometer measuring the flowtime. It should have a capillary sufficiently long and with a diameter of bore so that the flowtime of the sucrose solution at 23.9° C. will be about 120 sec., in order to reduce the kinetic flow error in calculating the centipoise values. The viscosity of the 40 per cent sucrose solution at 23.9° C. is taken as 5.41 centipoises.

In figure 4 the viscosity values of the control samples and the commercial samples are plotted against the absorption values. There is a very good correlation up to an absorption value of 1.20. For the higher absorption values the viscosity values vary considerably above and below the curve drawn through the points for the control samples. This shows that a sample of nonfat dry milk solids like J might have a very high viscosity without necessarily showing a correspondingly high absorption value. On the other hand, the sample T, with a total protein content of 41.3 per cent and an absorption value of 1.50, had a viscosity of only 20 centipoises. This indicates that there are qualities in milk proteins, other than viscosity, developed by heat treatment, which determine the effect on dough consistency. High initial viscosity of a 50 per cent dough and short time of development of the gluten structure undoubtedly are due to certain qualities of the milk proteins that favor high absorption values.

DISCUSSION

Soon after the preliminary Mixograph tests with increased concentration of nonfat dry milk solids in dough were undertaken, which indicated the possibility of using it as a test for absorption value, the paper by Hoffman *et al.* (2) was brought to the authors' attention. This paper deals with the evaluation of the baking properties of roller-process nonfat dry milk solids by a Farinograph procedure. They state that this method probably could be used for spray-process nonfat dry milk solids, but that a lower level of water would have to be used and a different rating worked out. Roller-process samples of nonfat dry milk solids usually have a relatively high-heat treatment and it is difficult to explain the relatively high percentage of soluble whey protein nitrogen of 5.37 to 10.63 per cent of total nitrogen. These values correspond to approximately 1.9 and 3.8 per cent soluble whey proteins on a dry solids basis for average total protein content. Possibly some roller-process milk powders might show partial degradation of proteins, which probably would result in a weaker gluten structure in the dough.

The advantage of the 50 per cent Mixograph test described above is that it is objective, eliminating the personal opinion of the technician. The test is recorded on graph paper, and the values for initial viscosity and time of development are taken from the mixograms. The mixograms serve as a permanent record. Low-heat samples causing low loaf volume will always give low initial viscosity and long time of development. As supplementary tests, the 6 per cent Mixograph test (6) and the measurement of viscosity of a 25 per cent reconstituted sample are recommended. The Mixograph should preferably be equipped with a ther-

mostatically controlled cabinet or be placed in a laboratory maintained at a constant temperature. It is advisable to have two identical mixing bowls to be used alternately. In that way about six to eight samples can be tested per hour, depending upon the absorption value. It is very important to have a fairly large quantity of a standard control sample of nonfat dry milk solids with a high absorption value. A low-heat control sample with an absorption value of 0.58 is also advisable. Tests with these standard samples should be run daily to check the calibration. The flour should be medium strong with about 12.5 per cent protein and should be well mixed for uniformity and kept in a metal container to maintain a constant moisture content. The water may be delivered from a burette or from a special automatic delivery pipette adjusted to a volume of water which will give reasonable mixograms with samples of high absorption values.

Although it might be desirable to check the baking quality of nonfat dry milk solids by actually baking loaves of bread in an experimental laboratory and measuring the loaf volume, reliable information probably can be obtained by simply testing the effect of nonfat dry milk solids on the bread dough, leaving out part or all of the other customary ingredients. The explanation for this is that a low loaf volume is caused by the weakening action of insufficiently heated milk solids on the gluten structure, and this action can be measured by suitable tests on the dough without actually baking a loaf of bread. As regards absorption value, a baker can tell by handling a dough whether or not he has added the optimum amount of water, but his judgment is based on long experience and constitutes an art rather than scientific measurement. A series of baking tests with increasing amounts of water will give information when judged at various steps of the baking procedure as to the maximum water which can safely be added, but again it is mainly dependent upon the personal judgment of an experienced baker what this maximum water amounts to, and such tests are very time consuming.

SUMMARY

Two methods for determination of the absorption value of nonfat dry milk solids in a 6 per cent bread dough are described. The modified mixing bowl method is recommended for use without subsequent baking tests for loaf volume. It determines the absorption value of samples of nonfat dry milk solids in comparison with control samples of known absorption value. It is suggested that low-heat nonfat dry milk solids be used as one of the controls to establish the lowest point of the absorption value scale at 0.58 or three and one-half parts of extra water to six parts of nonfat dry milk solids with 100 parts of flour. Other control samples with absorption values determined by other laboratories or preferably by tests in a commercial bakery should be used for checking the mixing bowl test. The absorption value as determined by the mixing bowl method is accurate probably only to approximately $\pm$ 0.05, and the results are recorded in increments of 0.08. The 50 per cent Mixograph method is recommended for routine tests of nonfat dry milk solids samples to check the effect of the heat

treatment on the absorption value. It is a purely empirical test based on absorption values obtained with the modified mixing bowl method. Calibration curves obtained by testing control samples are presented to show the correlation of initial viscosity and time of development with the absorption value as determined by the modified mixing bowl method.

There is a fairly good correlation between viscosities of 25 per cent solutions of nonfat dry milk solids samples and absorption values up to 1.20, but above that the viscosities vary considerably with samples of the same absorption value. Constant temperature of the room and ingredients ($\pm 1°$ C.) and a uniform composition of the flour used are very important factors in reproducibility of both methods. The calibration curves all refer to spray-process nonfat dry milk solids samples. Similar calibration curves for roller-process samples probably can be made with higher water levels.

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INHIBITION OF *STREPTOCOCCUS LACTIS* IN MILK BY FATTY ACIDS[1]

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The inhibitory action of rancid milk on the growth of *Streptococcus lactis* has been confirmed by Tarassuk and Smith (8). Costilow and Speck (2) have shown recently that such milk also inhibits the growth of a number of other microörganisms. The latter workers noted that the inhibition of bacterial growth was not due to slightly lowered pH, low surface tension of rancid milk or to any activity of lipase upon the bacterial cell. The inhibition of *S. lactis* was related directly to the degree of lipolysis which had occurred in milk and thus probably to some component of rancid milk. Owing to the increased concentration of free fatty acids in rancid milk, attention was directed to the effect of these compounds upon the growth of *S. lactis*.

METHODS

The fatty acids used were purchased commercially in as chemically pure a form as possible. These were dissolved in ethyl alcohol and added to homogenized milk before autoclaving. The fatty acids were prepared in a concentration so that only 1 per cent ethyl alcohol was added to the milk to be cultured. One per cent of the alcohol alone was added to the control samples to compensate for any possible effects of residual alcohol in the sterile sample.

The procedures for carrying the culture of *S. lactis* H-1-10, plating the samples and the measurement of pH and surface tension have been reported earlier (2).

RESULTS AND DISCUSSION

All of the fatty acids known to occur in milk fat (5) plus linolenic acid were observed for their effect upon the growth of *S. lactis* by culturing this organism in milk containing the individual acids. Lacking information on the actual concentrations of these acids present in rancid milk, an arbitrary concentration of 0.1 per cent of each acid was used unless otherwise specified. This concentration of any one acid undoubtedly exceeded that present in rancid milk, but it was expected to reveal more clearly the toxicity or non-toxicity of the individual fatty acids.

The acids which were observed to have no effect upon *S. lactis* were oleic, butyric and 0.05 per cent caproic[2] (fig. 1, 2); linoleic, linolenic and arachidic (fig. 3, 4); palmitic (fig. 5); and 0.5 per cent stearic (fig. 6, 7). Thus, neither

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[2] As 0.075 per cent caproic acid caused coagulation of the milk upon autoclaving, the concentration was reduced. Butyric acid caused coagulation at the 0.05 per cent level, but, since it is a water-soluble acid, a sterile solution was added aseptically to the sterilized milk.

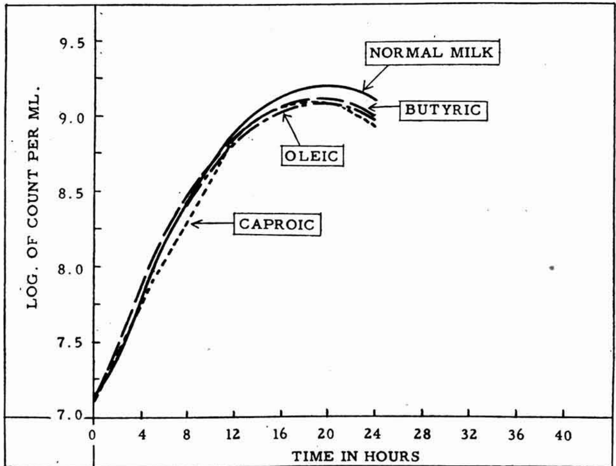

FIG. 1. The growth of *S. lactis* in milk containing oleic acid (0.1%), butyric acid (0.1%), and caproic acid (0.05%).

the low-molecular-weight fatty acids (butyric and caproic) nor the acids of higher molecular weights (palmitic, stearic, oleic, linoleic and linolenic) showed any inhibitory effect. In contrast, the intermediate acids, caprylic, capric and lauric, inhibited the growth of *S. lactis* (fig. 5). The inhibitory effect of myristic acid was somewhat doubtful even at the 0.5 per cent level (fig. 6, 7).

As the concentrations of capric and lauric acids were increased, their respective inhibitory effects also increased (fig. 6, 7). A concentration of 0.5 per cent capric acid almost completely inhibited growth of *S. lactis,* and the count actually

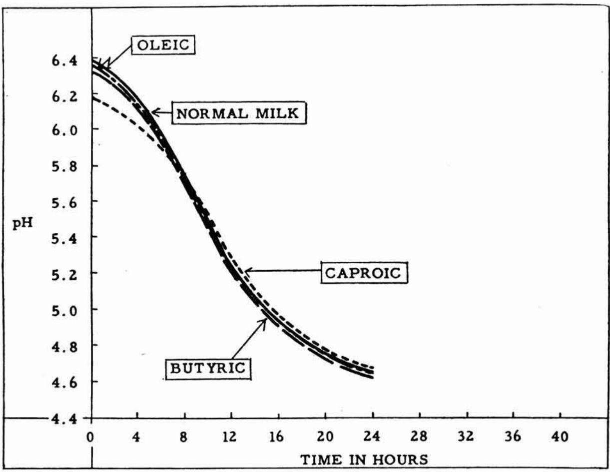

FIG. 2. The acid production by *S. lactis* in milk containing oleic acid (0.1%), butyric acid (0.1%) and caproic acid (0.05%).

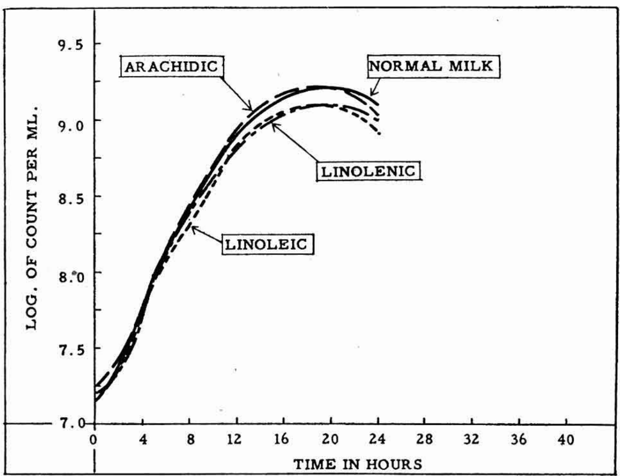

FIG. 3. The growth of *S. lactis* in milk containing 0.1% linoleic, linolenic and arachidic acids.

declined after 5 days; this sample failed to coagulate on continued incubation. A concentration of 0.5 per cent lauric acid delayed the development of the culture almost entirely for 11 hr., after which time growth proceeded fairly rapidly, and the milk coagulated within 36 hr.

Various reports dealing with the effects of fatty acids on the growth of different lactic acid bacteria in synthetic or semi-synthetic media have demonstrated toxicity of unsaturated fatty acids. However, the presence of lecithin, cholesterol or serum albumin eliminated the toxicity (3, 6, 9), and addition of oleic

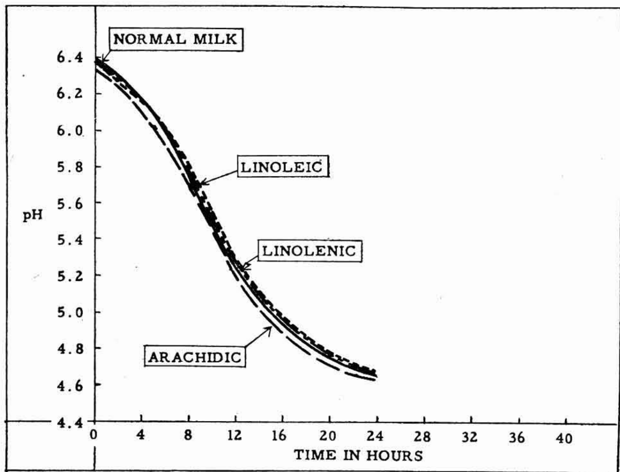

FIG. 4. The acid production by *S. lactis* in milk containing 0.1% linoleic, linolenic and arachidic acids.

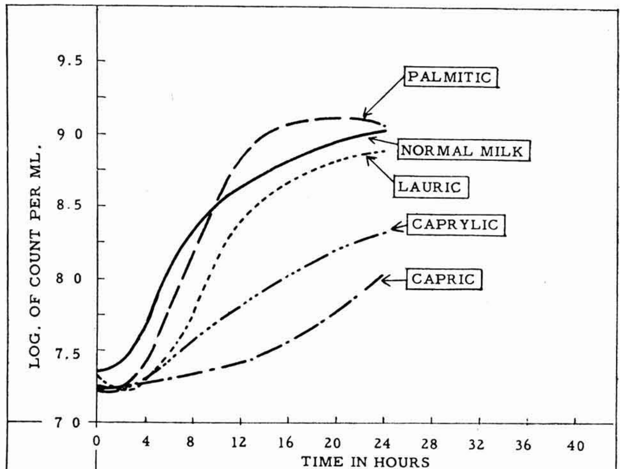

FIG. 5. The growth of *S. lactis* in milk containing 0.1% of palmitic, lauric, capric and caprylic acids.

acid in a combined form was stimulatory (1) or essential for growth in the absence of biotin (10). As milk contains cholesterol and lecithin, the observations that oleic, linoleic and linolenic acids were non-inhibitory for *S. lactis* in milk were anticipated.

There appears to be a critical relationship between the carbon chain length and the degree of the inhibitory property. Thus, capric acid was the most inhibitory, caprylic acid next and lauric acid the least inhibitory of the three. Hardwick *et al.* (4) noted a similar relationship in the carbon chain length of

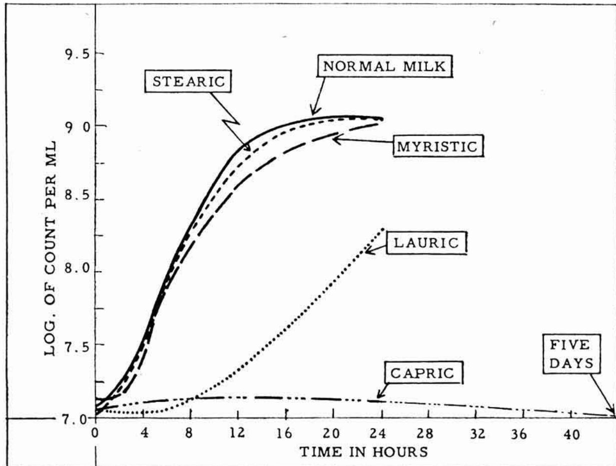

FIG. 6. The growth of *S. lactis* in milk containing 0.5% stearic, myristic, lauric and capric acids.

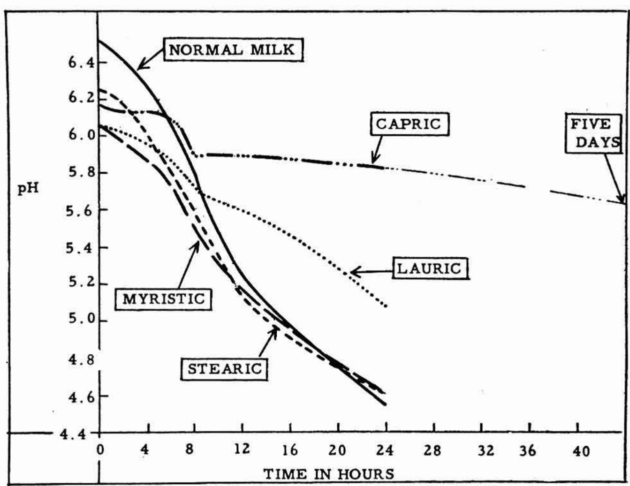

FIG. 7. The acid production by *S. lactis* in milk containing 0.5% of stearic, myristic, lauric and capric acids.

fatty acids to their antisporulation and growth inhibition activity on certain bacteria, with the C_{10} to C_{14} fatty acids having the greatest effect. Salts of capric, lauric and myristic acids also have been found to be toxic to resting cells of *Escherichia coli* (7). This toxicity for *E. coli* was considered to be the result of the accumulation of these diffusable compounds within the cell resulting from the inability of the organism to use them anaerobically. Cells stored aerobically were unaffected. Tarrasuk and Smith (8) also noted the inhibition by lauric

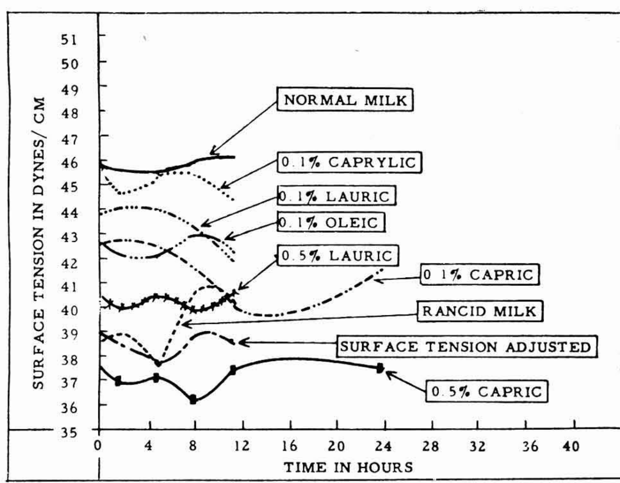

FIG. 8 The surface tension of cultures of *S. lactis* in normal milk, rancid milk, normal milk containing "Tween" 40 and normal milk containing various fatty acids.

acid and diglycol laurate of *S. lactis* in milk and attributed the inhibitory property of these compounds to their depression of the surface tension.

