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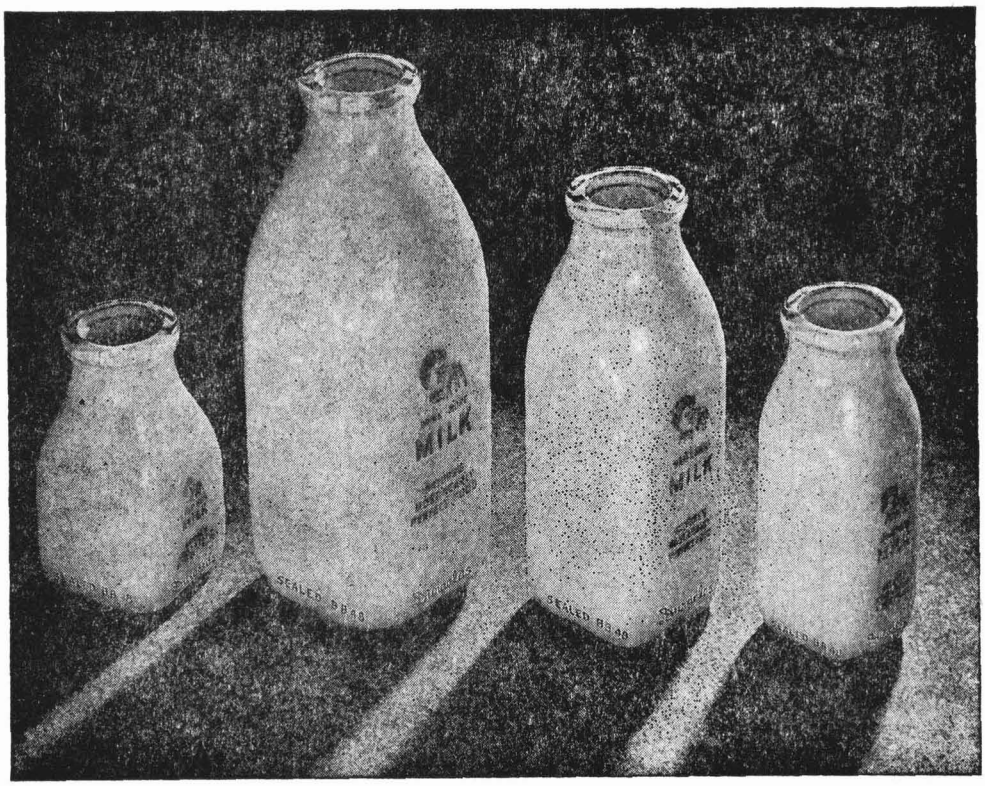

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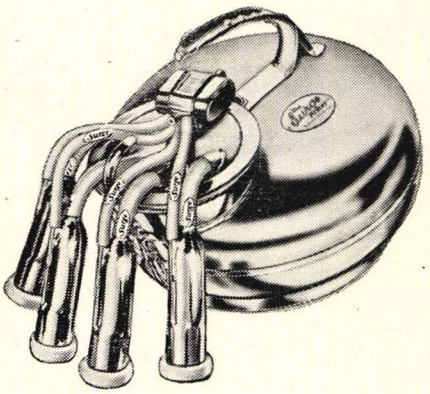

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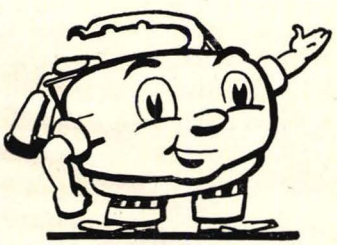

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THE RELATIONSHIP OF THE PREPARTUM DIET TO THE CAROTENE AND VITAMIN A CONTENT OF BOVINE COLOSTRUM

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The purpose of the experiments reported in this paper was to determine the relationship of the prepartum diet to the carotene and vitamin A content of bovine colostrum.

The literature revealed scanty information concerning the effect of the feed of the dry cow upon the carotene and vitamin A content of colostrum. Kramer *et al.* (5) reported values of 25 and 28 I.U. of vitamin A per gram of colostrum from two cows on rye pasture and values of 16 and 20 I.U. for two cows receiving winter rations. Henry *et al.* (3) noted that cows on pasture before calving produced colostrum containing more carotene than barn fed cows, but that vitamin A was not affected. Stewart and McCallum (11) were unable to increase the vitamin A content of colostrum by feeding 3 lbs. of carrots or one-seventh of a pint of cod-liver oil per day to cows on winter rations. The paucity of available information and the practical importance of colostrum seemed to warrant further study of this problem.

PLAN OF EXPERIMENT

Twenty-nine Holstein and four Guernsey heifers in the Cornell University dairy herd were used in this experiment. These heifers had been on excellent pasture prior to the experiment and were considered to be in good physical condition. Approximately 60 days before calving they were divided into four dietary groups. The experimental diets were as follows: A low-carotene ration composed of wheat straw and a commercial grain mixture containing 12 per cent of protein; a standard dry-cow fitting ration consisting of the same grain mixture plus good quality hay and corn silage; a carotene-rich ration made by supplementing the standard dry-cow ration with one million I.U. of carotene[1] daily; and a vitamin A–rich ration made by

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[1] Commercial concentrate containing 50,000 I.U. per gram, purchased from General Biochemicals, Inc., Chagrin Falls, Ohio.

adding one million I.U. of vitamin A[2] daily to the standard dry-cow ration. Periodic determinations of the potency of the carotene concentrate were made by measuring the absorption of a petroleum ether extract in an Evelyn photoelectric colorimeter at 440 mμ. Vitamin A potency of the shark-liver oil was determined by saponifying a weighed amount of the oil, dissolving the non-saponifiable portion in chloroform, and measuring the Carr-Price reaction in an Evelyn photoelectric colorimeter. The carotene content of the hay (U. S. No. 1 timothy-clover mixed) was 11.6 μg. per gram, and the corn silage contained 5 μg. of carotene per gram on the fresh basis as determined by a slight modification of the chromatographic method proposed by Charkey and Wilgus (2). Feed intakes were not recorded. The experimental rations were fed until the cows calved, after which a regular milking herd ration was fed.

Samples of colostrum were obtained on the first, third, and seventh days following parturition. The first sample, drawn shortly after parturition and before the calf had nursed, was obtained by pooling a pint of colostrum hand-milked from each of the four quarters of the udder. This sample was taken to represent the first colostrum as obtained by the calf following birth. After the first colostrum sample was taken, the cows were machine milked twice daily. All calves were removed from their dams at the end of the second day. The third and seventh day samples were taken from the sixth and fourteenth milkings postpartum. Carotene and vitamin A determinations were made according to the method of Boyer *et al.* (1).

RESULTS

The mean values with their standard error as well as the individual values in each dietary group are given in tables 1 and 2. The significance of mean differences was determined by a t test. P values of 0.05 arbitrarily are considered as significant, and P values of 0.01 are considered highly significant (6).

Carotene

The data presented in table 1 show the relationship of the prepartum diet to the carotene content of the colostrum. Carotene concentration is expressed per unit of volume and per gram of butterfat.

Pre-nursing sample. The difference between the means of the low-carotene group and the standard group is not significant, but the means of the carotene supplemented and the vitamin A supplemented groups are significantly higher when the results are expressed per gram of butterfat. The daily addition of one million I.U. of carotene to the standard fitting ration did not increase significantly the carotene content per 100 ml. of colostrum over that of the standard group. On the other hand, the mean value per

[2] Shark-liver oil, 41,000 I.U. per gram, generously supplied by National Oil Products Co., Paterson, N. J.

TABLE 1

The effect of the prepartum ration on the carotene content of colostrum

Cow no.	μg. per 100 ml.			μg. per gram butterfat		
	Pre-nursing	Third day	Seventh day	Pre-nursing	Third day	Seventh day
Low-carotene ration						
R–26	142	21	.....	22.9	14.0	.....
3	38	21	22	10.8	4.2	3.2
R–86	65	24	29	12.3	4.0	5.2
8	130	51	.....	9.5	5.8	.....
12	47	54	.....	10.8	6.1	.....
13*	187*	102*	29*	64.7*	20.6*	8.0*
17	45	19	15	14.5	4.4	2.9
R–95	169	12	5	18.2	4.5	1.0
24	42	28	5	11.3	2.6	0.9
Mean ± S.E.	85 ± 19	29 ± 5	15 ± 5	13.8 ± 1.6	5.7 ± 1.2	2.6 ± 0.8
Standard dry-cow ration						
1	208	50	21	35.4	9.6	3.9
7	32	61	19	21.4	9.2	3.7
14	170	57	19	22.9	8.1	2.5
R–74	121	40	13	33.5	9.2	3.4
23	70	27	25	15.0	6.5	3.1
34	38	58	38	23.0	8.9	9.2
Mean ± S.E.	107 ± 29	49 ± 4	23 ± 4	25.2 ± 3.1	8.6 ± 0.0	4.3 ± 1.0
Carotene-supplemented ration						
4	195	123	44	23.6	16.5	9.7
5	187	66	20	31.8	7.5	2.8
9	136	52	18	47.1	9.5	1.9
10	111	63	18	41.4	8.4	3.1
18	46	48	31	44.6	11.6	3.2
29*	198*	86*	41*	68.5*	16.0*	7.9*
30*	790*	127*	117*	173.9*	30.0*	19.5*
31	124	47	11	30.0	5.7	1.8
32	86	96	26	21.9	12.2	3.2
33	140	102	31	48.6	18.6	5.2
Mean ± S.E.	128 ± 17	75 ± 10	25 ± 4	36.1 ± 3.7	11.3 ± 1.3	3.9 ± 1.5
Vitamin A-supplemented ration						
2	56	105	26	40.2	12.7	6.2
R–64	100	61	15	28.5	11.4	3.1
6	72	38	26	24.1	6.3	3.1
16	400	25	1	61.5	3.5	1.7
20*	111*	80*	34*	71.7*	16.8*	5.4*
22	56	.....	17	18.1	.....	4.4
26	132	20	12	13.9	2.7	2.2
27	57	28	17	14.5	5.7	1.6
Mean ± S.E.	125 ± 32	46 ± 13	16 ± 0.04	28.7 ± 4.1	7.1 ± 1.6	3.2 ± 0.6