In the present study surface tension of the milk containing the fatty acids failed to correlate with the degree of inhibition of the growth of *S. lactis* (fig. 8). In addition to data for milk cultures containing various fatty acids, results also are included for cultures in normal milk with the surface tension lowered with "Tween" 40, in rancid milk and in normal milk. Previous work (2) had shown that rancid milk was inhibitory to *S. lactis,* while normal milk with its surface tension lowered with "Tween" 40 to the level of that of the rancid milk was not inhibitory. Further evidence of the independence of inhibition from surface tension can be noted from the cultures containing fatty acids. Milk containing 0.1 per cent oleic acid, which showed no inhibition, had a surface tension as low or lower than milk containing the same concentration of lauric or capric acid, each of which showed marked inhibition of *S. lactis.* Milk containing 0.1 per cent caprylic acid showed definite toxicity, whereas the surface tension of this milk was essentially the same as normal milk. These and similar observations indicate that the inhibition of *S. lactis* in milk by fatty acids is caused by some factor(s) other than the surface tension of the medium.

SUMMARY

In an effort to determine the cause of the inhibitory property of rancid milk on *S. lactis* all of the fatty acids common to milk fat were tested for their effect upon the growth of this organism. Caprylic, capric and lauric acids, inhibited growth of *S. lactis;* the degree of inhibition increased as the concentration of the acid increased. The effect of myristic acid was doubtful, and none of the other acids tested was found to inhibit the growth of this organism.

The inhibitory property of rancid milk and that observed in milk containing fatty acids is not the result of the low surface tension of the milk, but is due to some other unexplained toxic effect of the individual fatty acids.

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EFFECTS OF STORAGE TEMPERATURE ON PROPERTIES OF EVAPORATED MILK

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The physical condition of evaporated milk generally remains acceptable during the interval required to move it from manufacturer to consumer. However, it has long been recognized that high temperatures and long periods of storage cause undesirable changes. This study was made to evaluate quantitatively the effect of variations in the time and temperature of storage of evaporated milk on its viscosity, pH and color, and on the distribution of its fat and protein phases.

EXPERIMENTAL

Much experimental work was done with samples of milk prepared in these laboratories. Data obtained from the laboratory milks were confirmed by experiments with commercial samples. Results of storage tests on one of the commercial milks (no. 429), being typical, will be reported here in some detail. Most of the commercial and laboratory samples were sterilized in batch-type sterilizers, in contrast to the continuous machines which now are being used extensively.

Evaporated milk no. 429 was manufactured May 6, 1946, in a large Wisconsin condensery that was completely fitted with stainless steel equipment. The milk was of grade A quality. It was forewarmed to 195° F. for about 10 min. and concentrated in a single effect pan. Ten oz. of anhydrous Na_2HPO_4 per 1,000 lb. of evaporated milk were used as a stabilizer. The 14.5-oz. cans of milk were sterilized in a Fort Wayne sterilizer at 240° F. for 15 min. with the reel running for the first 10 min. Two cases of milk were removed from the same sterilizer rack and shipped to Washington, D. C., where they were received May 22. Batch 429 was taken from a regular factory run and was typical of the best quality commercial evaporated milk. Batches 882 and 935 were made in the same plant and under substantially the same conditions.

Evaporated milk samples were stored in rooms in which the temperature variation usually was within ± 1.5° F. The cans of milk were not moved or disturbed in any way during the storage period. Relative viscosity was determined on a MacMichael viscosimeter, using standardized wires (4). The tests were made in a room held at 86° F. The samples were placed in this room several hours before determinations were made. A Beckman meter was used for pH measurements. Color was determined by visual comparison with color standards evaluated in numerical terms according to the Munsell system and

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numbered from 1 to 12 in order of increasing color (5) or by direct comparison with standardized Munsell color sheets.

Separation of the fat and protein in the stored evaporated milk samples was determined by modification of an earlier method of freezing the samples and slicing and analyzing sections of the frozen cake (6). Upon conclusion of the storage period, cans to be examined were removed carefully, without tipping, and immersed in an alcohol bath held below – 25° F. with dry ice. A series of preliminary determinations showed that slower freezing in air or even in an alcohol bath at 0° F. permitted some migration of constituents and resulted in small discrepancies in the results. These discrepancies were eliminated when samples were frozen in alcohol at – 25° F., and held at about 0° F. until the cans were opened, and the frozen cake of milk was sawed into three parts of equal weight for analysis.

Fat was determined by the Mojonnier modification of the Rose-Gottlieb test. Micro-Kjeldahl determinations gave nitrogen values which were converted to protein by multiplying by 6.38.

RESULTS

Changes in the viscosity, pH and color of evaporated milk 429 during storage for 2 yr. at temperatures from 34 to 98° F. are shown in figure 1. The viscosity of the milk when it was received varied from 80 to 90 centipoises.

Measurements on many samples of batch-sterilized milk showed that a variation of 10 to 15 per cent in the viscosities of samples from cans from the same batch was common. This variation occurred because milk heated in a batch autoclave could be brought to the point of incipient coagulation with a minimum of agitation. At this point the viscosity increase was so rapid that differences in body between samples of the same batch were not unusual. Variations in the viscosity of fresh evaporated milk were minimized whenever the milk was of very high heat stability and the sterilization process did not bring it near its coagulation point or when sterilization was accompanied by constant agitation. These conditions were sometimes found during continuous sterilization and this explains in part why continuously processed milks often were more uniform but thinner in body than batch-processed milks.

The rate of change in viscosity, pH, and color is shown in figure 2. The reciprocals of the storage times in days required to produce definite changes in viscosity, pH and color have been plotted against the corresponding temperatures of storage.

The viscosity of evaporated milk has been shown to decrease during early storage to a minimum value (2). The effect of storage temperature on the attainment of this minimum viscosity value as determined on three milks of commercial origin is shown in figure 3. The points on the figure represent the average minimum viscosities reached by each milk during 1-yr. storage at the indicated temperatures. At high storage temperatures, particularly at 98° F., the low basic viscosity of evaporated milk was reached during the first few weeks

of storage. Thereafter, an increase in viscosity and even gelation often occurred.

Figure 3 shows that at temperatures from 34 to 50 or 60° F., the viscosity of evaporated milk remained relatively stable. At 70° F. or above, the milks dropped to low basic viscosity levels and this was conducive to the rapid onset of other physical changes. The most effective means of retarding phase separation and formation of an objectionable cream layer in evaporated milk was by maintenance of a high viscosity level.

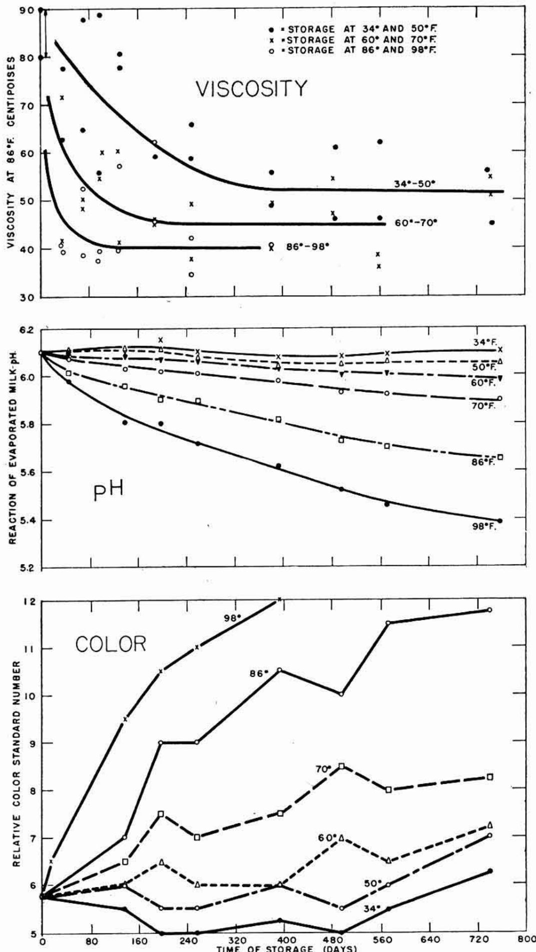

FIG. 1. The effect of time and temperature of storage on the viscosity, pH and color of a commercial evaporated milk (Batch 429).

The rate of decrease in the pH value of milk 429 varied directly with the temperature of storage. Determination of the pH of samples of evaporated milk appeared to give a rapid and reliable indication of their storage history. Fresh

evaporated milks usually had a reaction of about pH 6.1. The different milks under observation remained in good condition during storage until their reactions were below pH 6.0. When the milks examined reached pH 5.9 or less they no longer were acceptable. Measurements of pH were used as a means of determining if phase separation in an evaporated milk was a result of improper manufacture or of high temperature or long time storage. It was considered that a carefully prepared milk of good body should not show undesirable physical changes during storage until it became more acid than pH 6.0. If there was phase separation and if the reaction was pH 5.95 or lower, the state of the milk

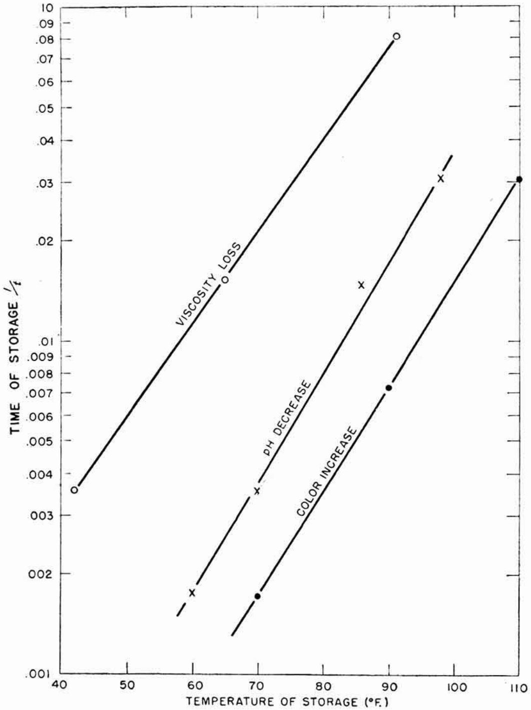

FIG. 2. Rate of change of evaporated milk no. 429 during storage at different temperatures. For viscosity, t = days for viscosity to drop from 85 to 55 c.p. For pH, t = days to reach pH 6.0. For color, t = days to reach color standard no. 8.

was considered to be caused by unfavorable storage conditions. Evaporated milks of much lower viscosity than milk 429 may show objectionable fat separation when they reach pH 6.0.

The effect of processing conditions on the color of evaporated milk has been studied previously (5, 1, 3). The data of figures 1 and 2 and table 1 show the effect of time and temperature of storage on color development in milk 429. The rate of color increase with temperature of storage parallels the loss in viscosity and the decrease in pH.

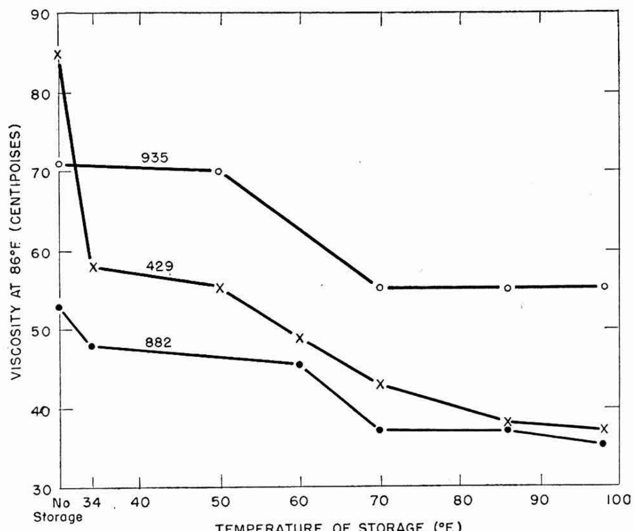

FIG. 3. The average minimum viscosities attained by three commercial evaporated milks during storage at various temperatures for 1 yr.

After milk 429 had been held in undisturbed storage for 757 days at various temperatures, samples were examined for fat and protein separation. The results are given in figure 4. There was no separation of either phase at temperatures below 50° F. and separation was slight at 60° F. Above 70° F. the cream layer was deep and it could not be satisfactorily redispersed by ordinary means. The fat globules were held together by adsorbed protein, which in turn had become denatured. Additional denatured protein settled to the bottom of the cans. As this precipitate increased, the high localized concentration of protein was conducive to gel formation.

TABLE 1

Munsell color notations on evaporated milk 429 after storage at various temperatures

Temperature of storage[a]	Munsell notation					
	555 days			741 days		
(° *F.*)	*Hue*	*Value*[b]	*Chroma*[c]	*Hue*	*Value*	*Chroma*
34	2.5Y[d]	8.0	2.5	2.5Y	7.9	3.0
50	"	8.0	2.9	"	7.8 +	3.4
60	"	7.9	3.0	"	7.8 –	3.5
72	"	7.9	3.1	"	7.7	3.7
86	"	7.8	4.0	"	7.3	4.2
98	"	7.2	4.5	"	7.0	4.7

[a] Color before storage: hue = 2.5Y, value = 8.0, chroma = 2.3.
[b] In the value scale black = 0, white = 10.
[c] No chroma = 0, strong chroma = 10.
[d] Y = yellow.

The insoluble top and bottom layers in aged evaporated milk could be broken sharply like a rennet curd. When the milk was stirred, these layers became non-dispersible lumps. The top third of the milk in the can contained slightly more protein than the bottom third. The protein in the top portion was evidently largely adsorbed on the fat and had been carried up with it. Only a

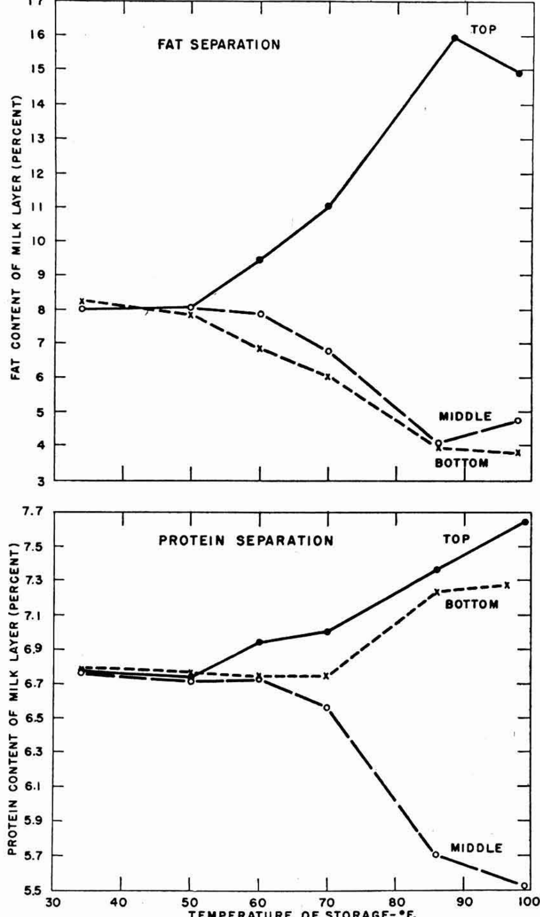

FIG. 4. The separation of fat and protein in normal evaporated milk (Batch 429) held in undisturbed storage for 757 days at different temperatures. The evaporated milk contained 8.06% fat, 6.77% protein, and 26.15% total solids. After storage, fat and protein determinations were made on the contents in the top, middle and bottom third of each can.

small quantity of fat was associated with the protein in the middle and bottom layers.

If the fat in evaporated milk could be further subdivided to provide new adsorption surfaces for the protein which now eventually settles to the bottom,

increased stability would be attained. By intimate binding together of finely dispersed protein and fat to form a particle of about the same density as the milk serum, it might be possible to maintain a relatively stable dispersion. With such a balance, separation would be retarded regardless of the viscosity of the milk.

SUMMARY

Quantitative determination of the deterioration of evaporated milk during storage showed that the pH and viscosity decrease and the color increases with an increase in the temperature of storage. Commercial evaporated milk of heavy body remained acceptable for 2 yr. when held in undisturbed storage below 60° F. Physical deterioration was rapid during storage above 70°.

Determination of the reaction of evaporated milk afforded a means for evaluating its previous storage conditions. The reaction of fresh evaporated milk was about pH 6.1. The product remained of good quality until the reaction became more acid than pH 6.0. The product was unacceptable when it was more acid than pH 5.9.

Separation of fat and protein in a commercial evaporated milk of heavy body was negligible during storage for 2 yr. below 60° F. At higher temperatures, protein not associated with milk fat gradually settled and the precipitate thus formed became an insoluble gel. The fraction of protein that was adsorbed on the fat phase slowly rose with the fat, and redispersion of this gel-like fat layer became almost impossible.

It is suggested that to attain improved stability in evaporated milk, the fat globules might be further subdivided to create new surfaces for adsorption of protein. If very small fat-protein particles could be formed, a balance might be obtained which would retard separation regardless of the viscosity of the milk.