* Guernsey not included in mean.

gram of butterfat of the carotene-supplemented group is significantly higher (0.01 level) than that of the other groups.

Third-day sample. The increase in carotene content per unit of volume and per gram of butterfat of the carotene-supplemented group over that of

the other groups is highly significant. In contrast to the pre-nursing sample, the mean value of the low-carotene group is significantly lower than that of the standard group.

TABLE 2

The effect of the prepartum ration on the vitamin A content of colostrum

Cow no.	μg. per 100 ml.			μg. per gram butterfat		
	Pre-nursing	Third day	Seventh day	Pre-nursing	Third day	Seventh day
Low-carotene ration						
R–26	220	32	.....	35.5	21.4	.....
3	180	90	66	51.3	18.2	9.7
R–86	320	116	36	60.8	19.4	6.5
8	694	205	.....	51.0	23.4	.....
12	231	214	.....	53.3	24.4	.....
13*	86*	174*	11*	29.7*	35.1*	3.0*
17	172	64	47	55.6	14.7	9.2
R–95	188	62	45	19.6	23.1	9.6
24	216	150	24	58.1	14.0	4.3
Mean ± S.E.	278 ± 58	117 ± 23	44 ± 6	48.2 ± 4.5	19.8 ± 1.3	7.9 ± 1.1
Standard dry-cow ration						
1	418	88	29	71.1	16.9	5.4
7	182	97	12	121.6	14.7	2.3
14	735	202	70	98.9	28.8	9.4
R–74	160	71	33	44.3	16.4	8.6
23	626	117	98	134.8	28.3	11.9
34	124	163	84	75.9	25.0	20.4
Mean ± S.E.	374 ± 107	123 ± 21	54 ± 14	91.1 ± 13.8	21.7 ± 2.6	9.7 ± 2.5
Carotene-supplemented ration						
4	757	201	92	91.7	26.1	20.2
5	608	192	39	103.4	21.9	5.4
9	318	95	50	100.5	17.4	5.4
10	229	182	54	85.4	24.2	9.3
18	55	78	225	53.3	18.9	23.4
29*	335*	103*	44*	115.9*	19.2*	8.5*
30*	397*	57*	24*	87.4*	13.5*	4.0*
31	226	87	17	54.7	10.5	2.7
32	142	169	65	19.6	21.6	8.1
33	89	93	37	30.8	17.0	6.8
Mean ± S.E.	303 ± 87	137 ± 19	72 ± 23	67.4 ± 11.3	19.7 ± 1.9	10.2 ± 3.2
Vitamin A-supplemented ration						
2	199	896	98	142.8	108.5	23.4
R–64	239	216	62	68.1	40.3	12.8
6	429	323	43	143.4	54.0	5.1
16	5760†	249	67	885.9†	35.5	11.5
20*	309*	358*	101*	199.6*	75.4*	16.0*
22	962	.....	79	310.7	.....	20.7
26	1750	181	64	184.3	24.3	11.9
27	540	396	139	137.7	80.8	13.4
Mean ± S.E.	687 ± 210 1411‡	377 ± 108	79 ± 11	164.5 ± 32.9 267.5‡	57.2 ± 12.9	14.1 ± 2.3

* Guernsey, not included in mean.
† Unusually high value not included in mean.
‡ Including value †.

Seventh-day sample. Significant differences in the mean values of these samples are found only between the low-carotene group and the standard and carotene-supplemented groups. There is no difference per gram of butterfat.

Vitamin A

The effect of the prepartum ration on the vitamin A content of colostrum is shown in table 2.

Pre-nursing sample. There are no significant differences in the mean values of the low-carotene, the standard, and the carotene-supplemented groups when the results are expressed on a volumetric basis. On the other hand, per gram of butterfat, the difference between the low-carotene group and the standard and carotene-supplemented groups is highly significant. Colostrum from the cows receiving the vitamin A supplement contained 409, 313, and 384 μg. more vitamin A per 100 ml. and 116, 73, and 97 μg. more per gram of butterfat than that from the low-carotene, the standard, and the

TABLE 3

The relationship of the diet of the dry cow to the vitamin A potency of colostrum

Ration	Pre-nursing	Third day	Seventh day
	*I.U./100 ml.**	*I.U./100 ml.**	*I.U./100 ml.**
Low-carotene ration	1245	516	201
Standard dry-cow ration	1674	574	254
Carotene-supplemented ration	1425	773	330
Vitamin A-supplemented ration	5850	1584	343

* Assuming 0.6 μg. carotene = 1 I.U.
Assuming 0.25 μg. vitamin A = 1 I.U.

carotene-supplemented cows, respectively. These differences are highly significant. No explanation is apparent for the extremely high vitamin A value of the pre-nursing sample from cow no. 16.

Third-day sample. There is no apparent difference between the low-carotene, the standard, and the carotene-supplemented groups. The third-day sample of colostrum from the cows receiving the vitamin A supplement contained significantly more vitamin A than that from the other groups.

Seventh-day sample. Seven days after calving little difference existed between the vitamin A content of the colostrum from the cows receiving the low-carotene, standard, and carotene-supplemented rations. However, the effects of vitamin A supplementation still were evident, as indicated by the significant increase per gram of butterfat of the vitamin A–supplemented group over the other groups.

The total vitamin A potencies of the colostrum samples, expressed as I.U. per 100 ml., are given in table 3. These results are expressed on a volumetric basis as being representative of the vitamin A intake of the newborn calf. Colostrum from the vitamin A-supplemented cows contained 5,850 I.U. as

compared to 1,245 I.U. for the low-carotene group, 1,674 I.U. for the standard group, and 1,425 I.U. for the carotene-supplemented group. Effects of the vitamin A supplementation still were evident on the third day, this colostrum being approximately three times more potent in vitamin A than that from the low-carotene or standard group.

It was thought that the form of vitamin A present in the diet might be a factor in the mammary transmission of vitamin A. The data shown in table 4 indicate that the vitamin A present in colostrum is entirely of the ester form regardless of the prepartum ration. The analytical method used was essentially that described by Kascher and Baxter (4) for separating the ester and alcohol forms of vitamin A.

TABLE 4

The relationship of prepartum diet to the form of vitamin A in colostrum

Ration	Breed	Carotenoids	Vitamin A	
			Alcohol	Ester
		μg./100 ml.	*μg./100 ml.*	*μg./100 ml.*
Standard dry-cow	H	191	0	335
Carotene (alfalfa leaf meal)–supplemented	G	440	0	163
Vitamin A (ester form)–supplemented	G	724	0	1307
Vitamin A (ester form)–supplemented	H	136	2	213
Vitamin A (alcohol form)–supplemented	H	132	0	325
Vitamin A (alcohol form)–supplemented	H	158	0	251
Vitamin A (alcohol form)–supplemented	H	172	0	558

The plasma carotene and vitamin A values of the experimental cows 60 and 18 days before parturition have been reported (10). Using these values, highly significant positive correlations of 0.509 ± 0.14 and 0.523 ± 0.139 were found between the plasma carotene and vitamin A 18 days before calving and the carotene and vitamin A content of the pre-nursing samples of colostrum.

DISCUSSION

The value of colostrum in combating scours and allied diseases of newborn calves was demonstrated by Smith and Little (9). Later workers (8, 11) have associated the protective characteristics of colostrum with its high vitamin A content. The data presented here show that the prepartum diet of the bovine may influence markedly the vitamin A activity of colostrum.