The results presented here were from a single batch of commercial evaporated milk but this was considered to be representative of a large group of laboratory and commercial milks that were studied. Milks manufactured under a variety of conditions, particularly those sterilized by continuous methods, could be expected to yield data in general, but not in close agreement with the results reported here. Since this work indicates the nature of the changes through which all evaporated milks pass during storage it may serve as a guide for additional studies on a wide variety of samples.

ACKNOWLEDGMENT

The authors wish to express their appreciation to P. L. Haymes, A. R. Davis and other executives of United Milk Products Co. for their interest in this work and to thank them for furnishing ample supplies of commercially produced evaporated milk.

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INHIBITORY EFFECT OF RANCID MILK ON CERTAIN BACTERIA[1]

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Owners of one to three dairy cows occasionally encounter failure of raw milk to sour normally. The milk has a rancid flavor shortly after removal from the mammary gland, and the surface tension drops to 30 to 35 dynes per centimeter within several hours as a result of lipolytic activity. In such milk acid formation is delayed or even completely inhibited by the repression of the natural flora of lactic acid bacteria or of added *Streptococcus lactis*. Pasteurization of the milk within 30 min. after milking, followed by inoculation with *S. lactis,* results in normal growth and acid development by the culture. Previous studies have shown that rancid milk is inhibitory to *S. lactis*. Tarassuk and Smith (10) have reviewed earlier studies on this subject and have confirmed the previous observations that milk which has undergone lipolysis is inhibitory to this organism.

The cause of such inhibition has not been clearly defined. Tarassuk and Smith (10) attributed it to the low surface tension of rancid milk and based their conclusions upon a comparison of the effect of rancid milk with milk containing diglycol laurate, or lauric acid, both of which are surface tension depressants. Since lauric acid is one of the fatty acids which would be liberated in rancid milk and diglycol laurate is an ester of the same acid, and since it was not demonstrated that these two compounds are not otherwise toxic to *S. lactis,* the explanation of the inhibition based on low surface tension appeared questionable.

This investigation was undertaken to demonstrate the effect of rancid milk upon a number of bacteria common to milk and milk products and to study the cause of such inhibition.

EXPERIMENTAL

Milk samples from several animals in the College herd were tested for tributyrinase activity by the method of Peterson *et al.* (6), and the milk from one cow which consistently was high in lipolytic activity was used. The milk was cooled to at least 5° C. immediately. One portion to be used for a control sample was heated to 76.5° C. in a water bath within the first hour after milking to destroy the lipase; the remaining portion was not pasteurized. Both heated and unheated portions were warmed to 30° C., homogenized at 2,500 lb. per sq. inch pressure, cooled to 10° C. and allowed to remain in a refrigerator at 70° C. for at least 16 hr. The milk then was tempered to 20° C. and its surface tension measured with a Cenco-du-Nuoy tensiometer to ascertain the degree of lipolysis

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in the unheated portion. Each lot was dispensed in 250-ml. Erlenmeyer flasks, using 150 ml. of milk per flask. Flasks were stoppered with cotton plugs, covered with aluminum foil and with parchment and sterilized by autoclaving at 15 lb. pressure (121° C.) for 20 min. These samples were stored at refrigerator temperature until used.

The cultures selected for observation of their growth in rancid milk were: *S. lactis, Streptococcus zymogenes, Streptococcus bovis, Lactobacillus bulgaricus, Lactobacillus casei, Escherichia coli,* and *Pseudomonas aeruginosa.* Stock cultures were transferred weekly in litmus milk, using about a 1 per cent inoculum at each transfer. The *S. lactis* cultures were incubated at 21° C. and all others at 35 to 37° C. for 18 to 20 hr. Eighteen-hr. skimmilk sub-cultures were prepared and 1 per cent used for inoculating the experimental samples. Periodically the samples were examined for (a) plate count, (b) surface tension, using the Cenco-du-Nuoy tensiometer with samples adjusted to 20° C. and (c) pH, using a Beckman pH meter.

The samples containing the streptococci were plated with trypticase soy agar (BBL); those containing lactobacilli with trypticase sugar agar (BBL); and samples with *E. coli* and *Ps. aeruginosa* with tryptone glucose meat extract agar (Difco). Plates from the *S. lactis* samples were incubated at 21° C. and those for all other organisms were incubated at 35° C.

RESULTS

The original plan was to use milk that became rancid spontaneously, particularly since preliminary observations had indicated a considerably more severe inhibition of *S. lactis* in such milk than had been observed by Tarassuk and Smith (10). Lacking this milk, the studies have dealt with less rancid milk in which lipolysis was induced by means of homogenization and heat activation of the lipase.

Effect of Rancid Milk and of Milk Containing a Surface Tension Depressant upon the Growth of Bacteria

Four different samples of milk were used in each experiment with each organism tested. Sample no. 1 in each was a normal milk control; no. 2, a control in which the pH of the normal milk was adjusted with lactic acid to approximately that of the rancid sample; no. 3, a control with the surface tension of the normal milk depressed by use of "Tween" 40[2]; and no. 4, the rancid sample. A concentration of 1.7 per cent of the "Tween" compound was used in control no. 3, as this was found to give about the maxium reduction in surface tension and no toxicity of this compound was noted.

Effect on Streptococcus lactis. Two strains of *S. lactis* (H and H-1-10) were used in duplicate experiments using a 1 per cent inoculum and another using a 0.01 per cent inoculum of strain H-1-10. Particular emphasis was placed on this species due to its great importance in the dairy industry. However, since the

[2] "Tween" 40 is a polyoxyethylene derivative of sorbitan esterified with palmitic acid.

results of all four experiments were very similar, the data of only one experiment will be presented.

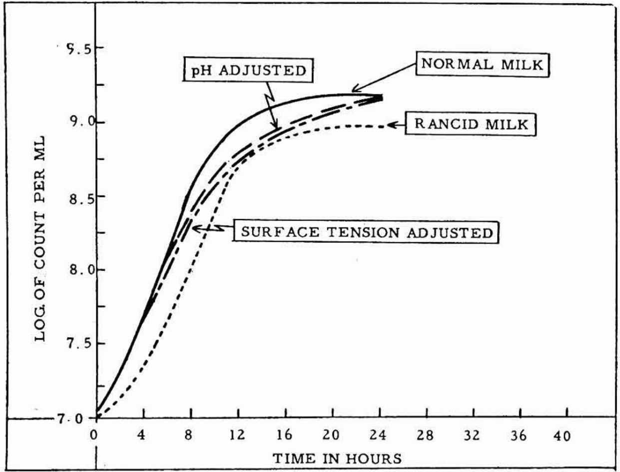

FIG. 1. The effect of rancid milk on the growth of *S. lactis* (No. H-1-10).

The effect of the rancid milk upon the growth of *S. lactis* was very pronounced, as may be noted in fig. 1. This strain was very active and the lag

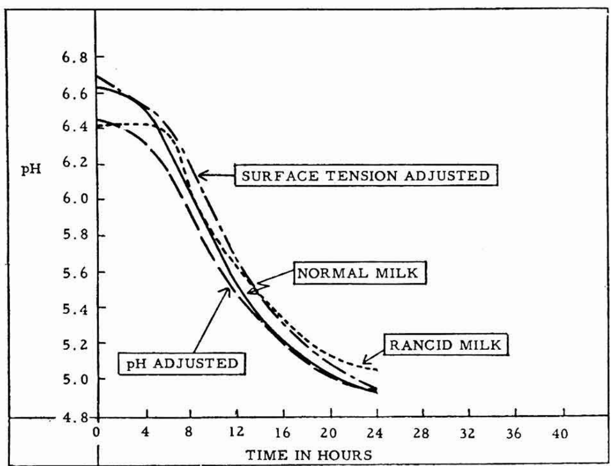

FIG. 2. The effect of rancid milk on the acid production by *S. lactis* (No. H-1-10).

phase of growth was hardly perceptible in the control samples, while growth was delayed to some extent for 5 hr. in the rancid milk sample. The rate of growth of the rancid-milk culture was approximately equal to that of the control during the logarithmic growth phase, but decreased thereafter, the final count being only about 60 per cent of that of the normal milk control.

The pH measurements (fig. 2) correlated well with those of the plate counts, showing an extended lag phase in growth and a definite retardation of acid production toward the end of the growth period in the rancid milk.

With the exception of the observations at 8 and 11 hr., there was little difference to be found in the three control samples. Some lessening of both growth and acid production was evident in both the pH and surface tension-adjusted controls, as compared to the normal-milk control sample at these times, but the final counts of all three were approximately equal. No reasonable explanation was found for this, and the other experiments with this culture of *S. lactis* indicated that the irregularities in the noted observations were experimental in nature. Therefore, no significant differences in the growth of *S. lactis* were believed to result from either the lowered pH or the depressed surface tension (table 1).

TABLE 1

The surface tension of milk cultures of different bacteria during growth

Experiment	Sample no.	Period of incubation (hr.)					
		0	2	5	8	11	24
					(*dynes/cm.*)		
S. lactis	1[a]	45.9	45.6	45.6	46.0	46.1	Coag.
(Figs. 1 & 2)	2	45.9	45.7	45.6	45.9	45.9	Coag.
	3	38.9	38.5	37.8	38.5	38.5	Coag.
	4	38.5	38.9	37.5	40.1	40.1	Weak Coag.
L. bulgaricus	1	45.9	45.9	45.9	Coag.		
(Fig. 3)	2	45.8	46.0	45.4	Coag.		
	3	38.2	38.6	37.3	38.1	Coag.	
	4	39.8	39.3	39.6	40.3	Coag.	
L. casei	1	46.1	45.5	45.6	46.2	45.4	Coag.
(Fig. 5)	2	45.8	45.5	45.5	45.7	45.6	Coag.
	3	38.5	37.5	39.1	37.4	37.6	Coag.
	4	37.1	35.4	36.8	36.6	34.9	Coag.
E. coli	1	46.1	46.0	45.4	45.2	45.4	Coag.
	2	46.2	45.8	45.3	45.2	45.2	Coag.
	3	37.8	39.2	38.2	37.9	36.5	Coag.
	4	37.0	37.1	36.3	36.0	35.2	33.8
Active lipase	1[b]	33.0	32.3	33.6	33.6	35.5	Coag.
and degree of	2	32.6	34.2	34.8	35.4	36.1	Coag.
rancidity	3	36.2	37.2	38.3	38.9	38.7	Coag.
(Fig. 9)	4	46.4	47.2	45.8	45.4	44.3	Coag.
	5	47.0	46.9	46.4	46.7	46.2	Coag.
	6	46.9	46.9	46.5	46.8	46.3	Coag.

[a] Sample 1 was the normal milk control, 2 the pH adjusted control, 3 the surface tension adjusted control, and 4 the rancid milk.

[b] Sample 1 was very rancid (active lipase), 2 very rancid (inactive lipase), 3 medium rancid (inactive lipase), 4 not rancid (active lipase), 5 not rancid (inactive lipase) and 6 normal milk (no added lipase).

The surface tension of the rancid milk inoculated with *S. lactis* H-1-10 showed a tendency to rise during the incubation period (table 1). This same tendency was present in the other experiments and had been observed in preliminary work in which raw rancid milk was inoculated with this same strain of *S. lactis*. This

indicates that perhaps some small portion of one or more of the surface tension-lowering agents were utilized by this organism. This was suggested by Tarassuk and Smith (10), who noted an even greater rise in the surface tension of rancid milk as the acid fermentation of *S. lactis* progressed. However, the significance of the rise in surface tension noted in the present study was doubtful and no rise whatsoever was found with *S. lactis* H.

Effect on S. zymogenes and S. bovis. The results of experiments using *S. zymogenes* and *S. bovis* were similar to those in which *S. lactis* was used. Although the inhibition of *S. bovis* by rancid milk apparently was not as great as with the other two streptococci, the results of duplicate experiments definitely indicated that some inhibition occurred in the lag phase and in the latter part of the logarithmic growth phase.

Effect on Lactobacillus bulgaricus. Albus and Holm (1) found that the growth of *L. bulgaricus* was inhibited in bouillon when the surface tension was

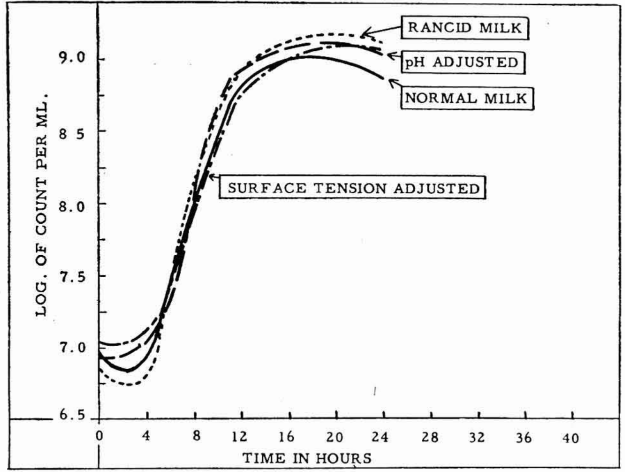

FIG. 3. The effect of rancid milk on the growth of *L. bulgaricus*.

depressed below 40 dynes per centimeter with sodium ricinoleate, while *L. acidolphilus* grew in this medium with a surface tension as low as 36 dynes per centimeter. In the present investigation, the strain of *L. bulgaricus* used apparently was unaffected by lowered surface tension in milk. It grew as well in the rancid milk and in surface tension-adjusted control samples, both of which had surface tension below 40 dynes per centimeter, as in the controls with normal surface tension (45 to 46 dynes per cm.) (table 1). The results of duplicate experiments, one of which may be noted in fig. 3, failed to yield evidence of any real inhibitory effect of rancid milk on *L. bulgaricus*, with the exception that the acidity possibly developed somewhat slower in the rancid sample during the first 5 hr. of growth than in the other three samples. The variations indicated in the growth curves at 11 and 24 hr. were not reproducible in the duplicate experiment and, therefore, were considered to be of no significance.

Effect on Lactobacillus casei. As may be noted in fig. 4, inhibition of *L. casei* was found to some degree in the initial growth period, and apparently extended into the logarithmic phase. The final count, however, was of about the same order in all samples. Acid production by this culture correlated with the observations on its growth in the different samples. The pH- and surface tension-adjusted control samples indicated that these factors were of no significance in the growth of this culture. Here again, there was no evidence of any significant change in the surface tension of the rancid milk (table 1).

Effect on Escherichia coli. It was not expected at the outset that rancid milk would display any appreciable effect toward an intestinal organism such as *E. coli.* However, the results of a single experiment indicated that both the growth and acid production of the strain of this organism used were inhibited in the lag and logarithmic growth phases, the results being similar to those for *S. lactis*

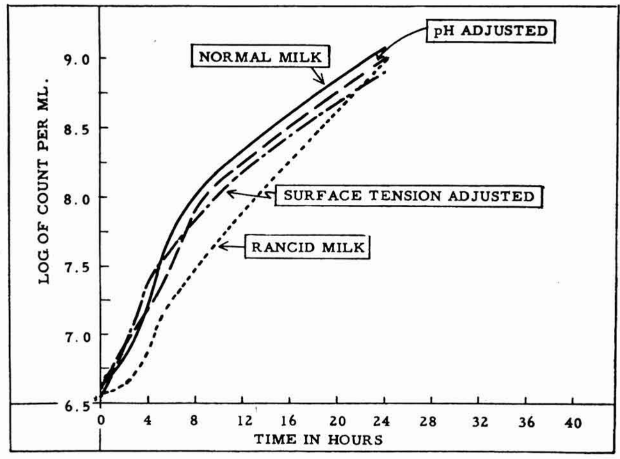

FIG. 4. The effect of rancid milk on the growth of *L. casei.*

(H-1-10, fig. 1 and 2). While all three of the other samples were coagulated in 24 hr., the rancid sample showed no coagulation at this period. No evidence of any inhibition was found in either the pH- or surface tension-adjusted control samples. There appeared to be a steady decline in the surface tension of the rancid milk sample throughout the incubation period (table 1).

Effect on Pseudomonas aeruginosa. Rancid milk was not found to inhibit *Pseudomonas aeruginosa* when a 0.001 per cent inoculum of the culture was used.

Effect of an Active Lipase and the Degree of Rancidity upon S. lactis

Although a definite inhibition of growth of this organism was found in sterilized rancid milk, it was not so great as that which had been observed to occur in spontaneously rancid raw milk. It seemed reasonable to believe that this might be the result of the products of lipolysis being different in nature, or the extent of lipolysis, or the action of the enzyme itself upon the bacterial cells in the raw milk. No means were readily available to test the first of these hypoth-

eses directly by chemical analysis of rancid milk The latter two possibilities were investigated by adding a sterile lipase and by controlling the degree of lipolysis.

Since no sterile milk lipase was available and attempts to recover it from milk were unsatisfactory, sterile concentrated lipase[3] prepared from *Mycotorula lipolytica* was used. This lipase had been found by Peters and Nelson (5) to attack the butterfat in pasteurized homogenized milk; thus, it was assumed that its activity would be comparable to the enzyme found in milk. This concentrated lipase was placed in sterile milk and incubated at 37° C. to permit lipolysis of the butterfat.

Six samples were used to observe the effect of the enzyme itself and the degree of rancidity on the growth of *S. lactis* H-1-10. Samples 1 and 2 were allowed to become very rancid by incubating the sterile milk containing 1 per cent of the lipase concentrate for 24 hr. before inoculation. The difference between these samples was that sample 2 was heated at 82.2° C. momentarily to destroy the enzyme before inoculation, while sample 1 was not. Sample 3 was incubated for only 12 hr. after adding the sterile lipase before it was heated to 82.2° C. and thus, less rancidity was produced. The sterile lipase was added to samples 4 and 5 immediately before inoculation. The enzyme was allowed to remain active in sample 4, but sample 5 was heated to 82.2° C. at the time of adding the lipase so that no lipolysis would occur. Sample 6 contained no lipase and served as the control.

The results of this experiment (fig. 5 and table 1) clearly demonstrated that inhibition of *S. lactis* varied directly with the degree of lipolysis. A definite inhibitory effect was found in the moderately rancid sample (no. 3), but it was only about half as great as that noted in the two very rancid ones (no. 1 and 2).

No direct effect of the enzyme upon the bacterial cell was indicated. The growth in the non-rancid sample (no. 4) containing the active lipase was about equal to that in the other two non-rancid samples (no. 5 and 6) during the first 8 hr. of incubation, but lessened thereafter. This lessening of bacterial growth probably was the result of the development of a slight rancidity in this sample, since a reduction in the surface tension indicated that lipolysis of the butterfat by the active lipase had occurred at this time. No significant differences were noted in the growth of the culture in the two very rancid samples, one of which contained the active enzyme. The presence of the inactive enzyme in the milk had no effect. These observations indicated that the inhibition of *S. lactis* correlated with the degree of rancidity and probably was not the result of any action of lipase *per se* on the bacterial cells. The rise in the surface tension of from 2.5 to 3.0 dynes per centimeter in the three rancid samples indicated the possible partial utilization of certain surface tension depressing compounds.

DISCUSSION

The present study has shown that rancid milk inhibits the growth of various bacteria, particularly the lactic acid types. The use of milk having varying degrees of rancidity may result in poor performance of lactic acid cultures used

[3] Generously supplied by F. E. Nelson, Iowa State College, Ames.

in the preparation of various fermented dairy products, such as buttermilk, cottage cheese and butter. In addition, rancidity in milk may contribute to inaccuracies at times observed when performing quality tests on raw milk by dye reduction methods.

In attempting to explain this inhibitory property of rancid milk the following possibilities have been eliminated: (a) a slightly lower pH than normal due to the release of free fatty acids, (b) low surface tension caused by the presence of surface active fatty acid and (c) action of the enzyme upon the bacterial cell.

The lower pH of rancid milk as compared to normal milk, although significant as demonstrated by Reder (8), is not a pronounced factor (0.2 to 0.3 pH unit in the samples used in this study). This hardly could be expected to bring about any great amount of inhibition of bacteria and the results showed it to be un-

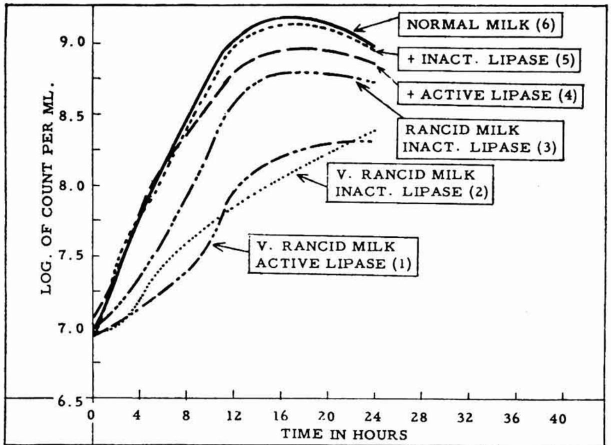

FIG. 5. The growth of *S. lactis* (No. H-1-10) in milk containing an active lipase and in milk of different levels of rancidity.

important. However, the low surface tension of rancid milk noted by Tarassuk (9), Doan and Minster (3) and Tarassuk and Smith (10) is significant and becomes progressively lower with continued lipolysis. While the results of this study, using "Tween" 40 as a surface tension depressant, showed no effect of the low surface tension, Ayers *et al.* (2) observed that the streptococci in general are inhibited in broth having a surface tension below 40 dynes per centimeter. Such conflicting evidence may be the result of differences in the media and in the nature of the surface tension depressants, as observed by Ayers *et al.* (2), Gibbs *et al.* (4) and Pizarro (7). These results are considered indicative that the inhibitory property of rancid milk cannot be attributed to the low surface tension.

It also can be reasoned that lipase does not adversely affect the cells of *S. lactis per se.* In the experiments in which active lipase was added to milk at the time of inoculation, no inhibition was noted until lipolysis had proceeded to the

extent that the surface tension of the milk was lowered. At such time inhibition was equivalent to that expected in milk showing this degree of lipolysis. If lipase had been acting on the bacterial cells, inhibition would have been expected to occur soon after inoculation.

Since rancid milk contains a higher concentration of free fatty acids than normal milk, the inhibition noted in rancid milk presumably may be caused by one or more of these compounds. Investigations concerning this will be reported later.

SUMMARY

Milk which had undergone lipolysis to the extent that a reduction of about 10 dynes per centimeter in the surface tension occurred was found to have a definite inhibitory effect toward *S. lactis, S. zymogenes* and *L. casei.* The inhibition of *S. bovis* and *E. coli,* though detectable, was definitely less than that of the above organisms. *L. bulgaricus* and *Ps. aeruginosa* were not measurably affected.

Use of control samples in connection with the rancid milk demonstrated that the inhibition displayed by the rancid milk was not caused by either the slight reduction in pH or the reduced surface tension noted in such milk. Sterile active lipase showed no direct effect on the growth of *S. lactis* other than through its lipolytic action on the fat in the milk. The data indicated that inhibition was caused by some component of rancid milk and that the extent of inhibition of *S. lactis* was in direct proportion to the degree of lipolysis occurring in the milk.

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SOME CHARACTERISTICS OF THE GROWTH INHIBITOR PRODUCED BY A LACTIC STREPTOCOCCUS[1,2]

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In earlier reports (1, 2) it was shown that several stock cultures of *Streptococcus lactis* and *Streptococcus cremoris* and a small number of commercial starter cultures produced a heat stable substance that inhibited the growth of *Lactobacillus casei.* The material also restricted growth of some strains of lactic streptococci as well as single strains of *Lactobacillus bulgaricus* and *Streptococcus liquefaciens.* The results were in general accord with those obtained by other workers with inhibitory strains of lactic streptococci.

The exact chemical composition of the inhibitory substances produced by lactic streptococci has not been determined. Whitehead (7) reported that the inhibitor studied by him was a protein derivative, possibly a polypeptide, which was destroyed by trypsin but not by pepsin. The inhibitor "diplococcin" was believed by Oxford (5) to be a protein-like substance of relatively small molecular weight, which was a part of the cell, possibly part of the somatic antigen. Oxford (5) and Hirsch (3) showed that the inhibitors studied by them could be produced in media containing nitrogenous compounds no more complex than the products of casein hydrolysis. Thus, the inhibitors must have been synthetic rather than decomposition products.

Several workers (4, 5, 6) have shown that the inhibitors studied by them were diffusible through collodion or similar membranes. Rogers (6) reported that his was partially removed by filtration through plaster of Paris or earthenware filters. Later, Hirsch (3) showed complete removal of the inhibitor "nisin" from culture fluids by Seitz filtration at pH 5.0 and above but none was removed at pH 2.0. Part of the inhibitor was taken out by filtering at pH 3.0–4.0.

All of the inhibitory substances produced by lactic streptococci described in the literature are relatively stable at an acid reaction but are unstable above neutrality. Hirsch (3) reported that a culture of *S. lactis* at pH 4.65 lost no inhibitory activity while standing at room temperature for 10 days. Similarly, Whitehead (7) showed that holding an inhibitory preparation overnight at 37° C. caused no loss of activity when the pH was 4.0, but at pH 8.0 much of the inhibitor was destroyed. Hirsch (3) showed that nisin in a culture fluid survived boiling for 10 min. at pH 2.0–3.0, but at higher pH values there was partial destruction. At pH 8.0–9.0 the nisin was completely inactivated. Whitehead (7) found that a large part of the inhibitor produced by one of his cultures was de-

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stroyed by boiling at pH 8.0, while that from another culture was completely inactivated by this treatment.

The purpose of this paper is to report some of the characteristics of the inhibitor produced by a stock culture of *S. lactis* and to compare them with the characteristics of inhibitory materials described by other workers.

METHODS

Methods of producing and testing the inhibitory material were essentially the same as those described previously (2). *S. lactis* 16, which was one of the most active producers of the cultures tried, was grown in casein hydrolysate broth for 24 hr. at 30° C. The culture was steamed for 5 min. to kill the organisms. One ml. of the inhibitory material was added to 9 ml. of sterile yeast extract-glucose-tryptone broth and the mixture was inoculated with 0.1 ml. of a 24-hr. culture of *L. casei* 65. For the control, 1 ml. of sterile casein hydrolysate broth was substituted for the heated culture. Growth of the rod in the two cultures was measured turbidimetrically. The results are expressed directly as optical densities or as per cent response by the method previously described (2). Per cent response indicates the amount of growth in the presence of the inhibitor as compared with the growth in its absence and represents the approximate amount of inhibitor present.

In these experiments the method of preparing the test material (*i. e.*, the heat-killed culture) was modified in one of the following ways: (a) The culture was centrifuged, the supernatant was decanted, and the cells were diluted back to the original volume of the culture with sterile casein hydrolysate broth. The supernatant and the suspended cells then were used as the test materials. (b) To increase its inhibitory effect the culture was concentrated to one-half its original volume by distillation at a partial pressure of approximately 65 mm. of mercury at 50 to 65° C. The concentrated culture then was steamed for 5 min. and stored at 5° C. until used. For the control, sterile casein hydrolysate broth was adjusted to pH 4.8–4.9 and concentrated in the same way.

RESULTS

Distribution of the inhibitor in a culture of S. lactis. Oxford (5) concluded that the inhibitor studied by him was attached to the cells of the organism producing it and was released by acid. Hirsch (3) agreed with Oxford's opinion and suggested that an equilibrium exists between the inhibitor associated with the cells and that in the medium, the equilibrium depending mainly on the pH of the culture. In a 24-hr. culture of *S. lactis* with a pH of 4.65 he found approximately half of the inhibitor in the broth and half associated with the cells, while at pH 2.0 all of the inhibitor was in the culture fluid.

To see if the same relationship existed in this work, a 24-hr. culture of *S. lactis* 16 was adjusted to pH 3.5 and steamed for 5 min. It then was readjusted to pH 6.8 and centrifuged The supernatant was poured off and the cells were resuspended to the original volume of the culture in sterile distilled water. The

supernatant and the cell suspension were used as test materials with *L. casei* 65 as the test organism.

The results in figure 1 show that both the supernatant and the cell suspension contained inhibitory material. As shown by the values at 38 hr., there was more in the supernatant than in the cell suspension. This would be expected because the *S. lactis* culture was adjusted to pH 3.5 before steaming, which, in view of Hirsch's results, should liberate over half of the inhibitor into the culture medium.

Effect of pH on inhibition of L. casei. In an earlier report (2) it was shown that *L. casei* started to grow normally in association with *S. lactis* but its inhibition became apparent at 12 to 18 hr. This time coincided with the production of sufficient acid to lower the pH to about 5.4 and the results can be interpreted

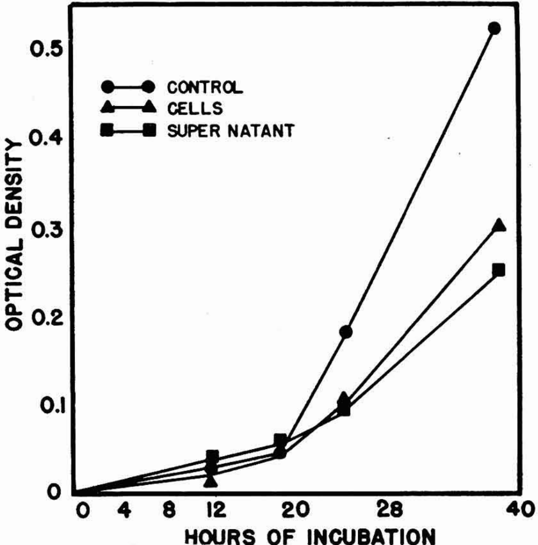

FIG. 1. Effect of the supernatant and cells of *S. lactis* 16 on growth of *L. casei* 65.

to mean that inhibition did not occur until sufficient acid was produced to liberate the inhibitor from the cells of the coccus. In trials with heat-killed cultures of *S. lactis,* however, *L. casei* also started to grow normally in broth at pH 6.8 to 7.0 and did not show inhibition until 24 to 36 hr., at which time it had produced considerable acid. This was true even when supernatant from an inhibitory culture was used as the test material.

The failure of an *S. lactis* culture or its inhibitory supernatant to restrict growth of *L. casei* until after the rod itself had produced some acid indicates that the inhibitor is more effective in the presence of acid. To demonstrate this, the supernatant and suspended cells of *S. lactis* 16 were added separately to tubes of yeast extract-glucose-tryptone broth containing 0.5 per cent K_2HPO_4 and 0.5 per cent sodium citrate as buffer. Aliquots of the medium were adjusted

with 2 N H_2SO_4 to reactions ranging from pH 6.4 to 5.1. All samples were inoculated with 0.1 ml. of a 1:100 dilution of a 24-hr. culture of *L. casei* 65 and incubated at 30° C. Control cultures were prepared in the same way but with the inhibitory material omitted. Earlier trials (2) had shown that *L. casei* grew equally well in milk adjusted to pH values from 5.25 to 6.3, but to eliminate any possible effect of pH, the control cultures were adjusted to the same reactions as those of the test cultures containing the inhibitory material.

Figure 2 shows that inhibition by the supernatant increased gradually as the pH dropped from 6.4 to about 5.4. Between pH 5.4 and 5.1, however, there was a marked increase in inhibition. Since all of the inhibitor was free in the medium, this increase must have been caused by enhanced activity of the inhibitor in this pH range. Significantly this was about the pH of a mixed culture of *S. lactis* and *L. casei* when inhibition of the rod first was noticed in earlier trials (2).

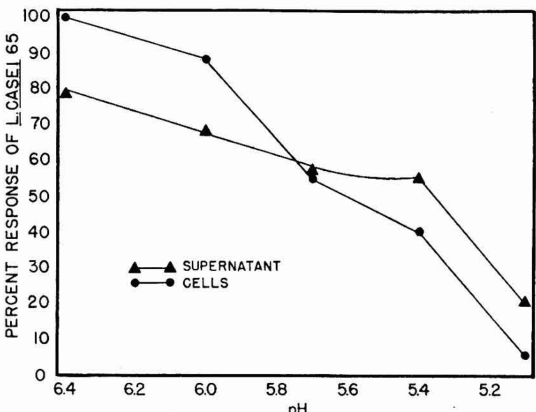

FIG. 2. Effect of pH on activity of the inhibitor produced by *S. lactis* 16.

Some of the increased inhibition observed as the pH was lowered might be explained merely on the basis of better stability of the inhibitor at the lower pH values. The marked increase in the range of pH 5.4 to 5.1, however, suggests a specific effect of pH on the inhibitor. No definite explanation of this effect can be given from the results, but it is possible that the inhibitor is more readily adsorbed by the *L. casei* cells as the pH is adjusted below pH 5.4.

The results for the cell suspension of *S. lactis* in figure 2 show practically no inhibitory activity when the culture medium was buffered at pH 6.4. There was only slight inhibition at pH 6.0, but between pH 6.0 and 5.7 there was a marked increase. This probably represents mainly liberation of the inhibitor from the cells. Between pH 5.7 and 5.4 inhibition again increased gradually as the acidity increased, similar to the response of the supernatant at this pH range. Below pH 5.4 there was a second marked increase of inhibitory activity just as was observed with the supernatant.

Effect of pH on stability of the inhibitor to heat. A concentrated culture of *S. lactis* 16 was used in these trials. The reaction before concentrating was pH 4.9; afterwards it was 4.8. Half of the concentrate was adjusted to pH 7.4, the other half was left at 4.8. Portions of the material at each reaction were heated at 100 and 121° C. for different times before being tested for their inhibitory activity .

As figure 3 shows, the samples at pH 4.8 exhibited a slight but steady loss of inhibitory activity as the time increased, but they retained most of it even when boiled for 2 hr. or autoclaved for 1 hr. At pH 7.4, however, there was rapid destruction of much of the inhibitor during the first 5–10 min. of heating, followed by a gradual further decrease in activity similar to that observed for the samples with the acid reaction. As might be expected, inactivation of the inhibitor was more rapid at the higher temperature regardless of the hydrogen ion concentration, although in no sample was inactivation complete in the time intervals used.

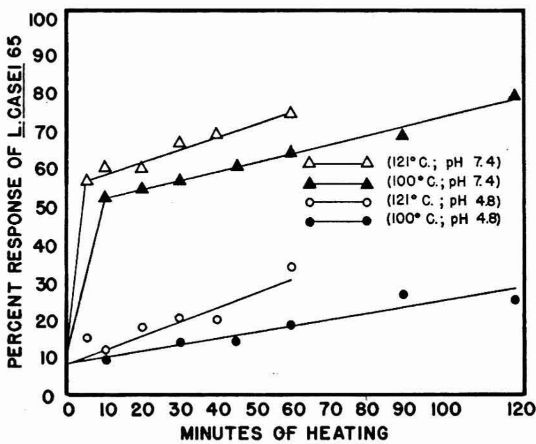

FIG. 3. Effect of heat and hydrogen ion concentration on the inhibitor produced by *S. lactis* 16.

The results in figure 3 indicate that the inhibitory material exists in two distinct forms. One is relatively stable to heat either at acid or near neutral reaction; the other is relatively stable at an acid reaction but is rapidly destroyed by heating at a pH slightly above neutrality.