Large variations in the carotene and vitamin A levels were observed among these samples of colostrum. Undoubtedly sampling procedure was a factor. However, it is not known whether the butterfat content and the associated fat-soluble vitamins of colostrum vary with the completeness of milking, as is the case with normal milk. By expressing the carotene and vitamin A content on a per gram of butterfat basis, the variations due to

sampling should be minimized, since it seems unlikely that the first drawn sample should contain more or less of the fat soluble vitamins per gram of butterfat than the last drawn sample. Further study of this problem is needed.

The second finding in these studies is the lack of increase of vitamin A in the colostrum when one million I.U. of carotene was added daily to the standard dry-cow ration. These results are an interesting corollary to the relatively poor fetal storage of vitamin A in the newborn calves from these cows as previously reported (10). Specific differences in the efficiency of conversion of carotene to vitamin A, the stability of carotene in the digestive tract, and the action of vitamin E in conserving carotene and vitamin A may well be contributing factors.

Although it has been shown that the prepartum diet and the concentration of carotene and vitamin A in the blood stream prior to the decline at parturition influences the level in the colostrum, the processes involved largely are a matter of speculation. Liver reserves of carotene and vitamin A probably play a part and may explain the relatively high colostral carotene and vitamin A of the low-carotene group, since these heifers were on excellent pasture prior to the experimental period. The accumulation of milk in the udder prior to parturition and the observation of Petersen and Rigor (7) that milk left in the udder for several days assumes the composition of colostrum may indicate a simple storage phenomenon.

The question warrants further study as to whether or not supplementing the dry-cow ration with extra vitamin A will result in superior performance by the newborn calf or the cow.

SUMMARY

A study has been made of the relationship of the prepartum diet to the vitamin A and carotene content of bovine colostrum. Four different rations were fed to 29 Holstein and 4 Guernsey heifers during the last 60 days of their gestation periods. The rations were a low-carotene ration of wheat straw and a concentrate mixture; a standard dry-cow ration of concentrate, mixed hay and corn silage; the standard ration supplemented with one million I.U. of carotene daily; and the standard ration supplemented with one million I.U. of vitamin A daily.

Colostrum from cows receiving the low-carotene ration contained significantly less vitamin A per gram of butterfat than did colostrum from cows receiving the standard dry-cow ration.

The carotene content per gram of butterfat in the colostrum from the carotene-supplemented cows was significantly higher than that from the other groups, although the vitamin A content was not increased.

Colostrum from cows receiving the vitamin A supplement contained an average of 687 μg. per 100 ml. or 164.5 μg. per gram of butterfat, while the

colostrum from the standard dry-cow ration group contained only 374 μg. per 100 ml. or 91 μg. per gram of butterfat, showing that the vitamin A content of colostrum may be influenced by the prepartum diet.

Regardless of the form of vitamin A in the ration, the ester form of vitamin A predominated in the colostrum.

Highly significant positive correlations were found between the plasma carotene and vitamin A of the cows 18 days before calving and the carotene and vitamin A content of the colostrum.

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A STUDY OF A CREAM SEPARATOR BOWL WHICH IS CLEANED BY CENTRIFUGAL FLUSHING

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In 1938 the W. and J. Whitehead, Ltd., in Laisterdyke, England, sent a centrifugal cream separator to the Illinois Agricultural Experiment Station. The bowl of this machine was designed to provide cleaning and drying by centrifugal force.[1] In 1945, another Whitehead separator was received by the Experiment Station. Both machines were investigated under various conditions of operation.

PRELIMINARY EXPERIMENTS

Tracy and Tuckey (8) in 1938 conducted a number of experiments with the English machine. The purpose of these early experiments was to study the automatic cleaning principle of this separator and ascertain its practical usefulness under farm conditions. The machine was used on two different farms near the Illinois Agricultural Experiment Station for periods of 5 to 7 days. Its operation also was studied in the dairy laboratories of the Experiment Station and results compared with those of a well-known American separator. The bacterial quality of the cream and skim milk was determined. The results of 24 separations, involving 12 comparisons, indicated that the English machine compared favorably with the American machine when both were operated under comparable conditions. This preliminary investigation indicated that the English separator was cleaned easily, that it was practical, that dairymen were interested in its development, and that the two farmers who used the separator were pleased with its performance. The separator was returned to England and World War II delayed further developments.

METHODS

In general, the washing procedure after each separation was as follows:

1. One pint of water at 100° F. was passed through the machine.
2. Next, 20 lbs. of water containing approximately 0.1 per cent of a

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[1] The bowl has a specially constructed water distributor which allows cleaning solutions to flow from the supply tank to all parts of the bowl while it is in motion. In the shell of the bowl there are three discharge ports, equal distance apart, and each one is closed by a valve which is held in place by a steel spring. The tension in the steel spring is overcome by the centrifugal force so that the ports are tightly closed when the bowl revolves at its normal speed. When the speed slackens to one-half that of normal, the valves open and cleaning solutions can be forced through with turbulent cleaning action.

detergent[2] was flushed through the machine. This flushing was accomplished by first allowing the bowl to decrease in speed, which permitted the port valves to open; then about one-fourth of the water was allowed to run through. The speed of the bowl was increased, but not sufficiently to close the ports, and another one-fourth of the water was flushed through the bowl. This was repeated a third and a fourth time until all the water had been used.

3. The speed of the bowl again was increased to force out the last particles of water.

4. The supply tank and spout assembly were brushed after each separation (beginning with separation 37), using the detergent solution which previously had been flushed through the bowl; and finally rinsed with water at about 100° F.

5. Before separating each lot of milk, 25 lbs. of water at 165–170° F. was flushed through from the supply tank in about four equal portions. Samples of the whole milk, cream, and skim milk were taken with sterile pipettes, placed in sterile tubes, and cooled to about 35° F. with iced water. Plate counts for bacteria were made in duplicate, using tryptone-glucose-extract agar. The plates were incubated at 37° C., for 48 hours. Mold and yeast counts were made in duplicate on potato-dextrose-agar, acidified to pH 3.5 with tartaric acid. The plates were incubated 4 days at room temperature.

Fat tests were made by the Babcock method on the milk and cream and by the American Association method (3) on the skim milk in trials 2, 3, 4, and 5.

EXPERIMENTAL

In July, 1945, a new model of the English separator, slightly different in construction from the first one, was sent to the Experiment Station and subjected to more detailed investigation. In construction, the shell of the bowl and the water distributor were made of aluminum, while the cream screw and valve springs were made of brass and steel, respectively. The supply tank, spout assembly, and discs were not heavily tinned because of the existing tin shortage.

Testing the centrifugal flushing principle. The inventor had claimed that this machine could be cleaned by centrifugal flushing and that it was unnecessary to disassemble the separator for cleaning except at infrequent

[2] This detergent is called "Vel" and is the sodium salt of a sulfated monoglyceride. The coco-glyceride is chiefly used. The sodium sulfo-monoglyceride constitutes about 30–55 per cent on a dry basis; moisture 1–2 per cent; tetrasodium pyrophosphate 8–9 per cent, and the balance is mostly sodium sulfate, resulting from the reaction in obtaining the sulfated monoglyceride salt. This detergent was used in all trials and the authors are indebted to the Palmolive-Colgate-Peet Company for the supply used in the experiments reported in this paper.

intervals. The objective in this series of separations was to operate the machine each morning and evening over a period of 2 weeks without disassembling it and to determine the effectiveness of centrifugal flushing by measuring the quality of the cream and skim milk with the plate count method. The results are given in table 1. The machine was operated

TABLE 1

The effect on the bacterial plate counts of the cream and the skim milk of operating the English machine for 28 separations (Trial 1)

Separation no.	Time	Counts per ml. of:		
		Milk	Cream	Skim milk
25	a.m.	37,000	94,500	65,000
26	p.m.	37,100	96,000	13,700
27	a.m.	7,900	188,000	10,400
28	p.m.	8,600	146,000	7,800
29	a.m.	7,000	65,000	54,000
30	p.m.	4,200	1,630,000	*
31	a.m.	13,800	251,000	6,400
32	p.m.	7,600	1,700,000	14,500
33	a.m.	7,500	35,000	10,400
34	p.m.	8,000	1,250,000	8,500
35	a.m.	2,600	50,000	4,700
36	p.m.	2,600	1,300,000	257,000
37	a.m.	240,000	115,000	110,000
38	p.m.	215,000	174,000	130,000
39	a.m.	4,800	2,100	3,900
40	p.m.	6,200	2,000	32,000
41	a.m.	7,300	1,000	2,000
42	p.m.	9,900	26,000	4,900
43	a.m.	7,400	151,000	76,000
44	p.m.	7,600	10,000	9,700
45	a.m.	3,900	64,000	3,900
46	p.m.	5,500	9,800	47,000
47	a.m.	2,900	6,400	5,900
48	p.m.	3,600	4,900	7,500
49	a.m.	7,000	4,900	7,900
50	p.m.	7,000	10,000	9,700
51	a.m.	471,000	250,000	321,000
52	p.m.	449,000	414,000	806,000

* Sample lost.

without diassembling it through separation 36. The spout assembly was inspected and sour and putrid cream was found in the cream spout. Evidently the force of the washing solution from the bowl was not sufficient to remove adhering cream. Beginning with separation 37, the supply tank and spout assembly were washed by brushing each morning and evening with the water that previously had been flushed through the bowl. Including separation 38 and through the remainder of this trial, with the exception of separation 43, the plate counts of the cream do not reveal any significant contamination from one separation to the next. The only cream samples that revealed any yeasts and molds were those from separation 50, which showed 1 yeast and 1 mold colony; separation 51, which showed 1 yeast and

9 mold colonies; and separation 52, which showed 17 mold colonies per ml. The plate counts of the milk separated each morning and evening agreed closely, as expected, since the milk was obtained from the same lot.

The bowl was taken apart after separation 52. The lock ring had a bad odor as the result of milk solids which had accumulated in the threads, for it had not been tightened sufficiently at the beginning of the trial. The shell of the bowl was clean, but the dividing disc had some milk solids at the points opposite the holes in the discs. The top disc had a slight deposit of milk solids around each hole, and the amount of this deposit became progressively greater from the top to the bottom disc. The lower surface of the milk distributor had some adsorbed milk solids.

The inventor had claimed that the centrifugal flushing action from the

TABLE 2

The effect on the bacterial plate counts of the cream and skim milk of operating the English machine for 14 separations without disassembling the bowl, using a combination of detergents (Trial 2)

Separation no.	Time	Counts per ml. of:		
		Milk	Cream	Skim milk
53	a.m.	9,500	4,200	16,000
54	p.m.	13,000	3,500	18,000
55	a.m.	8,300	5,600	11,000
56	p.m.	9,800	7,600	20,000
57	a.m.	20,000	4,800	25,000
58	p.m.	260,000	8,000	40,000
59	a.m.	300,000	15,000	10,000
60	p.m.	15,000	4,000	*2,600,000
61	a.m.	8,000	1,400	5,500
62	p.m.	4,200	1,400	4,800
63	a.m.	11,000	*4,500	5,800
64	p.m.	10,500	*14,000	10,000
65	a.m.	120,000	*75,000	150,000
66	p.m.	15,000	50,000	332,000

* Spore-forming bacteria were present.

bowl also would be sufficient to clean the spout assembly. However, this series of separations proved conclusively that it is necessary to wash the supply tank and spout assembly after each separation. This was done in all the remaining trials.

In a second trial, the detergent "Vel" was combined equally by weight with another detergent and the mixture used in a concentration of about 0.1 per cent. The washing procedure was the same as that used in the previous experiment. The results are recorded in table 2. The machine was disassembled at the termination of the trial, and the underneath surfaces of all the discs had an adsorbed layer of white material which was, in all probability, a precipitate from the combined detergents. There was no separator slime on the inner wall of the bowl, but small amounts of solid materials were deposited near two of the discharge ports.

Washing the bowl without a detergent. A third trial was conducted to determine if the bowl could be washed without a detergent. Following the flushing of the bowl with the hot water, a pint of cold water was run through the machine to cool it so that milk would not adhere to the metal. The supply tank and spout assembly were washed as in previous trials, with the detergent solution. The milk separated each morning and evening was from the same lot.

The results in table 3 do not reveal any great increases in plate counts of the cream that could be attributed to contamination in the bowl from the preceding separation. The plate counts for bacteria in the cream and skim

TABLE 3

The plate counts of the cream and skim milk obtained with the English machine when no detergent was used to wash the bowl during 14 separations (Trial 3)

Separation no.	Time	Counts per ml. of:				
		Milk	Cream			Skim milk
		Bacteria	Bacteria	Yeasts	Molds	Bacteria
67	a.m.	49,000	42,000	.....	5	96,000
68	p.m.	63,000	36,000	.....	8	114,000
69	a.m.	142,000	106,000	1	16	170,000
70	p.m.	180,000	93,000	.....	11	210,000
71	a.m.	10,000	4,200	2	6	14,000
72	p.m.	11,000	5,300	4	7	14,000
73	a.m.	4,500	4,100	2	4	6,600
74	p.m.	6,100	2,300	.....	3	6,800
75	a.m.	71,000	4,200	1	5	86,000
76	p.m.	70,000	6,600	.....	6	87,000
77	a.m.	2,800	9,200	.....	9	51,000
78	p.m.	36,000	11,000	.....	7	47,000
79	a.m.	230,000	129,000	1	2	389,000
80	p.m.	276,000	142,000	.....	3	328,000

milk obtained from the duplicate lots of milk that were separated each day are in close agreement.

Examination of the various parts of the bowl revealed a very slight deposit of milk solids near the holes on the lower side of the first disc. This formation became progressively greater on each disc from top to bottom, whereas the upper surface of every disc was free from milkstone. The base of the bowl spindle was covered with a thin film of white material which was lightly adsorbed. The outer surface of the binding ring had a layer of milkstone, but there was no other deposit on the outside of the bowl.

Comparing the English and the American separators. A fourth trial was conducted to compare the quality of the cream and skim milk obtained by separating duplicate lots of milk with the English and the American separators. The American machine was washed after each separation by the rapid method suggested by Rudnick (7) except that 25 lbs. of water at

165–170° F. was passed through it immediately before each separation. The procedure for the English separator was the same as that outlined. Two 10-gallon cans of milk were obtained from the University dairy. The milk from one can was mixed well and divided equally each morning; one half of it was separated with the American and the other half with the English machine. The same procedure was repeated in the evening with the other can of milk, which had been held at 35–40° F. The results are summarized in table 4. The plate counts of the cream from separations 82 and 106 with the English machine are higher than the comparable plate counts from separations 81 and 105 with the American machine. The plate counts for

TABLE 4

Comparison of the bacterial plate counts of the cream and skim milk obtained by separating duplicate lots of milk with the American and English machines (Trial 4)

Separation nos.	Time	Counts per ml. of:				
		Milk	Cream		Skim milk	
			American	English	American	English
81,* 82†	a.m.	3,700	3,800	11,000	3,400	5,600
83, 84	p.m.	2,300	1,100	1,500	2,000	2,200
85, 86	a.m.	6,800	3,200	2,000	4,800	5,300
87, 88	p.m.	10,600	2,500	700	7,500	6,800
89, 90	a.m.	2,700	1,200	1,500	2,400	2,900
91, 92	p.m.	900	900	600	2,000	900
93, 94	a.m.	10,400	‡	‡	9,800	11,000
95, 96	p.m.	2,000	600	1,500	2,800	1,900
97, 98	a.m.	1,700	4,200	900	2,600	2,500
99, 100	p.m.	5,500	500	2,400	2,100	2,600
101, 102	a.m.	2,000	400	1,900	2,400	2,900
103, 104	p.m.	1,100	600	800	1,600	1,700
105, 106	a.m.	1,700	500	38,000	1,100	1,000
107, 108	p.m.	3,500	400	4,800	1,600	1,200

* Odd nos. represent separations with the American machine.
† Even nos. represent separations with the English machine.
‡ Samples lost.

the cream in the other 12 comparisons and those for all the skim milks obtained with both machines agreed closely.

The bowl of the English machine was sanitary at the end of the trial except for the three bottom discs, which showed slight traces of dried milk solids. There was not the slightest trace of milk solids on either the inner shell or the outer surface of the bowl. The rapid procedure used to wash the American machine did not remove all milk solids from the lower surfaces of all the discs.

Separating milk of inferior quality. The milk separated with the English machine in the preceding trials was of good quality, as indicated by relatively low plate counts. It was deemed advisable to determine the effect of separating milk of poor quality on the plate counts of the cream

and skim milk and on the centrifugal flushing ability of the bowl in the English machine. Two 10-gallon cans of milk were obtained and dumped into a vat, mixed well, and poured back into the same cans. One can of milk was separated immediately and the other was held at 36–40° F. and separated in the evening. The washing procedure was the same as that outlined.

With the exception of separation 112 (table 5) there was little evidence of contamination from one separation to the next, as indicated by the bacterial plate counts of the cream. Except for separations 109 and 110, there is fairly close agreement in the plate counts of the skim milk each morning and evening from the duplicate lots of milk. The milk used in separations

TABLE 5

The effect on the plate counts of the cream and skim milk of separating milk of poor quality with the English machine (Trial 5)

Separation no.	Time	Counts per ml. of:				
		Milk	Cream			Skim milk
		Bacteria	Bacteria	Yeasts	Molds	Bacteria
109	a.m.	12,500,000	1,650,000	2	6	2,220,000
110	p.m.	16,000,000	3,100,000	.....	.....	23,000,000
111	a.m.	20,000,000	6,400,000	2	12	21,500,000
112	p.m.	32,500,000	18,400,000	100	2	14,700,000
113	a.m.	1,200,000	1,500,000	130	100	4,700,000
114	p.m.	5,800,000	1,450,000	80	10	4,100,000
115	a.m.	4,200,000	9,300,000	4	7	5,500,000
116	p.m.	4,400,000	7,200,000	4	5	3,900,000
117	a.m.	300,000	250,000	9	2	330,000
118	p.m.	430,000	220,000	4	5	450,000
119	a.m.	1,000,000	460,000	12	7	1,300,000
120	p.m.	1,500,000	420,000	250	3	1,000,000
121	a.m.	5,800,000	8,000,000	12	2	8,200,000
122	p.m.	3,200,000	6,500,000	5	3	8,600,000

119 and 120 showed evidence of mastitis, as indicated by the presence of dark-colored coagulated material on a sediment disc. The inner shell of the bowl was inspected and found to be clean before proceeding with separation 119. Immediately after completing separation 120 and after washing by the prescribed procedure, dark material was found adsorbed on the inner shell of the bowl. This material had accumulated during separations 119 and 120 and was not removed by the washing procedure. A sediment test was made of the milk used in separations 119 to 122, inclusive, and the discs scored 6.5 and 7, which is indicative of the poor quality of the milk. At the conclusion of the trial the three bottom discs had small amounts of adsorbed milk solids, but the others were bright and clean on the upper and lower surfaces.