To determine more exactly the effect of hydrogen ion concentration on stability of the inhibitor to heat, a culture of *S. lactis* 16 was adjusted to pH 2.0 and concentrated to one-half its original volume. Portions were adjusted to pH values from 2.0 to 8.0, heated at 100° C. for 10 min., and cooled immediately. All samples then were adjusted to pH 6.9 to 7.0 and tested for activity of the inhibitor.

Figure 4 shows little or no loss of activity at pH 2.0, but inactivation proceeded rapidly as the pH increased to 7.0. These results agree with those of

Hirsch (3). The material that survived heating at pH 7.0 to 8.0 presumably represents the part of the inhibitor that is relatively stable at both acid and alkaline reactions.

The results in figures 3 and 4 can be interpreted to mean that the two forms of the inhibitor actually represent the material in the bound state (*i.e.* attached to the cells) and in the free or unbound state. When attached to the cells, the material is somehow protected from rapid inactivation by heating at an alkaline reaction. When liberated into the medium, however, it becomes highly unstable to this treatment. If this is true it would be expected that heating a culture supernatant at an alkaline reaction would completely inactivate the inhibitor.

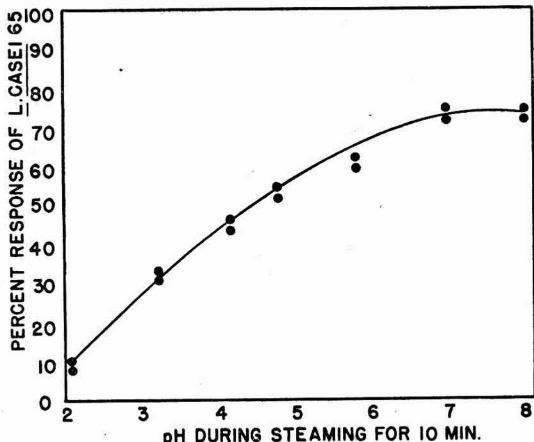

FIG. 4. Effect of hydrogen ion concentration on heat stability of the inhibitor produced by *S. lactis* 16. Results of two trials are shown. Each point represents the average of duplicate determinations.

Heating a whole culture, however, would inactivate only the portion of the material that was free in the culture medium.

Hirsch (3) demonstrated complete inactivation of nisin in culture fluid by heating to 100° C. for 10 min. at pH 8.0 to 9.0, thus showing that all of the inhibitor is unstable to this treatment after liberation from the cells. Figure 3 shows that approximately half of the inhibitory material in a culture of *S. lactis* was rapidly destroyed by steaming for 5 to 10 min. at pH 7.4. This is exactly as would be expected because, from Hirsch's results, half of the inhibitor in a culture of *S. lactis* at its final pH should be in the medium and half attached to the cells. Figure 4 shows about 75 per cent of the inhibitor to be destroyed by heating at pH 7.0 to 8.0, indicating that most of it was free in the culture medium. This is to be expected because the culture used for figure 4 was concentrated at pH 2.0, which should free more of the inhibitor from the cells than would concentrating at pH 4.8 to 4.9 as was done with the material used for figure 3.

DISCUSSION

Whether all lactic streptococci produce the same inhibitory material remains an intriguing question. Because so many different methods of studying and evaluating the effects of inhibitors have been used, it is difficult to conclude whether different investigators have worked with the same materials or not.

Some of the similarities between the inhibitors that have been reported are summarized at the beginning of this paper. Certain specific differences also have been reported and they may or may not be of sufficient importance to separate the substances. Oxford (5), for example, stated that diplococcin was insoluble in absolute alcohol, whereas Whitehead (7) found that his material was soluble, although he admitted that it might not be freely so. Whitehead further noted that the inhibitor produced by one lactic streptococcus inhibited a strain of *Lactobacillus acidophilus,* while that produced by another streptococcus did not. He also recorded differences in the stability of these substances at pH 8.0. Oxford (5) stated that diplococcin contained no sulfur, whereas Hirsch (3) reported that nisin contained 5–7 per cent of this element. In spite of these differences, however, there are enough similarities in the substances that have been described to justify the belief that, if not identical, all are at least closely related.

The findings of Oxford (5) and Hirsch (3) that the inhibitor produced by *S. lactis* is associated with the cells and is released by acid is confirmed by this study. The results also provide indirect evidence to support Hirsch's suggestion that an equilibrium exists between the inhibitor attached to the cells and that in the culture medium, the equilibrium depending on the pH of the medium. The existence of a form of the inhibitory material that is stable to heating at an alkaline reaction has not been reported before. From the amounts of the inhibitor that survived heating in cultures adjusted to different pH values, it is believed that the material which survives heating at alkaline reaction is that which is bound to the cells and in some way is protected from inactivation. When it is liberated into the culture medium, however, it is rapidly destroyed by heating, the destruction becoming progressively more rapid as the reaction is raised above pH 2.0.

The finding that the inhibitory material was more effective at pH values below 5.4 probably explains why *L. casei* was not inhibited in earlier trials until it had grown and produced some acid. Apparently the growth of this organism was largely unaffected by the inhibitor until the reaction of the culture had dropped to the vicinity of pH 5.4.

SUMMARY

The inhibitory substance produced by one strain of *Streptococcus lactis* was associated with the cells that produced it but was liberated from them by acid. Boiling or autoclaving a culture of *S. lactis* for 10 min. at pH 4.8 had little effect on its inhibitory activity but heating at pH 7.4 rapidly destroyed about half of the inhibitor in the culture. It is believed that the inhibitor is somehow protected from destruction by heating at neutral or alkaline reactions when it is at-

tached to the cells, but when released into the culture medium it is rapidly destroyed by this treatment. At acid reactions it is relatively stable to heat whether bound to the cells or not.

The inhibitory material was not only more stable but it was also more active at acid reactions. Its ability to inhibit a test culture of *Lactobacillus casei* was markedly increased when the reaction of the culture medium was below pH 5.4.

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THE PRODUCTION OF A GROWTH INHIBITOR BY LACTIC STREPTOCOCCI[1, 2]

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Rogers (11) probably was the first to show that lactic streptococci may inhibit growth of another lactic organism. He found that *Streptococcus lactis* inhibited acid production by *Lactobacillus bulgaricus* when the two organisms were grown together in making buttermilk. Whitehead and Riddet (14) and later Meanwell (8) isolated from "non-acid" or "slow" milks lactic streptococci which, when grown in normal milk, rendered it unsuitable for growth of starter organisms. Oxford (9) studied two of the cultures isolated by Meanwell and concluded that they produced a "protein-like substance of small molecular weight" which caused the inhibition of other organisms. He called the substance "diplococcin." Hunter and Whitehead (5) observed that most strains of *Streptococcus cremoris* and most of the commercial mixed starter cultures tested by them grew poorly in milk in which inhibitory lactic organisms had previously grown, although certain strains grew normally. About one-fourth of the cultures of *S. cremoris* isolated from commercial starters by Hoyle and Nichols (4) were inhibitory to a test culture of *S. cremoris*. These workers also found that about one-third of the cultures of *S. lactis* isolated by them from raw milk were inhibitory, and to a greater degree than were the cultures of *S. cremoris*.

Mattick and Hirsch (6) concentrated the inhibitory material produced by certain lactic streptococci and used it to treat mice and guinea pigs infected with hemolytic streptococci. Later, (7) they called the substance "nisin" and described a method for its large scale production. Factors affecting its production have been studied further by Hirsch (3).

The relatively slow appearance of lactobacilli in ripening cheddar cheese suggests that they may be inhibited to some extent by the starter organisms that grow during manufacture. For this reason the main purpose of this study was to test cultures of lactic streptococci for their ability to inhibit *Lactobacillus casei*.

METHODS

Organisms. All pure cultures were obtained from collections maintained in the Department of Bacteriology, University of Wisconsin, and were carried in litmus milk containing $CaCO_3$. The starter cultures were from commercial sources. All cultures were transferred at weekly intervals, grown at 30° C., and stored at 5° C. between transfers.

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Culture media. Reconstituted skimmilk was prepared by shaking 100 g. of powdered skimmilk with 1 l. of distilled water and filtering through coarse paper.

For the preparation of whey, 1 ml. of commercial rennet extract was added to 250 ml. of fresh skimmilk at 40° C. The resulting curd was cut and the whey was poured off, filtered and sterilized. Sterilization caused a precipitate which settled out, leaving a clear supernatant.

Casein hydrolysate broth was made by dissolving 2.5 g. of K_2HPO_4, 1.5 g. of $MgSO_4$ and 10 g. of glucose in 100 ml. of casein digest prepared from crude casein by the method of Roberts and Snell (10). The slight flaky precipitate that formed when the glucose and salts were added to the casein digest was removed by filtration through paper.

Yeast extract-glucose broth was prepared by dissolving 1 g. of Difco yeast extract and 1 g. of glucose in 100 ml. of distilled water. Yeast extract-glucose-tryptone broth contained these ingredients plus 0.5 g. of Difco tryptone and 0.5 g. of K_2HPO_4.

To make carrot liver broth, 1 g. of glucose, 1 g. of Difco peptonized milk, 0.5 g. of Difco neopeptone and 0.5 g. of K_2HPO_4 were dissolved in 80 ml. of water. To this were added 10 ml. of carrot extract and 10 ml. of liver extract prepared as described by Garey *et al.* (2).

All media were adjusted to pH 6.8 and sterilized at 121° C. for 20 min.

Measuring the effect of lactic streptococci on test organisms

Microscopic method. In the early experiments, *S. lactis* and *L. casei* were grown separately and together in reconstituted skimmilk. One per cent of a 24-hr. culture of each organism in casein hydrolysate broth was the inoculum. At intervals during incubation, aliquots were removed for pH measurements and for estimating numbers of cells of each organism by the direct microscopic method of Frazier *et al.* (1). Inhibition or stimulation was determined by comparing the counts of each organism in the mixture with those from the corresponding pure culture.

Turbidimetric method. After growth for 24 hr. in casein hydrolysate broth the lactic streptococcus culture, which had a pH of 4.8 to 4.9, was steamed for 5 min. to kill the organisms and 1 ml. was added to 9 ml. of sterile yeast extract-glucose-tryptone broth. As a control, 1 ml. of sterile casein hydrolysate broth was added to a second tube of the other medium. Both tubes were inoculated with 0.1 ml. of a 24-hr. culture of *L. casei,* or other test organism, before incubation. Growth was measured turbidimetrically at intervals with an Evelyn photoelectric colorimeter using a 620 mμ filter.

Inhibition or stimulation of the test organism was determined by comparing the increase in turbidity (optical density) in the tubes with and without the heated streptococcus culture. This comparison is expressed as per cent response, which is calculated as follows:

$$\frac{\text{Optical density of test culture}}{\text{Optical density of control}} \times 100 = \text{per cent response}$$

Replicate tests by this method with the same heated coccus culture agreed within

± 5 per cent. In all of the tests inhibition or stimulation was apparent by or before the 38-hr. reading. Therefore, the per cent response at this time is recorded in later sections of this report.

In some of the experiments whey and curd samples taken during cheddar cheese making were tested for their effect on *L. casei.* The samples of whey were adjusted to pH 3.5 with lactic acid, autoclaved for 10 min. and centrifuged. The

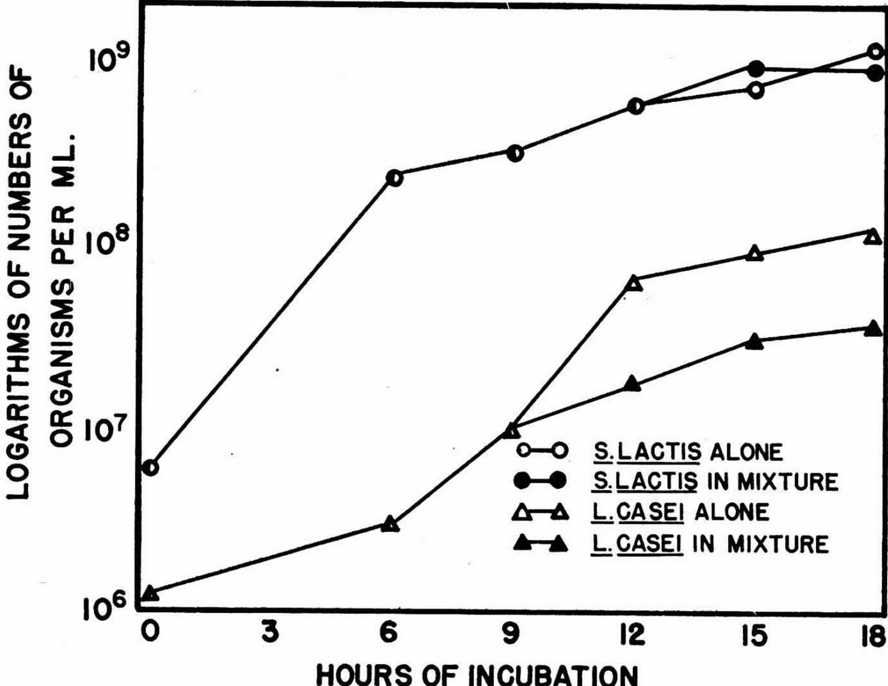

FIG. 1. Numbers of *Streptococcus lactis* DS and *Lactobacillus casei* DS in pure and in mixed cultures.

clear supernatant was used as the test material. The curd samples were suspended in 2 per cent sodium citrate solution as described by Frazier *et al.* (1), then acidified and treated as were the samples of whey. Portions of whey from the original milk used for the cheese were added to the control tubes.

RESULTS

Inhibition of L. casei by S. lactis. Figure 1 shows the result obtained when *S. lactis* strain DS and *L. casei* strain DS were grown together and separately in reconstituted skimmilk. Growth of the rod culture was unaffected by the coccus for the first 9 hr., but after that time it was inhibited. *S. lactis,* on the other hand, grew equally well in the mixture and in the pure culture.

The acidity of the mixed culture increased rapidly and the pH values coincided almost exactly with those for the pure culture of *S. lactis.* At 12 hr., when inhibition of *L. casei* first was noticed, the reaction of the mixed culture was

pH 5.4, while that in the pure culture of *L. casei* was pH 6.3. To show that inhibition was not caused by the acid formed by the coccus in the mixture, *L. casei* was inoculated into samples of reconstituted skimmilk adjusted to pH values of 6.30, 6.01, 5.65, 5.55 and 5.25. The rod grew equally well in all samples.

In another experiment the ability of a heat-killed culture of *S. lactis* DS to inhibit *L. casei* DS was determined by the turbidimetric method. The growth

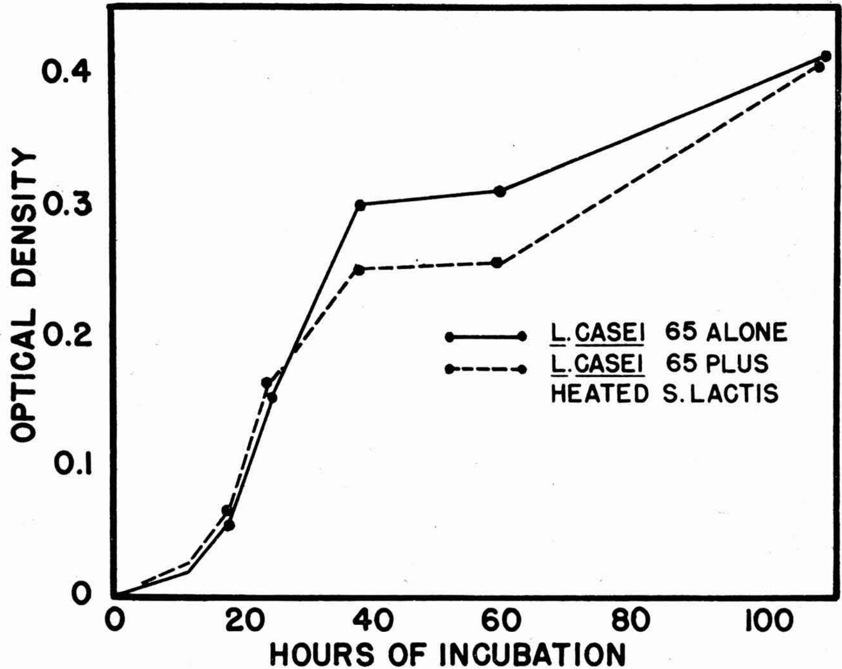

FIG. 2. Growth of *Lactobacillus casei* 65 alone and in the presence of heat-killed culture of *Streptococcus lactis* 16.

response of the rod at 38 hr. in the presence of the heated *S. lactis* culture was only 73 per cent of its response in the absence of the heated culture, thus indicating that the coccus produced a heat-stable substance capable of inhibiting growth of *L. casei.*

Effect of prolonged growth of L. casei in the presence of the inhibitor

The length of time which an inhibitory culture of *S. lactis* restricted the growth of *L. casei* is shown in figure 2. The differences in optical density indicate that inhibition of the rod first occurred between 24 and 36 hr. and persisted for over 60 hr. By 110 hr., however, growth in the test culture was approximately the same as that in the control. It would appear that either the inhibitory material disappeared during incubation, or the rod gradually became adapted to growth in its presence. Whitehead (13) and Hoyle and Nichols (4) observed a similar effect.

Production of the inhibitory material by different cultures of lactic streptococci. Eight strains of *S. lactis,* one of *S. cremoris* and three commercial starter cultures were tested for their ability to produce an inhibitor active against several strains of *L. casei.* The tests were made by the turbidimetric method. Table 1 shows the averaged results of two trials with four strains of *L. casei* as test organisms. All strains of *S. lactis* and *S. cremoris* had about the same effect on *L. casei* strains 9, 61 and 65, but varied widely in their ability to inhibit strain 25. The reason for this variation is not known. Of the pure cultures of lactic cocci, *S. lactis* Rogers and 16 were slightly more inhibitory in general than were the others. The commercial starter cultures had about the same effect as the pure cultures except that they inhibited *L. casei* strain 65 to a considerably greater extent. Of the lactobacillus cultures, strain 65 was much more sensitive to the inhibitor than were the other strains.