In trial 5, the fat content of the milk averaged 4.6 per cent and that of the resulting cream, 57.43 per cent. The cream contained a higher per-

centage of fat in this trial because the screw on the bowl did not permit a sufficient range of adjustment for milk of high fat content to obtain cream of what might be called normal fat content. In spite of the high fat content of the cream, the bowl was remarkably well cleaned up to and including separation 118, as indicated by the appearance of the inner shell.

Distribution of bacteria in the cream and the skim milk. This study provided data on the distribution of the bacteria in the cream and skim milk. The results from trials 2, 3, 4, and 5 are shown in table 6; trial 1 was excluded because it was preliminary. In 85 per cent of the separations with the English machine, the cream had a lower plate count for bacteria than the skim milk; in 80 per cent of the separations the cream had a lower count than the original milk. Similar results were obtained by Tracy and Tuckey (8). The results in table 4 also show that the cream obtained with

TABLE 6

Comparative distribution of the plate counts of the milk, cream, and skim milk for 55 separations with the English separator (Trials 2, 3, 4, and 5)

Plate counts of:	Total	
	No.	%
Cream lower than skim milk	47	85
Cream higher than skim milk	8	15
Cream lower than milk	44	80
Cream higher than milk	11	20

the American machine had a lower plate count than the skim milk in 11 of 13 comparisons. These data agree with the results of seven separations reported by Ulvin and Cree (9). The other data reported in the literature do not agree on the distribution of bacteria in cream and skim milk obtained by centrifugal separation. Eckles and Barnes (2) and Anderson (1) reported higher plate counts in cream than in skim milk. The results of Lamson (5) indicate less or only slightly more bacteria in separated cream than in the whole milk, but that the skim milk contained fewer bacteria than the whole milk. Leete (6) in 100 separations did not find much difference in the bacterial counts of cream, skim milk, and whole milk, the average counts being 501,000, 313,000, and 435,000 per ml., respectively.

The factors which determine the distribution of bacteria in cream and skim milk obtained by centrifugal separation should be studied in more detail because of the implications in determining bacterial standards for market cream. It is probable that the physical state of the fat globules and their membrane materials, the temperature of the milk, and the types, numbers, and specific gravity of the microorganisms are factors which determine their retention by either the cream or the skim milk.

Skimming efficiency. The skimming efficiency of the English machine was determined in trials 1, 2, 3, 4, and 5. The results assembled in table 7

TABLE 7

The milk fat content of the skim milk from 84 separations with the English machine

% milk fat in skim milk	No. of separations
0.03	1
0.04	2
0.05	8
0.06	11
0.07	20
0.08	14
0.09	11
0.10	6
0.11	2
0.12	3
0.13	1
0.14	0
0.15	3
0.16	0
0.17	0
0.18	0
0.19	0
0.20	2
Mean 0.08	84

indicate an average of 0.08 per cent fat in the skim milk, which compares favorably with results reported in the literature (4) of 0.06 to 0.08 per cent fat in the skim milk for a factory separator. In trial 5, 14 comparisons were made with the American and English machines. While the fat tests of the skim milk were slightly lower for the American machine, the data are insufficient to draw definite conclusions.

Time required to wash the English separator. Washing this separator by centrifugal flushing of the bowl, including brushing and rinsing the supply tank and spout assembly, varied from 3.5 to 5.5 minutes, depending on the experience of the operator. A person accustomed to the procedure can wash this machine after each separation in about 3 minutes and assemble it for operation in about 0.5 minute. When each experimental trial was terminated, the supply tank, spout assembly, and the bowl were disassembled, the discs inspected and washed, and the machine reassembled. The time required for this operation for one person for whom records are available was 17 minutes.

DISCUSSION

The experiments reported indicate that the English separator cannot be cleaned entirely by centrifugal flushing, as originally claimed by the inventor. However, the bowl can be washed easily and quickly without disassembling it; the supply tank and spout assembly must be washed after each separation. The bowl was cleaned remarkably well by the flushing action of the washing solutions, even though it was necessary to use a mild detergent of low alkalinity because of the aluminum bowl and some of its

parts and because of the inadequately tinned discs, supply tank, and s assembly.

The fact that the plate counts of the cream were lower than those o skim milk in 85 per cent of the separations is of interest, but it is ques able if these results can be attributed to any superiority of the En machine as similar results were obtained in a few trials with the Ame machine and in a limited number of trials by other investigators (9).

The skimming efficiency of the English machine of 0.08 per cent f the skim milk, using the American Association method (3), compares f ably with results reported for a factory separator.

This separator has been demonstrated to be practicable for farm Furthermore, the useful life of the bowl will be prolonged because it have to be disassembled and washed less frequently than present mach

CONCLUSIONS

1. The bowl of the English separator can be washed properly by trifugal flushing with two separations daily over a period of 1 to 2 w

2. In the great majority of cases, there was no significant contamin from one separation to the next, as indicated by the plate counts o cream and of the skim milk.

3. Presence of 0.08 per cent fat in the skim milk from the En separator as determined by the American Association method com favorably with results obtained with a factory separator.

4. The plate counts of the cream were lower than the skim milk per cent of the cases and lower than the milk in 80 per cent of the cases

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THE EFFECT OF SULFANILAMIDE IN THE DILUENT UPON FERTILITY OF BULL SEMEN

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Since 1939, when the possible effects of bacteria on the results of metabolic studies with spermatozoa or on the results of artificial insemination were first discussed by Salisbury *et al.* (9), this laboratory has been engaged in seeking a solution to that problem (3, 4, 5, 8). Recently, Knodt and Salisbury (5) have shown that 300 mg. of sulfanilamide added per 100 ml. of yolk-citrate diluent resulted in significant improvements in the livability of bull spermatozoa stored in this diluent. The sulfanilamide effectively controlled most of the bacteria normally found in semen and also depressed carbohydrate and oxygen utilization, but it increased the accumulation of lactic acid. Whether or not the stimulation of the spermatozoa to greater livability was due to the control of bacteria or to the effect on their metabolism was not proved.

The present paper deals with the fertility of bull spermatozoa when diluted with yolk-citrate to which sulfanilamide was added at the rate of 300 mg. per 100 ml.

EXPERIMENTAL

Three different experiments were conducted to determine the effect of sulfanilamide on fertility. Each was planned so that the diluent treatments were effectively randomized among the samples of semen collected and used from each experimental bull. The bulls used were those owned by the New York Artificial Breeders' Cooperative, Inc., and inseminations were made by the regularly employed inseminators on cows owned by the members.

The first experiment. The first study was conducted in June, 1945. It was designed to compare the regular yolk-citrate diluent with a yolk-citrate-sulfanilamide diluent containing 300 mg. of added sulfanilamide per 100 ml. and, also, a yolk-citrate-glucose diluent containing 540 mg. of added glucose per 100 ml. The latter diluent was used to determine the effect of added glucose upon fertility, for it had been shown earlier (10) that glucose effectively increased livability of and lactic acid production by bull spermatozoa.

The citrate buffer containing 3.6 g. of sodium citrate (dihydrate) per 100 ml. was prepared with water distilled in glass and autoclaved for 20 minutes at 15 lbs. pressure. Glucose was made up in approximately

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isotonic solutions (5.4 per cent) and sterilized in the same way as the citrate solutions. The yolk-citrate-glucose diluent was prepared by adding 20 ml. of isotonic glucose solution to 80 ml. of citrate buffer, and this mixture was added to 100 ml. of yolk to give a level of 540 mg. of glucose per 100 ml. Various multiples of these proportions were utilized, depending upon the total need for diluent.