TABLE 1

Per cent response[a] at 38 hr. of four strains of L. casei in the presence of different heat-killed lactic streptococci

Lactic streptococcus	Test strains of *L. casei*			
	9	65	25	61
S. lactis 16	87	71	88	74
" " E	95	76	101	88
" " Hammer	88	79	78	88
" " Iowa	89	82	95	96
" " DS	91	73	101	91
" " A-1	92	76	87	91
" " 23	87	75	82	86
" " Rogers	84	72	56	84
S. cremoris 144F	91	85	95	86
Commercial starter E	89	50	74	......
" " F	88	53	82	......
" " H	90	51	88	......

[a] Per cent response is an expression of the amount of growth of the test culture in the presence of 10% of the heat-killed coccus compared with its growth without the coccus. Values less than 100 indicate inhibition by the killed coccus culture; the smaller the number, the greater the inhibition.

After they had shown that certain strains of *S. lactis* and *S. cremoris* produced a heat-stable inhibitor active against *S. cremoris* 144F, Hoyle and Nichols (4) pointed out the importance of selecting noninhibitory strains for inclusion in mixed starter cultures. To see whether the stock cultures used in this study also were inhibitory to strains of the same species, an experiment similar to that described above was run but with strains of *S. lactis* and *S. cremoris* as the test organisms. The results in table 2 show considerable variation between cultures, but *S. lactis* strains Rogers and DS were slightly more inhibitory in general than were the others. The two test strains of *S. lactis* were inhibited to a very slight extent by their homologous killed cultures, but *S. cremoris* 144F was unaffected by its. In this connection Hirsch (3) found that much more of the inhibitor "nisin" was necessary to inhibit the organism that produced it than was ever found normally in a culture. Rogers and Whittier (12), however, concluded

that the final population of *S. lactis* cultures may be limited by a substance that they produce. The substance was not lactic acid. In general, the test strains of lactic streptococci were inhibited less than were those of *L. casei* (table 1).

In other trials one strain each of *L. bulgaricus* and *S. liquefaciens* were inhibited by a heat-killed culture of *S. lactis* 16.

Effect of the culture medium on production of the inhibitor. Hirsch (3) and Hoyle and Nichols (4) reported that more inhibitor is produced by lactic streptococci in certain media than in others. Furthermore, Hirsch's results show that this difference between media is not necessarily correlated with amount of growth, expressed as optical density, because nutrient additions that stimulated growth did not necessarily increase "nisin" production by a corresponding amount. A similar difference between media was observed in this study. *S. lactis* strains Rogers and 16 were grown in casein hydrolysate broth, yeast extract-glucose broth, yeast extract-glucose-tryptone broth and carrot-liver broth. They were killed by heating and tested for their ability to inhibit two strains of *L. casei*.

TABLE 2

Per cent response[a] at 38 hr. of three strains of lactic streptococci in the presence of heat-killed strains of the same species

Strains of lactic streptococci	Test cultures of streptococci		
	S. lactis 16	*S. lactis 23*	*S. cremoris 144F*
S. lactis 16	94	83	104
" " E	99	93	91
" " Hammer	89	101	104
" " Iowa	95	79	91
" " DS	95	73	72
" " A-1	104	81	105
" " 23	94	93	105
" " Rogers	75	85	81
S. cremoris 144F	84	105	102

[a] See footnote to table 1.

The rods were inhibited most by coccus cultures grown in casein hydrolysate broth and least by those grown in carrot-liver broth. In general, there was a tendency for the amount of inhibitor produced by the lactic cocci to decrease as the media were enriched.

Effect of time of incubation on production of the inhibitor. S. lactis strains Rogers and 16 were grown in casein hydrolysate broth and samples were removed at intervals up to 60 hr. These were heated and tested for their ability to inhibit *L. casei* 65 in yeast extract-glucose-tryptone broth. Table 3 shows that both organisms produced most of the inhibitor during the first 24 hr., but some was formed for as long as 60 hr. These results are not in complete agreement with those of Hoyle and Nichols (4), who found that practically all of the inhibitor appeared in the first 24 hr. Hirsch (3) also observed that maximum concentrations of "nisin" were reached in less than 18 hr.

Production of the inhibitor during cheese making. Samples of whey from cheddar cheese made with an ordinary commercial mixed starter culture became

inhibitory early in the manufacturing process, as is shown in table 4. The concentration of the inhibitor increased during the making process and at least until the cheese was removed from the press. The inhibitor appeared much more quickly in the cheese than it did in broth cultures of *S. lactis* Rogers and 16 (table 3). This difference may be explained by the fact that the commercial mixed starters tested were much more inhibitory to *L. casei* 65 than were the

TABLE 3

Effect of time of incubation of two strains of lactic streptococci on production of the inhibitor in casein hydrolysate broth

Time of incubation	Strain of *S. lactis*:	
	Rogers	16
(*hr.*)		
12	93[a]	91[a]
18	93	87
24	81	79
60	70	73

[a] Figures represent per cent response (see footnote, table 1) at 38 hr. with *L. casei* 65 as the test organism.

pure cultures (table 1). Also, it is possible that the inhibitor is produced more quickly and in greater quantity in milk (or cheese) than in broth cultures. Hoyle and Nichols (4) found this to be true in comparing milk and yeast-dextrose broth as media for their inhibitory organisms.

DISCUSSION

The slow growth of lactobacilli in cheese no doubt can be attributed to some extent to their low initial numbers and to the fact that milk is not a particularly favorable medium for their growth. The results of this study show that their development also may be inhibited by a product or products of the starter organisms. The ability to produce the inhibitory substance is common to many strains of lactic streptococci, as shown by these results and by those of Hoyle and Nichols (4), although certain strains produce considerably more of it than do others. In addition, certain strains of *L. casei* are inhibited to a much greater extent than are others. These variations in ability to produce the material by

TABLE 4

Effect of whey and curd from cheddar cheese on growth of L. casei 65

Test material	Per cent response[a] of *L. casei* 65 at 38 hr.:	
	Trial 1	Trial 2
Whey 1.5 hr. after starter added	101[b]	101[b]
" 3 " " " "	74	66
" 5 " " " "	38	46
Curd at 24 hr.	....	33

[a] See footnote to table 1.
[b] Result secured with whey from the original milk was considered as 100%.

lactic streptococci and in susceptibility to it by different cultures of the rod may indicate the cause of variations that have been noticed in the time and extent of growth of lactic rods in cheese during ripening.

It is impossible to assess with certainty the importance of the inhibitory substance produced by the starter organisms in cheese making without more experimentation. There seems to be little doubt, however, that this material can exert a selective action on the lactobacillus flora and thus might be expected to permit better growth of the less sensitive strains than of those more easily inhibited. Whether this is desirable cannot be said until more is known of the importance of individual strains of lactobacilli in cheese ripening. Probably the lactobacilli would develop more rapidly if the inhibitor were not present and this might indicate that attention should be paid to selecting starter organisms that are least inhibitory. The time required by lactobacilli to overcome the effect of the inhibitor no doubt depends on the amount of the inhibitor present as well as upon the numbers and kinds of rods in the milk.

SUMMARY

Nine strains of *Streptococcus lactis* and *Streptococcus cremoris* and three commercial starter cultures were found to produce a heat stable substance that inhibited growth of *Lactobacillus casei.* The material also inhibited certain strains of lactic streptococci, although to a lesser extent than it restrained growth of the rods. The lactic streptococci varied considerably in their ability to produce the inhibitor; also, strains of *L. casei* differed markedly in their susceptibility to inhibition. When given sufficient time, the rod cultures overcame the effect of the inhibitor.

Most of the inhibitor was formed by the lactic streptococci during the first 24 hr. of their growth in broth, although its concentration continued to increase slowly for as long as 60 hr. The culture medium used for growing the streptococci had a marked effect on the amount of inhibitor produced.

Whey and curd obtained during the manufacture of ceddar cheese was inhibitory to the one strain of *L. casei* tested. Thus, the production of an inhibitory substance by starter organisms may delay growth of lactobacilli in cheese during ripening. It also may exert a selective action that favors the predominance of less susceptible strains of lactic rods in the flora that develops.

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ABSTRACTS OF LITERATURE

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BOOK REVIEWS

602. Annual Review of Biochemistry, vol. 20. J. M. LUCK, editor. Annual Reviews, Inc., Stanford, Calif. 648 pp. $6.00. 1951.

The chapters in this new volume are: Biological Oxidations, by R. Wurmser; Nonoxidative, Nonproteolytic Enzymes, by W. R. Frisell and L. Hellerman; Carbohydrate Chemistry, by H. O. L. Fischer and D. L. MacDonald; The Polyuronides, by C. L. Hinton; Chemistry of the Lipids, by K. F. Mattil; The Chemistry of Amino Acids and Proteins, by K. Bailey and F. Sanger; X-ray Crystallographic Studies of Compounds of Biological Interest, by R. B. Corey; Nucleic Acids, Purines, and Pyrimidines, by J. Baddiley; Lipid Metabolism, by S. Gurin and D. I. Crandall; The Metabolism of Proteins and Amino Acids, by H. Borsook and C. L. Deasy; Biochemistry of Steroids, by S. Lieberman and K. Dobriner; Fat-soluble Vitamins, by H. Dam; Nutrition, by H. J. Almquist; Biochemistry of Cancer, by A. M. Brues and E. S. G. Barrón; Biochemistry of Antibiotics, by R. L. Peck and J. E. Lyons; Immunochemistry, by M. M. Mayer; The Metabolism of Drugs and Toxic Substances, by R. T. Williams; Biochemical Genetics, by N. H. Horowitz and H. K. Mitchell; Biochemistry of Natural Pigments (Exclusive of Haeme Pigments and Carotenoids), by T. R. Seshadri; Carbohydrate Metabolism, by S. P. Colowick and N. O. Kaplan; and Water-soluble Vitamins, by G. Emerson and K. Folkers.

The editors are to be complemented on again assembling an outstanding group of reviews on subjects of great current interest. The reviewers represent not only both academic and industrial groups in this country but also a group of distinguished foreign contributers. The indexing is adequate and the quality of editing and printing up to the high standards one has come to expect of this series. The preface contains an interesting discussion of some of the problems of printing references for publications such as this. The volume certainly should be available for reference to all in biological work. F. E. Nelson

603. Agricultural Marketing. F. L. THOMSEN. McGraw-Hill Book Co., Inc., New York. 483 pp. $6.00. 1951.

This text views marketing from the standpoint of the farmer and presents a broad view of the marketing problem. It has been well organized and lends itself to easy reading. It is not a handbook of marketing nor does it give practical pointers. It does give beginning students of agricultural marketing a general idea of agricultural production conditions in the United States. The use of practical examples to explain points greatly aids in the presentation as well as in the understanding of the material. Footnotes are included to aid in finding additional information. Almost a third of the text is devoted to the evaluation of the weaknesses in the marketing system with potential improvements.

The text is divided into 6 parts as follows: (a) The Marketing Job, (b) The Marketing System, (c) Prices and Margins, (d) Auxiliary Services in Marketing, (e) Potential Improvements in Marketing and (f) Means of Effecting Improvement. Contained in its 22 chapters are 18 tables and 64 figures. G. A. Claybaugh

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

604. Ring test for bangs. Anonymous. Milk Dealer, **40,** 9: 56, 58. June, 1951.

The ring test was developed in Germany and first announced in 1937. Much work has been done by the Univ. of Minnesota with this test and it has been found accurate in a varying percentage of cases on cows in production. However, it is not considered usable for a careful check and must be followed by the standard blood or agglutination test in order to verify the results. The test works most accurately on mixed milk from 5–10 cows and thus may be used at a receiving plant more accurately than on individual milk samples. The cost of a county-wide ring test is estimated at about 10% that of a county-wide blood test. This test is actually an agglutination test and depends on the same basic principle as the blood agglutination test. The agglutinins or antibodies, which the infected animal develops in attempting to rid itself of the disease, can be found in the milk as well as in the blood. A comparison of the milk and cream ring test with the blood test on slightly over 6,000 herds gave an efficiency of 75% for the ring test in locating infected herds. This compares favorably with an average of 82% efficiency for the ring test obtained in similar studies in Denmark. General conclusions from the proceedings of the 52nd Annual meeting of the U. S. Livestock Sanitary Association for 1948, and Public Health

Reports indicate that too much faith must not be put into the test as it now stands.

C. J. Babcock

605. Studies on the pathogenicity of Brucella suis for cattle. II. F. V. WASHKO and L. M. HUTCHINGS, Purdue Univ., Lafayette, Ind. Am. J. Vet. Research, **12,** 44: 165–174. July, 1951.

Suspensions of *Br. suis* were injected into the teats of 10 cows. Complete case histories of the development of brucellosis in each of these cows are presented. Applying *Br. suis* to abraded skin of the udder did not establish an infection. Placing *Br. suis* into the teat orifice did establish an infection. Acute mastitis occurred in the affected quarters, followed by considerable destruction of the mammary epithelium. *Br. suis* also was found in the lymph nodes, but not in the blood. Very high agglutination titers resulted. Pregnant cows and heifers did not abort following the infection and no *Br. suis* were found in the fetal membranes. These studies indicate that brucellosis can be produced in cattle through *Br. suis* invasion by way of the teat, which may develop into mastitis with *Br. suis* eliminated in the milk.

E. W. Swanson

606. The use of ether-killed Brucella abortus in saline-in-oil emulsion in a herd of cattle with brucellosis. I. LIVE and A. G. DANKS, Univ. of Pennsylvania, Philadelphia. Am. J. Vet. Research, **12,** 44: 175–182. July, 1951.

Immunizing efficiency of ether-killed *Br. abortus* was compared with strain 19 vaccine in a herd of 73 cows and heifers which had been exposed to a natural outbreak of brucellosis. All cattle were negative at the start of the experiment. 28 cows were vaccinated with strain 19, 27 with ether-killed *Br. abortus* combined with falba and mineral oil and 18 were left as controls. 2 from each group were challenged with a virulent *Br. abortus* strain and only 1 of the controls aborted. 8 of the strain 19-vaccinated animals aborted, including 1 apparently due to an infection with strain 19. Only 1 animal vaccinated with the ether-killed organisms aborted, and it was concluded that she had been infected at the time of vaccination. Only 1 of the control animals aborted, and this was a set of twins not infected with *Br. abortus.* The ether-killed vaccine produced a higher and more persistent blood titer than the strain 19 vaccine. No other undesirable effects were produced. These preliminary trials indicate that the ether-killed *Br. abortus* in falba and mineral oil may be of value as an immunizing agent in an infected herd.

E. W. Swanson

BUTTER

O. F. HUNZIKER, SECTION EDITOR

607. Continuous apparatus for making butter. C. E. NORTH. U. S. Patent 2,564,715. 2 claims. Aug. 21, 1951. Official Gaz. U. S. Pat. Office, **649,** 3: 691. 1951.

Cream is introduced into 1 end of a cylindrical casing, where it is agitated, churned and propelled toward the other end by 2 independently rotating shafts, consisting of a series of flutes and discs. The churned cream leaves the device in the form of small butter granules floating in buttermilk.

R. Whitaker

608. How proper packaging can increase butter sales—and profits. G. R. JOHNSON. Am. Dairy Prod. Mfg. Rev., **13,** 8: 14, 15, 42, 43. Aug., 1951.

Advantages of the paraffined carton in the merchandizing of butter are reviewed. These include consumer demand, better keeping quality, ease of handling, cleanliness, competition, sales appeal, appearance and brand promotion.

T. J. Claydon

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

609. Preparation of modified skimmilk. B. K. HOLLAND and W. C. WINDER, Univ. of Wis., Madison. Milk Dealer, **40,** 11: 43, 93–96. Aug., 1951.

A study is reported on fortification of skimmilk with vitamins A and D and the modification of the product with various quantities of milk solids-not-fat and fat. Skimmilk modified with milk solids-not-fat from either condensed skim or nonfat dry milk solids and vitamins A and D is a palatable product; 3% solids from condensed skim and 2% solids from nonfat dry milk solids produce better results than additions at other levels. Fortification with 2,000 U.S.P. units of vitamin A and 400 U.S.P. units of vitamin D/qt. produces a slight flavor change which is not objectionable to most consumers. Addition of 0.5% butterfat improves the product markedly. Pasteurization at 150° F. for 30 min. produces the best flavor during the winter season, but higher temperatures are desirable during other seasons. Homogenization pressures of 2,500 lb. result in the best flavored and smoothest product and minimize sedimentation of the added solids. The viscosity increases as the level of added solids is increased but is not affected by any other factor. Curd tension can be lowered by using a pasteurization temperature of 165° F. for 30 min. or by including at least 1% fat in the milk followed by homogenization. Curd tension increases after homogenization when condensed skim is used but decreases when nonfat dry milk solids are used.

C. J. Babcock

610. Apparatus for reconstituting dried powders. S. T. COULTER and J. L. BECKER (assignors to Maple Island, Inc.) U. S. Patent, 2,566,555. 19 claims. Sept. 4, 1951. Official Gaz. U. S. Pat. Office, **650,** 1: 140. 1951.

A device for reconstituting powders, such as skimmilk, consisting of a centrifugal pump, with a screen fitted inside the housing, so that the revolving blade forces the reconstituted material through the screen just prior to the pump outlet, is described.

R. Whitaker

611. New flavor evaluation method. D. R. PERYAM and R. J. REMALEY, Q. M. Food & Container Inst., Chicago, D. V. JOSEPHSON, Penn. State College, and H. L. FENOLD, Kraft Foods Co., Chicago. Food Eng., **23,** 8: 83–86, 167. Aug., 1951.