The citrate-sulfanilamide solution was prepared by heating water distilled in glass to approximately 80° C. and then dissolving in it the desired amount of sulfanilamide and citrate to give a final concentration of 3.6 g. sodium citrate (dihydrate) and 600 mg. of sulfanilamide per 100 ml. This solution was made up to a predetermined volume before autoclaving at 15 lbs. pressure for 20 minutes. Equal volumes of yolk and citrate-sulfanilamide solution then were mixed for the final diluent. In the first investigation, all diluents were made up before the investigation started and no precautions were taken to prevent direct light from falling on the citrate-sulfanilamide diluent during the 26 days which were required to complete the collections.

Ten bulls were used, 5 Holsteins and 5 Guernseys. Three collections at intervals of 1 week to 10 days were taken from each bull, and the diluent to be used for any particular collection for any one bull was assigned at random. Thus each bull was a block and the design was that of a randomized block.

All semen was diluted so that each milliliter of diluted semen contained 30 million spermatozoa. This was done in order not to have dilution rates as a confounding factor. Later (7) it was found that this precaution was unnecessary within the limits of dilution used and it was not continued in the two subsequent experiments. Semen was shipped to 47 member units of the Cooperative.

Table 1 shows the data for the average quality characteristics of the semen used with each diluent. Statistical analysis of the data showed no significant differences between the semen samples used for each treatment. As can be seen, additions of glucose apparently increased methylene blue reduction time, but this difference was not statistically significant. Also, the results of the inseminations are shown in table 1. It is obvious that no difference in fertility was found between the yolk-citrate and the yolk-citrate-sulfanilamide diluents in this experiment.

In view of our more recent investigations, the authors are of the opinion that as a consequence of storing in clear bottles on the laboratory desk, the sulfanilamide in the alkaline citrate (pH about 7.45) was oxidized by the action of light, as evidenced by a slight browning, and thereby lost its effectiveness. This view is supported by data from a later experiment in which a limited number of ejaculates were treated with freshly prepared citrate-sulfanilamide as compared to the citrate-sulfanilamide diluent which

TABLE 1

Average semen quality characteristics and fertility results in the first experiment

Semen character	Diluent used		
	Yolk-citrate	Yolk-citrate-glucose	Yolk-citrate-sulfanilamide
Concentration 1,000's/mm.^{3}	1,232	1,280	1,190
Motility, %	80.0	79.0	79.0
Methylene blue reduction time, min.	3.88	4.68	4.08
Fertility			
Services, no.	786	720	767
5-mo. N.R.,* no.	377	352	368
5-mo. N.R., %	48.0	48.9	48.0

* N.R. = non-returns to service.

had been exposed to light. The action of light brought about changes in the sulfanilamide solution so that it failed to stimulate spermatozoan livability.

The addition of glucose to the citrate resulted in a slightly smaller proportion of returns to service during the 5 months following service, but the difference was not mathematically significant. There was some evidence in the data that the samples to which the glucose was added had been used more days in the field than the other samples, but again this difference was not statistically significant.

The second experiment. The second fertility experiment was conducted in November, 1945. The design was essentially the same as in the first experiment. Collections from ten bulls, 5 Holsteins and 5 Guernseys, were made twice with an interval of 7 to 11 days. To one or the other of these collections the diluent to be used, yolk-citrate or yolk-citrate-sulfanilamide,

TABLE 2

Average semen quality characteristics and fertility results in the second experiment

Semen character	Diluent used	
	Yolk-citrate	Yolk-citrate-sulfanilamide
Concentration 1,000's/mm.^{3}	1,189	1,187
Motility, %	79.5	78.5
Methylene blue reduction time, min.	4.82	4.88
Fertility		
Services, no.	676	765
5-mo. N.R.,* no.	379	476
5-mo. N.R., %	56.1	62.2

* N.R. = non-returns to service.

was assigned at random. In this investigation the two buffers for the diluents were made up and sterilized as described above, but were stored in the dark during the 18 days required to complete the semen collections. The diluted semen was shipped to 66 local units of the Cooperative. Table 2 gives the average semen quality characteristics and the results of the inseminations.

In this experiment the results were strikingly different from those obtained in the first one. An analysis of covariance, using the number of services as the independent variable and 5-month non-returns as the dependent variable, indicated that the 6.1 per cent difference in level of fertility for the diluents was mathematically significant at the 5 per cent level of probability.

The varying results of the first and second experiments suggested that the different fertility levels observed might be due simply to the random variability typical of biological experiments. The number of sulfanilamide-treated semen samples was small, only 10, and the level of odds for the differences in fertility was just barely over the 5 per cent point.

The third experiment. A third experiment was planned to test the matter more thoroughly. It was desired to study not only the effect of sulfanilamide on fertility, but to study certain other questions as well. Work by Gunsalus *et al.* (4) had indicated that first ejaculates contained a higher concentration of bacteria than did second ejaculates collected a few minutes later. Mercier *et al.* (6) found that first ejaculates were more concentrated, but were of lower volume and contained spermatozoa of which a somewhat smaller percentage were motile. As far as was known, no controlled investigation had been conducted to determine whether first ejaculates were less fertile than second ones, though such an opinion was known to be held by some workers in this field. If such opinions were found to be based upon fact, the question arose as to whether sulfanilamide, supposedly by bacteriostatic action, would eliminate the difference between first and second ejaculates.

The third experiment was conducted during June, 1946, and 16 bulls, 12 Holsteins and 4 Guernseys, were used. The bulls within a breed were paired at random. Two collections, spaced on the average 14 days apart, were made from each bull. At each collection two consecutive ejaculates of semen were obtained and used separately. For any one bull at each collection period one or the other of the ejaculates was diluted with the diluent containing 300 mg. of sulfanilamide per 100 ml. At the next collection period either the first or second ejaculate, whichever had not been treated with sulfanilamide the previous time, received it. The design was thus a $2 \times 2 \times 2$ replicated 8 times and is illustrated thus:

Design of the third experiment

Ejaculate no.	Collection	
Bull 1	1	2
1	Y-C-S*	Y-C†
2	Y-C	Y-C-S
Bull 2		
1	Y-C	Y-C-S
2	Y-C-S	Y-C

* Y-C-S = Yolk-citrate-sulfanilamide.
† Y-C = Yolk-citrate.

In order to record the identity of each ejaculate, only one of each collection could be shipped to a particular inseminator. Thus, the number of inseminating units to which semen was shipped (76 different circuits) was divided into two approximately equal groups. For example, at one collection one group of circuits received one ejaculate, the second group the other ejaculate. At the next collection the same circuits received the same ejaculate number but the treatment was different. This design involved the willful confounding of the groups of circuits to which the semen was shipped, with a comparison of first and second ejaculates. This fact did not invalidate the test of whether or not first ejaculates responded differently to sulfanilamide than did second ejaculates, for the design was orthogonal in this respect.

The citrate-sulfanilamide buffer was prepared by bringing a measured

TABLE 3

Average semen quality characteristics and fertility results in the third experiment

Semen character	Diluent used			
	Yolk-citrate		Yolk-citrate-sulfanilamide	
	Ejac. 1	*Ejac. 2*	*Ejac. 1*	*Ejac. 2*
Volume, ml.	5.30	5.78	5.38	5.97
Concentration 1,000's/mm.^{3}	1,397	1,178	1,409	1,188
Motility, %	71.9	73.8	70.6	72.2
Methylene blue reduction time, min.	4.2	5.1	4.3	5.3
Fertility				
Services, no.	1,392	1,092	1,149	1,151
5-mo. N.R., no.	821	661	716	761
5-mo. N.R., %	59.0	60.5	62.3	66.1
Combined				
Services, no.	2,484		2,300	
5-mo. N.R.,* no.	1,482		1,477	
5-mo. N.R., %	59.7		64.2	

* N.R. = non-returns to service.

quantity of water distilled in glass to a boil and adding the predetermined quantity of citrate and of sulfanilamide. The solution immediately was removed from the heat source, poured directly into sterile bottles, and stored in the dark.

The data on semen quality and results of insemination are shown in table 3. The data are arranged to give a comparison of first and second ejaculates and to show the effect of diluents on the fertility of the first and the second ejaculates. If one were interested only in a semen-quality comparison of first and second ejaculates before treatment in this study, the proper comparison is between first ejaculates for yolk-citrate and the second ejaculates for yolk-citrate-sulfanilamide. The other consecutive paired ejaculates are the first for yolk-citrate-sulfanilamide and the second for yolk-citrate.

Statistical analysis of the semen quality characteristics showed that the bulls differed significantly only in volume of semen produced. The specification that two consecutive ejaculates which were satisfactory for use be collected at each of two collection periods was responsible for this result. No semen sample was considered acceptable which contained less than 800,000 spermatozoa per mm.^{3}, or which contained less than 60 per cent motile spermatozoa. A total of 20 bulls was sampled for this experiment, 4 of which failed to meet the above specifications.

The spermatozoa of the second ejaculate were significantly more motile than those of the first ejaculate. Highly significant differences were found between first and second ejaculates in concentration and methylene blue reduction time. The first ejaculates were somewhat more concentrated and reduced methylene blue faster than did the second ejaculates. Other differences, as between collection periods, were not of important magnitude and were not mathematically significant.

It should be mentioned that the insemination data were recorded separately for cows being bred for the first time and those which had failed to conceive on first service and were being returned for a second service. The same procedure was followed for the two earlier experiments discussed. In the first two experiments the number of observations was too small to determine whether or not there was a real difference in fertility level for these two groups of cows. In the third, where more observations were made, the difference found was only 1.0 percentage unit, but this small difference was statistically significant. However, the data showed that the addition of sulfanilamide to the diluent increased fertility the same average amount in both groups of cows. In each experiment an analysis of covariance was made on the length of time after collection semen diluted with each diluent was used for insemination. No significant differences were found. For the three experiments combined, 1 per cent of the inseminations were made on the first day (the day of collection), 71 per cent on the second day, 24 per cent on the third, 3 per cent on the fourth, and 1 per cent of all inseminations on the fifth day or later.

As can be seen in table 3 the difference in favor of sulfanilamide in this experiment amounted to a total of 4.5 percentage units. That is, of the 2,300 cows bred with the semen to which sulfanilamide was added, 4.5 per cent more cows apparently conceived than was the case with the 2,484 cows which were bred with the normal yolk-citrate diluent. Statistical analysis of covariance indicated that this difference was greater than was required to show significance at the 1.0 per cent level of probability. Thus, it is concluded that sulfanilamide added to the diluent does increase significantly the probability that a cow will conceive from artificial insemination.

In spite of the fact that no significant differences in semen quality were found between bulls, the differences in fertility among the bulls used was highly significant. However, there was no evidence to indicate that the semen of the several bulls reacted differently to the treatments employed, even though it is known that bacterial contamination varies exceedingly from bull to bull. This fact suggests that the effects of sulfanilamide are largely through the metabolism of semen, and that all normal bull semen would tend to react similarly.

Though first ejaculates tended to be slightly lower in fertility than second ones, an observation bearing out popular belief, the difference observed was not large enough to be mathematically significant when subjected to a test of significance. To test this item effectively a carefully planned and larger experiment should be conducted. The averages given in table 3 suggest that first ejaculates responded less to sulfanilamide treatment that did second ejaculates. However, the sum of squares for error of estimate for the interaction was slightly smaller than the error term, which result forces the authors to conclude that the response to sulfanilamide was not proved different between first and second ejaculates. This observation strengthens the interpretation that the effects of sulfanilamide are largely metabolic ones, for if the effects were due to bacterial control alone one might expect the fertility of the first ejaculates, which contained more bacteria, to be increased more than second ejaculates.

DISCUSSION

It should be mentioned that none of the bulls used in these investigations were known to produce semen containing *Pseudomonas aeruginosa.* This bacterial species is difficult to control and is known to reduce fertility of semen in which it is found as the predominant type (3, 4). Though it was not possible to examine each of the semen samples used in the investigations reported here, the semen of the bulls used had been examined from time to time for bacterial numbers and types, and *Pseudomonas aeruginosa* had not been found.

Reports of the teratogenic effects of sulfonamides when brought into direct contact with certain of the lower animals in the early stages of their

development have appeared in the literature (1, 2, 11, 12). While the authors have not examined each calf produced by the sulfanilamide-treated semen, a number of such animals have been examined and no abnormalities observed. Neither have reports of the birth of abnormal calves been received. When one considers the fact that each milliliter would contain only 3.0 mg. of sulfanilamide, such an amount inseminated into the uterus probably would be absorbed quickly and little would find its way to the developing egg.

The practical value of the results of these studies in routine artificial insemination is obvious. However, in the storage of diluents containing sulfanilamide, care must be taken to keep them out of the light. Preferably, the buffer should be made fresh. However, it is difficult to do so and in large operations this may be a nuisance. Sulfanilamide goes into solution slowly. The authors believe that the simplest way to make the diluent is to bring a required volume of properly distilled water to a boil, add the required amounts of both sodium citrate and sulfanilamide, remove the source of heat, and, when the material has been dissolved and thoroughly mixed, pour the buffer thus prepared into sterile, dark bottles and store it in the dark until used. Buffers so prepared have been used for 2 weeks with no observable influence on their effectiveness. How much longer they can be used satisfactorily has not been determined.

SUMMARY

Three separate experiments involving a total of 8,498 inseminations were conducted to determine the effect on fertility of bull semen of adding sulfanilamide to the yolk-citrate diluent at the rate of 300 mg. per 100 ml. In the first experiment no benefit was observed. In the next two investigations, where the citrate buffer containing sulfanilamide was protected from direct light rays, an increase in fertility by use of the sulfanilamide was obtained. This improvement amounted to 6.1 per cent of the cows inseminated in the second experiment and 4.5 per cent in the third.

The sulfanilamide appeared to influence all semen samples in the same direction, for, in the third experiment, no significant interactions were observed between the treatments and either bulls or first and second ejaculates. These results are interpreted as indicating that the beneficial effects of sulfanilamide on fertility largely are metabolic ones, rather than due to bacterial control alone.

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THE EFFECT OF REFRIGERATOR STORAGE ON THE KEEPING QUALITIES OF PASTEURIZED MILK

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Considerable attention has been directed recently to the problem of the keeping quality of milk in the household refrigerator. The economy of alternate-day or 3-day-a-week delivery of milk was amply demonstrated during the war years with the result that most distributors would prefer to retain this practice. On the other hand, some public health officials have questioned the advisability of maintaining this system of milk distribution because of the highly perishable nature of the product. Under the 3-day-a-week system of delivery the product is consumed in from 1 to 4 days after pasteurization. Therefore, to justify such a practice, we must assure the consumer that the product delivered will retain its initial high qualities for at least 4 days.

Several investigators have demonstrated that good quality, commercially processed milk can be held in a household refrigerator for a considerable period of time without impairment to its nutritional qualities or bacteriological safety.

Nicholas and Anderson (8) studied the keeping qualities of regular pasteurized, pasteurized-homogenized, and raw milks under household refrigerator conditions (40° F.). They found that pasteurized milks could be stored at 40° F. for a period of 2 weeks or more before spoilage would occur. Furthermore, milks that were removed from the refrigerator each day, shaken, and allowed to stand at room temperature for 1 hour retained high quality for approximately 10 days. These investigators based their conclusions on the results of standard plate counts, titratable acidity, and flavor observation, but did not take into account the psychrophilic or coliform organisms. Since the standard plate counts at the time of souring of most of the milks used in their studies were very little different from those of the original fresh milk, it would appear that a consideration of psychrophilic organisms would have been helpful in interpreting their data.

Mott and Mayer (7) conducted a study involving the collection of retail milks in Boston. They found that the standard plate count of grades A and B pasteurized milks increased after the samples were stored at 40° F., and had average counts of 1,300,000 and 1,700,000, respectively, after a storage period of 5 days. Twenty-two samples of certified-pasteurized milk had an average count of 770 per milliliter after 5 days' storage at 40° F.

A far more comprehensive study was reported by Dahlberg (3), who obtained samples from six milk plants in the New York metropolitan area

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and stored them at various temperatures. The freshly pasteurized milks had an initial, arithmetic average plate count of about 12,000 colonies per milliliter. Allowing these milks to stand at room temperature for 6 hours had the immediate effect of increasing the plate count slightly and giving more positive coliform tests than were observed with samples not exposed to room temperature. Storage temperatures of 35–40° F. decreased the standard plate count of the milk, while the coliform bacteria remained constant. Samples stored at 45–50° F. showed reduced standard plate counts after 1 day, but considerably higher counts after 4 days. At a storage temperature of 55–60° F. a slight decrease in the standard plate count was observed after 1 day, but thereafter the count increased rapidly. All samples in the group were sour after 4 days. With the exception of this latter group, all samples exhibited good flavor after 7 days' storage. Dahlberg concludes that to insure good keeping quality, milk should not be stored at temperatures above 50° F.

Weber (9) reported an analysis of 10,000 coliform tests collected over a 3-year period for three health departments. These data showed that 21.5 per cent of all pasteurized milks tested were positive to the coliform test during the period of January, February, and March, while 48.0 per cent of the tests were positive during July, August, and September. The higher incidence of coliform-positive milk during the warm months was attributed primarily to a greater degree of contamination of the milk from equipment which is exposed to flies, insects, and air-borne sources.

Dahlberg (4) demonstrated more rapid development of coliform bacteria in refrigerated milk during the summer than during the month of October.

A preliminary report (2) covering certain phases of the present study showed that most commercially pasteurized milks could be held for at least 7 days during the summer months if refrigerator temperatures were held at 45° F. or lower.

The present study was undertaken in order to obtain a more complete picture of the changes that take place during the refrigerator storage of commercially processed market milks, under both summer and winter conditions. It was felt that much of the work reported previously was far too narrow in scope and did not present a complete picture of the bacteriological changes characteristic of stored milk.