A new method of evaluating flavor was developed for dried milk and is based on a quality index termed a dilution number (DN). The DN of a sample is defined as "per cent of reconstituted milk in a mixture of that material with a fresh whole milk standard such that the difference in taste between it and the standard lies just above the threshold." It is determined by the concentration that can be detected by 15 correct judgments out of 20 by several judges. The method has good reproducibility and good correlation with consumer preference. The weaknesses of the regular panel method of quality evaluation also are considered. T. J. Claydon

612. Baked goods with cheese flavor. PETER KASS (assignor to Inter-chemical Corp.). U. S. Patent 2,564,763. 6 claims. Aug. 21, 1951. Official Gaz. U. S. Pat. Office, **649,** 3: 1951.

A pronounced cheese flavor is produced in baked goods by introducing milk-flavored cheese fortified with leucine, prior to fermenting the dough. R. Whitaker

613. Milk food product. P. F. SHARP, W. H. HOECKER (assignors to Golden State Co., Ltd.). U. S. Patent 2,565,098. 17 claims. Aug. 21, 1951. Official Gaz. U. S. Pat. Office, **649,** 3: 792. 1951.

A concentrated milk product is described containing 30–60% water and having a heavy body, suitable for use as a spread. The proportion of solids-not-lactose to lactose is about 1:2. The lactose not in solution in the water is present as crystals of impalpable size. R. Whitaker

614. Process for producing sweetened condensed milk and similar products. D. D. PEEBLES (assignor to Golden State Co., Ltd.). U. S. Patent 2,565,085. 6 claims. Aug. 21, 1951. Official Gaz. U. S. Pat. Office, **649,** 3: 788. 1951.

To produce sweetened condensed milk having a thin body, the milk is heated to 265–310° F. prior to the addition of sugar and concentration to 70–75% total solids. The milk is not held at the high preheating temperature long enough to darken the color. R. Whitaker

615. Varied food uses opened up for cheese whey protein. Anonymous. Am. Dairy Prod. Mfg. Rev., **13,** 8: 2–3, 39–41. Aug., 1951.

The method of Malkames, Walter and Sager, Bur. Dairy Ind., U.S.D.A. for hydrolysis of protein from Swiss cheese whey gives a smooth product that can be used in preparing spreads and as an ingredient in other foods. After precipitation and recovery of the whey protein, a commercial proteolytic enzyme (Rhozyme P11) is used for partial hydrolysis of the product which subsequently is homogenized at 2500 lb. pressure. Procedures are given for the preparation of various spreads and the incorporation of the hydrolyzed protein. T. J. Claydon

616. Process for the treatment of lacteal sera. E. ABRAHAMCZIK, H. PETROVICKI, F. SCHAFFERNAK. U. S. Patent 2,566,477. 12 claims. Sept. 4, 1951. Official Gaz. U. S. Pat. Office, **650,** 1: 121. 1951.

A whey concentrate is prepared by removing weak acids and parts of weak acids by suitable ion exchange, concentrating, hydrolyzing the concentrate at 115° C. with a mineral acid and deacidifying by means of hydroxyl ion exchange. R. Whitaker

617. Method of crystallizing crystallizable substances. P. F. SHARP (assignor to Golden State Co., Ltd.). U. S. Patent 2,565,097. 6 claims. Aug. 21, 1951. Official Gaz. U. S. Pat. Office, **649,** 3: 792. 1951.

Products containing crystallizable substances, as for example, concentrated dairy products supersaturated to lactose, are made smooth and of very fine texture by causing the lactose to crystallize in numerous impalpable crystals. This type of crystallization is induced by flowing the product supersaturated to lactose, over large lactose crystals, through beds or pills composed of compressed lactose, or in contact with a rotating roll, the surface of which is impregnated with lactose crystals. R. Whitaker

618. Coating of paper. J. A. MONTGOMERIE (assignor to Chemical Mfg. Co.). U. S. Patent 2,566,529. 11 claims. Sept. 4, 1951. Official Gaz. U. S. Pat. Office, **650,** 1: 133. 1951.

A paper coating composition consists of a vegetable resin and casein in the ratio of 1:1 to 1:3, the resin being dispersed in an aqueous alkaline solution of the casein. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

619. Variations in the characteristics of E. coli induced by quaternary ammonium compounds. C. K. CROCKER, Diversey Corp. Research Lab., Chicago, Ill. J. Milk & Food Technol., **14:** 138–141. July–Aug., 1951.

Induced resistance may be acquired by *Escherichia coli* and *Serratia marcescens* when exposed to quaternary ammonium compounds. *E. coli* failed to produce gas in liquid culture media and gave atypical small colony variants on desoxycholate agar. Furthermore, the variant strains grew slower and reduced methylene blue very slowly. These observations alter the value of the coliform test when the organisms tend to develop a resistance to quaternary ammonium compounds. H. H. Weiser

620. La formation du diacétyl dans les levains de beurrerie. (The formation of diacetyl in creamery fermentations). A. CAMNS, P. LANIESSE and J. BURDIN. Lait, **31,** (305–306): 225–233. May–June, 1951.

The formation of diacetyl, acetoin and 2,3 butanediol in skimmilk cultured with *S. lactis* was

studied under a variety of conditions. The nature of curves concerning the formation of these compounds indicated that diacetyl is produced rather feebly (6–8 mg./l.) reaching a maximum at 18 hr. after which time it gradually disappears. Acetoin is produced in much greater quantity (105 mg./l.), reaching a peak at about 18 hr., after which it slowly disappears. Butanediol steadily increases over a 65-hr. incubation period and the data suggest that it is formed at the expense of acetoin, at least in part. Dilution of skimmilk to be inoculated has no appreciable effect on the shape of the curves. However, the amounts of acetoin and butanediol were substantially reduced by this treatment. Incubation at 17 rather than 25° C. merely appeared to delay the production of diacetyl, but did not reduce the quantity formed. Use of greater or lesser amounts of inoculum was observed to hasten or delay the time of maximum production. Incubation of cultures at 25° C. followed by holding at 10° C. retarded destruction of both diacetyl and acetoin. The practical significance of these findings as they related to butter manufacture is discussed. S. Patton

621. Method and agent for controlling undesirable fermentation in cheese. H. Jergensen (assignor to Aktieselskabet Dansk Gaerings-Industri). U. S. Patent 2,566,941. 5 claims. Sept. 4, 1951. Official Gaz. U. S. Pat. Office, **650,** 1: 242. 1951.

Anaerobic bacterial spoilage of cheese in sealed containers is prevented by incorporating a small amount of a soluble chlorite R. Whitaker

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

622. Effect of preheating time on the inactivation of phosphatase in milk. S. A. Lear and H. G. Foster, Rutgers Univ., New Brunswick, N. J. J. Milk & Food Technol., **14:** 131–133 and XI. July–Aug., 1951.

Experimental data were obtained that meet the mathematical equations used in the food canning industry to calculate total lethal heat effect of a process.

Preheating 1 min., with straight-line heating, will add 0.40 and 29.91% of the total lethal heat when the holding temperatures are 146.3 and 163.4° F., respectively. H. H. Weiser

623. Contribution a l'étude de la lipase du lait (Contribution to the study of milk lipase). M. Loncin and D. Jacqmain. Lait, **31,** (305–306): 233–250. May–June, 1951.

Lipase activity of mother's and cow's milk was followed by means of titratable acidity. During the period of study, it was shown that microorganisms were making no significant contribution to developed acidity. Agitation was observed to activate the lipase of mother's milk much more readily than that of the cow. Degree of lypolysis in mother's milk was found to increase with time of agitation and to be proportional to the extent of fat globule deformation. The enzyme was shown to be in the serum phase of the milk.
S. Patton

624. A note on temperature measurement in Babcock test centrifuge. L. M. Lampert, State Dept. of Agr., Sacramento, Cal. J. Milk & Food Technol., **14:** 137. July–Aug., 1951.

More uniform results may be obtained in the Babcock test if the temperature is properly controlled. By using a special thermometer, a temperature of 170° F. was recorded in the centrifuge cups as compared to 152° F. as shown by ordinary methods.

The author recommends that the temperature be checked by placing 2 maximum recording thermometers in opposite cups of the centrifuge and noting the temperature equilibrium when the tests are removed from the machine.
H. H. Weiser

625. Determination des teneurs en matierè sèche, en calcium et en phosphore de diverses variétés de fromages a pate ferme ou demi-dure, et a croute résistance (Determination of the amounts of dry matter, calcium and phosphorus in several varieties of hard paste or semi-hard cheeses with a hard rind). L. Randoin and C. Jourdan-Vatinel. Lait, **31,** (305–306): 250–256. May–June, 1951.

Dry matter, Ca and P values for certain soft cheese, from a preceding report are reviewed. The present paper reports these values for 4 varieties of semi-hard cheese.

The mean dry matter values are 61.11, 62.50, 51.36 and 66.15% for Hollande, Cantal, Saint-Paulin and Gruyère, respectively. The Ca values (g./100 g. dry matter) were 1.271, 1.246, 1.258 and 1.528, respectively, and the P values were 0.543, 0.735, 0.691 and 0.912, respectively. The nutritional and compositional significance of these values are discussed. S. Patton

626. Hardening casein artificial fibers with titanium, zirconium or tin salts. J. F. Corwin, J. R. Calhoun, T. M. Buzzo (assignors to The Borden Co.). U. S. Patent 2,567,184. 8 claims. Sept. 11, 1951 . Official Gaz. U. S. Pat. Office, **650,** 2: 402. 1951.

Alkaline casein is coagulated in an acid bath and hardened in a formaldehyde bath containing between 0.5% and saturation of salts of titanium, zirconium or tin at pH 0.8–3 and at a temperature of 120–200° F. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

627. Method of standardizing cream. P. H. Staaff (assignor to Aktiebolaget Separator). U. S. Patent 2,567,898. 6 claims. Sept. 11, 1951. Official Gaz. U. S. Pat. Office, **650,** 2: 593. 1951.

Cream of about 80–82% fat is produced by passing a low-fat cream from a separator through a 2nd separator. Uniformity of fat content is achieved by a regulating system which maintains a constant fat content in the low-fat cream, in spite of variations in the fat content of the milk

or rate of flow of milk to the 1st separator.
R. Whitaker

628. Operation and service tips on dairy equipment. N. J. Peters, Damrow Brothers Co., Fond du Lac, Wis. Milk Dealer, **40,** 11: 50, 107–110. Aug., 1951.

Instructions are given on the operation and care of pasteurizers, milk can washers, conveyors, storage tanks, trailer or truck tanks, cheese vats, electrical equipment and on the care of stainless steel.
C. J. Babcock

629. Protect your equipment. D. Gillespie, Creamery Package Mfg. Co., Chicago, Ill. Ice Cream Field, **57,** 3: 24, 77–101. Mar., 1951.

The necessity of carefully selecting and training all plant personnel is stressed in order to insure a proper equipment maintenance program. Proper cleanup and lubrication are emphasized. Special instructions are given regarding freezers and homogenizers.
W. C. Cole

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

630. Hood plants now work smarter. Anonymous. Ice Cream Field, **57,** 3: 36–38, 40, 44, 45, 46, 48, 50. Mar., 1951.

An account is given of the work simplification program at H. P. Hood & Sons under the direction of H. G. Dunlap. Dunlap believes that an effective work simplification program must begin at the top; accordingly managers and superintendents became students first; later foremen and lower-level supervisors were invited to class.

The technique of work simplification is to eliminate unnecessary parts of a job. Hood's slogan is "Be alert; work smarter, not harder." Dunlap says "Work simplification isn't a list of procedures; it's a way of thinking."

As part of the program various charts are used, *e.g.,* flow chart, lick the problem, job progress chart. Films are used extensively to study problems and illustrate improvements. Several examples accompanied by illustrations are used to show benefits from this program. Both management and employees are enthusiastic about the program—the employees because their work is made easier and their suggestions are welcome, the management because of the resulting over-all benefits. No company is too small to initiate a work simplification program.
W. C. Cole

631. Have you a bottleneck in your front yard? A. Bradfield and R. Carter, Univ. of Vermont, Burlington. Milk Dealer, **40,** 10: 47, 48–101. July, 1951.

Studies show that in many plants men on the receiving decks are idle 50% of the time. The cause for the idleness is discussed and tables are presented showing: (a) Use of time on receiving deck during milk receiving period; (b) relationship of type of hauler to arrival at milk plant; (c) cans received at 2 average plants during receiving period according to type of hauler; (d) characteristics of various vehicle types which relate to unloading efficiency. Figures are presented showing: (a) Comparison of receiving time/load and waiting time between loads under present delivery system and under a system of scheduled deliveries and (b) time needed/can declines as loads get larger up to the point that they become so large that extra time and effort are needed.
C. J. Babcock

632. Bulk milk dispensers. Anonymous. Milk Dealer, **40,** 10: 43, 77–81. July, 1951.

A survey representing a cross section of the country showed that bulk milk dispensers increased sales 10–100%. In addition, they brought about saving of time, labor and handling in the plant. Locating the machines at points of greatest advantage is discussed. One report shows that dispensers immediately increased the consumption of milk in schools 50–100%. Increases ranging from 15–25% in the sale of milk to restaurants and hotels also are reported. It is concluded that especially in those markets using paper containers the dispenser, in many cases, is the answer to high packaging costs in 0.5-pt. containers.
C. J. Babcock

633. Growing market for milk. Caroline Menuez, Paper Cup and Container Inst., Inc., New York. Milk Dealer, **40,** 10: 46, 105–107. July, 1951.

A survey of the Paper Cup and Container Inst., Inc., shows that ¾ of the new factory buildings will have cafeterias, and 20% of the 240 companies queried are making plans to feed more of their workers. The survey also shows that 59% of the employees in plants having food service are customers. This is an increase of 40% over 1944. Of the plants responding to the queries, 79% had cafeterias but most had more than one type of food service. The trend in most companies is to decentralize the actual distribution of food. In other words, they will use food carts, snack bars, canteens or carry-out systems which bring food to the worker at the job. This decentralization is bringing into use the vending machines, especially for milk. In fact, vending machines are 75% more popular than they were during the war. One report on 15 plants, which provide milk between meals, shows that workers interpreted the availability of milk as an indication of management's interest in their health and one manager felt that the milk dispensing machine was an important factor in labor relations.
C. J. Babcock

634. Problems of pricing milk. D. E. Hirsch, Farm Credit Admin., USDA. Milk Dealer, **40,** 11: 52–58. Aug., 1951.

The classified price plan involves the systematic segregation of milk of bottling quality in such a way as to reflect the form or manner in which it was used and to recognize differences in the value of milk according to its use. Ideally, the supply and demand for fluid milk would be equal at all times in all markets. This is impossible because the supply of milk and the demand both vary. A few specific objectives of a classified

price plan are: (a) To maintain an adequate supply of milk at all times for use in fluid milk products; (b) to reflect to producers the highest practicable blend price under current demand and supply conditions, giving proper consideration to regional and national conditions as well as local, and to keep dairymen's incomes in line with general economic changes; (c) to distribute equitably among producers the proceeds from the sale of their milk; (d) to facilitate the utilization in other dairy products of all milk of bottling quality not needed for use in fluid milk products; (e) to encourage the utilization of milk in those products which will reflect highest returns to producers; (f) to compensate handlers adequately for functions performed in the disposition of milk not needed for fluid consumption; (g) to encourage efficiency in the marketing of milk of all classes. The problems which are concerned in developing the mechanics of the plan and those which involve human relations are discussed. The arguments in support of placing concentrated milk in class I also are given.
C. J. Babcock

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

635. Inflation member for milking machines. L. T. CONDE. U. S. Patent 2,565,721. 1 claim. Aug. 28, 1951. Official Gaz. U. S. Pat. Office, **649,** 4: 1054. 1951.

A teat cup is described which employs a flexible casing within a rigid cylindrical cup, of such design that the inner casing collapses from the top progressively to the bottom or teat end.
R. Whitaker

636. Milk metering device. E. B. KNAGGS. U. S. Patent 2,566,729. 4 claims. Sept. 4, 1951. Official Gaz. U. S. Pat. Office, **650,** 1: 185. 1951.

A pulsator-type milker has a metering device in the milk line to the milker pail for measuring the amount of milk passing through the tube.
R. Whitaker

637. Sealing can closure. R. W. SAXTON. U. S. Patent 2,565,269. 4 claims. Aug. 21, 1951. Official Gaz. U. S. Pat. Office, **649,** 3: 837. 1951.

A closure for milk cans of the flared-top type, consists of a dish-shaped lid which fits into the flared section and is held securely to the can by bolts attached to a ring which encircles the neck of the can.
R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

638. Faster mix handling. R. K. LAWHORN, Abbott's Dairies, Inc., Philadelphia, Pa. Ice Cream Field, **57,** 3: 56, 105. Mar., 1951.

A report is given of economies obtained resulting from more efficient handling of mix ingredients. Abbott's have aided in developing a machine that will shave 80% plastic cream rapidly enough and in an economical and sanitary manner. The use of proportioning meter devices has aided in streamlining their operation.
W. C. Cole

639. HTST pasteurization of ice cream mix. F. N. BARBER, Natl. Dairy Research Lab., Inc., Oakdale, L. I., N. Y. Ice Cream Field, **57,** 4: 42, 64, 65, 68, 69. Apr., 1951.

There is considerable interest in HTST pasteurization of ice cream mix because of economies resulting from streamlining manufacturing processes. Certain types of HTST equipment have the additional advantage of improving quality of the finished product by removal of off odors and flavors during the pasteurization process, although it is claimed that choice of equipment is now a matter of personal preference.

The author reports using an unidentified micrococcus designated by the Natl. Dairy Labs. as MS-102 for evaluating the efficiency of pasteurization by the HTST methods. This microorganism has a greater resistance to heat than the tubercle bacillus. MS-102 required slightly longer holding times or higher temperatures for its destruction in ice cream mix than were required in milk.

Experimental data showed that heating ice cream mix to a temperature of 175° F. for 25 sec. gave slightly greater destruction of the microorganism than resulted from 155° F. for 30 min. The laboratory findings were confirmed under commercial conditions. As a result of these findings, as well as the work of others, the U.S.P.H.S. has granted tentative approval for pasteurization of ice cream mix at 175° F. for 25 sec. The author urges commercial cooperation in establishing this as a permanently approved standard for HTST pasteurization. W. C. Cole

640. Quality nuts mean quality ice cream. C. A. PETERSON, Cleveland, O. Ice Cream Field, **57,** 6: 132–133. June, 1951.

A brief review is given of the sources as well as the selection and care required for the following nuts used in ice cream: Pistachio, walnut, pecan, almond, cashew, filbert and peanut.
W. C. Cole

641. Texture control. T. A. NICKERSON, U. of Cal., Davis. Ice Cream Field, **57,** 5: 68, 70, 88. May, 1951.

A brief discussion is given of the essentials in controlling ice cream texture. Mix composition, processing, freezing and hardening as well as storage are considered. W. C. Cole

642. Sweetening agents in ice cream. J. J. SAMPEY, Director of Research, Abbotts Dairies, Inc., Philadelphia, Pa. Ice Cream Rev., **34,** 12: 105, 106. July, 1951.

The various sweetening agents suitable for use in ice cream are discussed. It is pointed out that corn sweetening agents have been used successfully to substitute as much as 45% of the sucrose in ice cream. The use of corn sweeteners has resulted in a slight improvement in body and texture of the ice cream and they are less expensive than sucrose. Bacterial, yeast and mold checks are suggested on both sucrose and corn syrups, as well as on the storage tanks, pumps and sanitary lines used for their distribution.

The importance of a high sugar ratio in fruit

ice cream is stressed as an effective aid in preserving the flavor of the fruit and in preventing hard icy particles of fruit in the ice cream. A ratio of 2 parts of fruit and 1 part of sugar has been found most satisfactory for this purpose.
W. J. Caulfield

643. How much vanilla flavoring should ice cream contain? Anonymous. Ice Cream Rev., **35,** 1: 78. Aug., 1951.

Results of a study, conducted at 6 state colleges, to determine the amount of vanilla flavor which will produce the most desirable vanilla flavor in ice cream, indicate that there is a definite trend in consumer preference for ice cream with more vanilla than is customarily recommended. Forty per cent of the group examining the ice cream preferred the sample of ice cream prepared with 6 oz. of a single strength pure Bourbon bean vanilla extract/5 gal. of mix, one-third of the group favored the sample containing 4 oz., 17% preferred the sample containing 8 oz. of vanilla and 10% thought the sample containing no vanilla had the most desirable flavor. It was the opinion of the group conducting the experiments that either too little or too much vanilla would be equally undesirable. W. J. Caulfield

644. Ice cream problems. C. H. MINSTER, Greenbrier Dairy Products Co., Beckley, W. Va. Ice Cream Field, **57,** 3: 22, 93–96. Mar., 1951.

Three main problems of the ice cream industry are discussed. Maintaining desirable qualities until products reach the consumer is considered of primary importance. The main personnel problem consists of providing the individual with knowledge of what he is to do, when he is to do it, how it is to be done and why it is to be done, and giving full recognition to a job well done. The 3rd problem is defined as coordination of equipment for the most economical operation. Most of this phase of the discussion deals with refrigeration equipment. W. C. Cole

645. Quality survey of Ohio's fruit and nut ice creams. T. D. HARMAN, Dairy Technology Dept., Ohio State Univ. Ice Cream Rev., **35,** 1: 100. Aug., 1951.

Examination of 50 factory-filled pt. packages each of fruit and nut ice creams revealed the need for greater uniformity and improved quality in the production of fruit and nut ice creams. In several samples examined there were no fruits or nuts visible in the ice cream. More than half of the strawberry ice creams had a preserve flavor, whereas others varied widely in color and flavor quality. Artificial and imitation nut flavors were common. There was a noticeable lack of uniformity with respect to weight of the packages and in the fat and total solids content.
W. J. Caulfield

646. What I saw in Scandinavia. L. A. CARLINER, Better Ice Cream Co., Baltimore, Md. Ice Cream Field, **58,** 1: 28, 82. July, 1951.

In Scandinavia the author reports that he saw more modern and more automatic ice cream equipment than we have in the United States. He describes a completely automatic stick confection machine that takes ice cream from the freezer, molds it, inserts a stick, hardens, coats, wraps and expels the product automatically.

The author concludes that Scandinavia rates as an equal and in some instances excels us in production machinery. We are ahead in sales and delivery equipment. Both countries are equal in quality merchandise, it is stated.
W. C. Cole

647. Ice cream softening apparatus. K. E. BEMIS. U. S. Patent 2,566,651. 3 claims. Sept. 4, 1951. Official Gaz. U. S. Pat. Office, **650,** 1: 164. 1951.

This device softens ice cream by means of a motor-driven beater. The motor is located on the top of an ice cream cabinet and a shaft extends down into a specially constructed chamber where the beater softens and maintains the ice cream in a soft condition. R. Whitaker

648. Mounting means for bulk ice cream containers. M. J. WEST (assignor to Irving King). U. S. Patent 2,567,458. 5 claims. Sept. 11, 1951. Official Gaz. U. S. Pat. Office, **650,** 2: 474. 1951.

Ice cream in cylindrical bulk paper containers is forced out of the container by a piston pressing on the bottom. The container is rolled back as the ice cream is extruded. R. Whitaker

649. Rural retail delivery of ice cream. Anonymous. Ice Cream Rev., **34,** 12: 50, 75. July, 1951.

The operation of rural retail delivery routes has proved to be a profitable venture of the Sunlight Dairy, Oshkosh, Wis., and Dairyland Ice Cream Co., Sheboygan, Wis. The Sunlight Dairy started operation with a panel truck and a 12-hole refrigerated cabinet in the summer of 1950. Since that time an additional truck has been added and a 3rd truck is contemplated. The company reported that the drop in winter sales amounted to only 5%. Although packages ranging in size from pints up to 2.5 gal. are carried on these routes, the 0.5-gal carton has proved to be the most popular seller.

The Dairyland Ice Cream Co. of Sheboygan has been operating 2 trucks on their rural delivery routes. This company reports daily sales ranging from \$75–\$90/day during the summer and \$30–\$35/day during the winter with a greatly reduced routing. Both firms are of the opinion that the rural business will continue to increase as better facilities are provided in the farm homes for ice cream storage. W. J. Caulfield

650. Selling thru supers. Anonymous. Ice Cream Field, **58,** 1: 26, 27, 80. July, 1951.

According to Food Fair's ice cream specialist, Harry Blask, their ice cream volume remains relatively constant throughout the year, although there is a seasonal switch from one variety of ice cream to another. In their 129 stores, all of which sell ice cream, in-store merchandising is the

key to their success. Cabinet placement, point-of-sale material and tie-in sales are the 3 major factors in their promotion program. Free insulated bags are supplied with ice cream purchases.

The Eastern Div. of the Great Atlantic and Pacific Tea Co. offered ice cream for sale in 300 super markets in June, 1951. Four brands of ice cream are being featured. Natl. Dairy Products is supplying 2 brands—Sealtest and Wallesley Farms. The Borden Co. is supplying the other 2 brands, *viz.,* Borden and Dairy Made. Insulated bags are given free to each purchaser of ice cream.

W. C. Cole

651. Portal to portal pays. Anonymous. Ice Cream Field, 57, 5: 95. May, 1951.

With the large number of home freezers and mechanical refrigerators now in use there is an opportunity to sell ice cream to both city and country dwellers. Selling ice cream on the retail milk route is a natural outlet, according to the report. House-to-house selling of ice cream should include the following: (a) company advertising, (b) monthly ice cream specials, (c) having the milk driver carry an empty ice cream carton on all deliveries, (d) having ice cream of good quality and (e) delivery of clean and neat packages.

W. C. Cole

652. The factory as outlet. Anonymous. Ice Cream Field, 57, 6: 126. 1951.

A survey by Paper Cup and Container Institute, Inc., in which 240 factories participated, showed that over half of the companies who told what they would do if they had to expand, mentioned plans which decentralize food service. 23% said they would add mobile food carts, 17% said they would put snack bars in the plant and 12% would try to give their workers more supplementary food in vending machines which make no demand on factory management. Only 10% would build a new cafeteria.

The survey shows that decentralized food services are more apt to break even financially and require fewer hands to operate. It is further claimed that one food service worker can handle 159 customers at a snack bar or canteen, 127 from a food cart, 61 in a cafeteria and 28 in a waiter service restaurant.

Ice cream is a popular favorite on food carts but individual portions in paper containers are essential to this type of service, it is claimed.

According to the survey there are more types of vending machines now than there were at war peak production. Providing more food in vending machines is a good way to get nourishing food into the diets of employees who bring their lunches from home.

W. C. Cole

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

653. Canned milk. J. MEYER, Minneapolis-Honeywell Regulator Co. Brown Instrument Div., Philadelphia, Pa. Milk Dealer, **40,** 9: 45, 108, 112. June, 1951.

A description of the method using high-speed continuous sterilization and canning of milk, perfected by Dr. Martin of Redwood City, Calif., is given. The method has shown great promise in both pilot plants and in full-scale installations. The Martin Aseptic Fill Method is designed to sterilize, cool and can milk in seconds. The milk is raised to a temperature of about 300° F., and other dairy products to even higher temperatures. With instantaneous heating and immediate cooling, there is little change in flavor. In sterilizing, all of the milk is heated to the same temperature. There is no localized over-heating. This makes it possible to process milk at a higher temperature and for a longer time than the minimum safe limit, so that the sterilization treatment can be several times as stringent as the minimum requirement without affecting quality. Milk, concentrated milk and dairy drinks processed in this manner probably will serve to supplement rather than replace present fluid milk deliveries and may do much to put our surplus milk production into use.

C. J. Babcock

654. Milk dispenser with quantity regulator. W. TAMMINGA (assignor to Monitor Process Corp.). U. S. Patent 2,567,683. 8 claims. Sept. 11, 1951. Official Gaz. U. S. Pat. Office, **650,** 2: 535. 1951.

Measured amounts of milk are dispensed from milk cans by operating this sanitary piston-type pump which is mounted in the lid of the milk can.

R. Whitaker

655. Milk bottle handle. F. L. FESSNER. U. S. Patent 2,565,806. 2 claims. Aug. 28, 1951. Official Gaz. U. S. Pat Office, **649,** 4: 1077. 1951.

A wire handle for carrying glass milk bottles, which is easily snapped on and removed from the bottle, is described.

R. Whitaker

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

656. The germicidal effectiveness of a new chlorine compound. C. K. JOHNS, Div. of Bact., Canada Dept. of Agr., Ottawa, Ontario. J. Milk & Food Technol., **14:** 134–136. July–Aug., 1951.

Antibac 25 and Lo Bax 21 were not superior in germicidal effectiveness to BK and Dalglish. *Escherichia coli, Micrococcus pyogenes var. aureus* and *Pseudomonas aeruginosa* were the test organisms used.

H. H. Weiser

657. Permanent sanitary pipe lines. Anonymous. Milk Dealer, **40,** 9: 48, 72–74. June, 1951.

The design, installation and cleaning instructions are given for cleaning permanent stainless steel lines by employing a recirculating system. The following cleaning instructions are given: (a) Remove all connections between permanent lines and processing equipment. (b) Rinse lines thoroughly with clear warm water. (c) Circulate for 30 min. an acid solution heated to 145–165° F. (d) Rinse with warm water. (e) Circulate for

30 min. alkali solution heated to 140° F. (f) Rinse lines, remove all caps, cuts and plugs to hand clean and examine lines and brush when necessary. (g) Reassemble and rinse lines with hot water, gradually reducing to cold water temperature. (h) Circulate for 20 min. sterilizing solution 200 ppm. available chlorine. (i) Rinse with clear cold water. (j) Clean outside of pipes. Advantages of permanent sanitary pipe lines are as follows: (a) Lines are cleaned in place with no dependence on a variable amount of attention to each individual piece of pipe, resulting in better cleaning. The possibility of contamination after sterilization is eliminated, and thus permanent lines can be kept more sterile than assembled lines. (b) Labor costs of cleaning permanent lines are less than for assembled lines. (c) There is a considerable saving in product losses resulting from faulty gaskets, nuts and caps. (d) There is a marked saving in depreciation and maintenance of pipes and fittings. (e) Better plant appearance results. C. J. Babcock

658. Controlled cleaning. D. H JACOBSEN, Cherry-Burrell Corp., Chicago, Ill. Ice Cream Field, **57,** 3: 28, 103. Mar., 1951.

The essentials of cleaning ice cream equipment are outlined. Chemical "sterilization" is recommended for most ice cream equipment. Even when heat "sterilization" at 180° F. for 5 min. has been used on heaters, pasteurizers and sanitary lines, chemical "sterilization" prior to use is recommended. W. C. Cole

659. Power washing centrifugal separator. W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,564,899. 3 claims. Aug. 21, 1951. Official Gaz. U. S. Pat. Office, **649,** 3: 738. 1951.

A cream separator designed to permit washing of the bowl and discs without disassembly is described. The cleaning fluid is discharged through a valve, responsive to centrifugal force, at the bowl periphery. R. Whitaker

660. Good housekeeping means dollars and cents. C. B. WELLS, Whitson Div., The Borden Co., New York. Ice Cream Field, **51,** 3: 30, 32, 34, 104. Mar., 1951.

Good housekeeping in a plant includes every room as well as surroundings of the plant and delivery equipment. The roles of the plant superintendent and laboratory director are stressed. The author considers plant housekeeping as an index of plant profits. Cooperation of labor and management is essential to the success of any housekeeping program, and will improve products and increase sales, it is claimed. W. C. Cole

661. Communities awarded milk sanitation ratings of 90 per cent or more, July 1949–June 1951. Anonymous. Pub. Health Reports, **66,** 34: 1086–1090. 1951.

A revised list is presented of 211 cities and counties awarded milk sanitation ratings of 90% or more by the public health service from July 1, 1949 to June 30, 1951. A total of 51 communities have been added and 21 dropped from the previous list. Some communities with high-grade milk supplies have not been included because (a) no arrangements have been made for the State milk sanitation authority concerned to determine their rating, (b) some ratings submitted had lapsed since they were over 2-yr.-old and (c) certain communities showed no desire for rating or inclusion in the list even though some may have had high-grade milk supplies.

D. D. Deane

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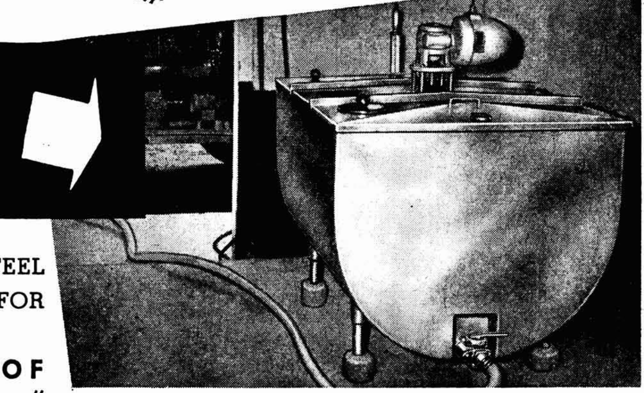

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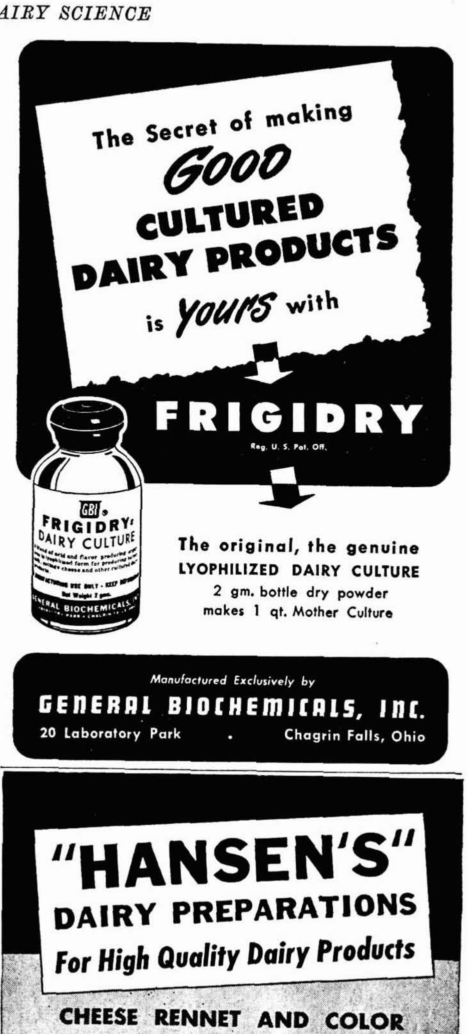

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Sample of journal citation: (1) JONES, L. W., AND SMITH, J. D. Effect of Feed on Body of Butter. J. DAIRY SCI., 24: 550–560. 1941.

Sample of book citation: (1) LANDSTEINER, K. The Specificity of Serological Reactions. Rev. Ed. Harvard University Press, Cambridge, Mass. 1945.

For Experiment Station publications, the citation should be as follows: (1) COULTER, S. T., AND JENNESS, R. Packing Dry Whole Milk in Inert Gas. Minn. Agr. Expt. Sta. Tech. Bull. 167. 1945.

The more common abbreviations used in the text are: cm., centimeter(s); cc., cubic centimeter(s); g., gram(s); mg., milligram(s); γ, microgram(s); ml., milliliter(s); mμ, millimicron(s); C., Centrigrade; F., Fahrenheit; lb., pound(s); oz., ounce(s).

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