METHODS

Two separate studies were undertaken, one under summer conditions (July and August) and the other during the winter (February and March). In each study eight different lots of milk were collected from five different commercial plants immediately after the bottling operation. One-half pint and quart samples of the same milk were collected, and where both homogenized and regular milks were available, a set of each was studied. Samples

were iced and transported to the University laboratories, where exposure treatments, bacterial analyses, vitamin analyses, acidity tests, and flavor observations were conducted prior to storage.

As a standard control, one-half pint samples were stored under the cold unit of a large household-type refrigerator. A sufficient number of these samples was stored so that a different bottle could be removed for analysis and observation at each interval of the storage period.

Of the replicate quart samples, one was placed in the refrigerator immediately, a second was allowed to stand at room temperature (summer, 81–89° F.; winter, 74–79° F.) for 1 hour prior to storage, and a third was exposed to room temperature for 2 hours before refrigeration. All quart samples were placed in the refrigerator on the side away from the cold unit. A careful study of temperature variations was made, using recording and maximum-minimum thermometers. The one-half pint samples stored under the cold unit were held at temperatures ranging from 36–42° F. (2.2–5.5° C.) during the summer study and 37–39° F. (2.8–4° C.) during the winter study. The temperature of storage for quart samples held on the side away from the cold unit ranged from 41–45° F. (5–7.2° C.) during the summer study and 39–42° F. (4–5.5° C.) during the winter study. Therefore, the temperature differential between the samples at the two positions of storage was about 3–5° F. during the summer and about 2–3° F. during the winter.

When quart samples were removed for periodic analyses, they were exposed to room temperature for 5 to 7 minutes before being returned to the refrigerator. During this time the temperature of the samples increased about 2–4° F. in the summertime and about 2° F. in winter.

Bacteriological, vitamin, acidity and flavor analyses were made daily for the first 4 days of storage and then at 7 and 10 days. After 10 days, analyses were run every other day until samples showed evidence of acidity change. At this point, samples were examined daily until 0.03 per cent acid had developed. Samples in which 0.03 per cent acid had developed were considered to be "sour," although the flavor of the product was usually quite good and definitely not sour to the taste.

The standard plate count at 37° C. (98.6° F.) (1) was employed for the detection of mesophilic organisms. Dilutions of 1:100 and 1:1000 were employed at the beginning of storage and gradually increased as storage progressed and counts increased. Psychrophilic organisms were similarly determined with standard tryptone-glucose-extract-milk agar plates incubated at 8–10° C. (46.4–50° F.) for 10 days. One milliliter and 0.1 ml. samples were plated for the first few days of storage, after which time high counts necessitated considerable sample dilution.

Coliform organisms were determined on desoxycholate agar plates incubated at 37° C. (98.6° F.) for 24 hours prior to counting; a 5 ml. sample was plated, using one plate with 2 ml. and one with 3 ml. When counts became high, smaller amounts or dilutions were plated.

Acid development was measured by the standard titration technique using phenolphthalein as the indicator and is expressed as lactic acid.

Riboflavin analyses were conducted according to the method of Hand (5), using a Coleman photofluorometer for measuring fluorescence. Ascorbic acid was determined by the conventional titration procedure employing sodium 2,6-dichlorobenzenoneindophenol dye.

RESULTS

Trend of Bacterial Development during Storage

The regular pasteurized milk usually displayed somewhat better keeping qualities than the homogenized product from the same plant. For the first 7 days of storage the differences usually were insignificant, but thereafter the homogenized milks appeared to support the growth of mesophilic and psychrophilic bacteria better than the unhomogenized product. The type of growth and growth curves were very similar. It was considered justifiable to average the counts of both types of similarly treated milk for the first 7 days of storage. Also one plant participating in the study produced only homogenized milk, while another sold only regular unhomogenized milk.

Since most milk is consumed before it is 7 days old, it seemed unnecessary to make a complete tabulation of bacteriological data taken after this time. Therefore, a geometric mean average was tabulated for each class of samples which had been subjected to the same treatment during the same season.

A graphic presentation of bacteriological data is shown in figure 1. It is very evident that all types of bacterial development were more rapid during the summer season. One factor which undoubtedly contributed to this condition was the slightly higher temperatures that prevailed in the refrigerator during the summer months. Another factor which may have influenced this trend was the greater "temperature shock" given quart samples during the warm summer months both before storage and on days when analytical samples were removed. A difference in the type of microflora present in summer and winter milks also might have contributed to this difference.

These data demonstrate the fact that any treatment which allows the milk to warm up will be reflected in the rate of development of all types of bacteria. This is especially evident from the data taken during the summer months. Furthermore, it can be seen that the more extensive the period of warming before storage, the poorer was the bacteriological keeping quality.

For the most part, mesophilic counts went down or remained rather constant for the first few days of storage, after which their rate of development increased according to the degree of exposure to room temperature (samples 2, 3, and 4). In summer milks which were subjected to warming either before storage or on days when samples were analyzed, the mesophilic count did not show any evidence of increasing until after 2 days of storage.

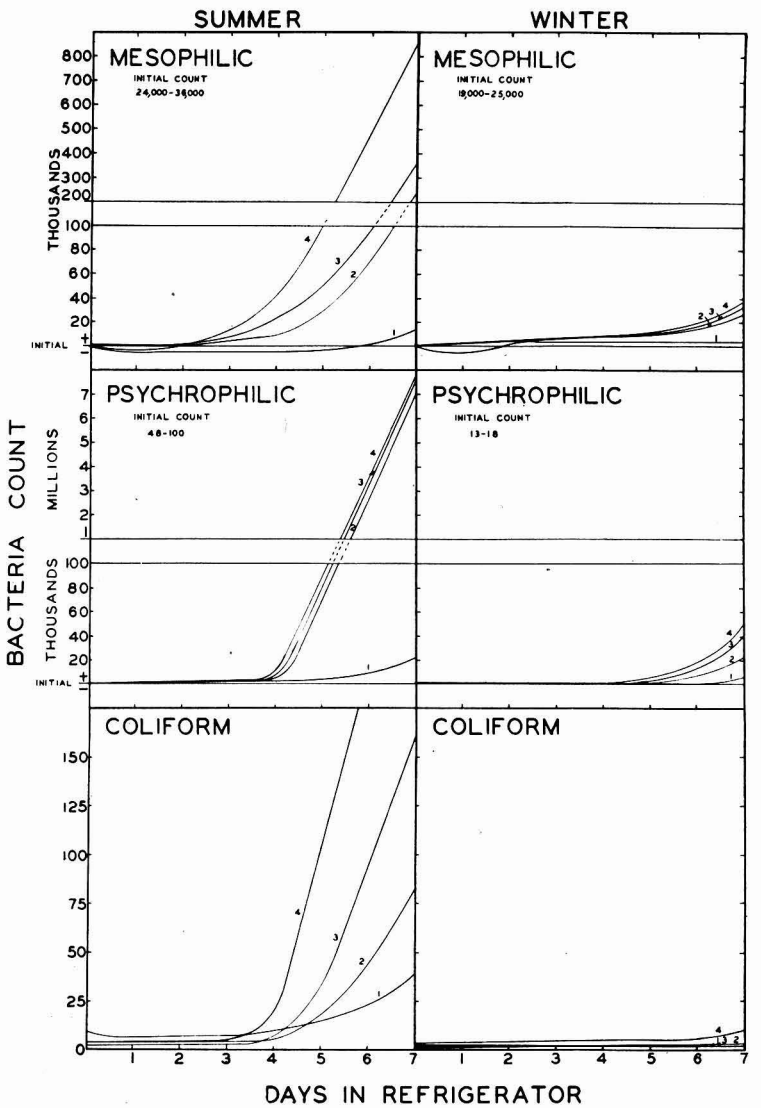

FIG. 1. The effect of refrigerator storage on the micro-flora of commercial market milks (geometric mean averages of all data taken). (1 = one-half pints left in refrigerator continuously; 2 = quarts removed from refrigerator each day for sampling; 3 = quarts allowed to stand out for 1 hour prior to storage, also removed each day for sampling; 4 = quarts allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.)

Undisturbed one-half pint samples (sample 1) showed very little change during 7 days of storage. All samples in the winter study, regardless of treatment, showed very little change during the first 7 days of storage, although the effect of warming before storage was becoming evident in samples 2, 3, and 4 at the end of this period.

The development of the psychrophilic organisms followed a similar pattern, but in the summer series their numbers far exceeded those in the mesophilic class after 6 days of storage. These organisms did not increase appreciably during the first 4 days of storage, but after this time they multiplied at a rather rapid rate. In the winter study the psychrophilic counts did not become significant until after 7 days of storage.

Possibly some mesophilic types may be facultative in the sense that they develop psychrophilic activity after adaptation to prolonged low temperature storage. Support for this view may be found in the fact that mesophilic counts frequently decrease during the first few days of storage. Actually these organisms may be going through a temperature adaptation period which eventually brings them into the active and rapidly growing psychrophilic group. Further support for this possibility is the fact that initial psychrophilic counts of the fresh pasteurized milks in this study usually were very low (0–30). If all growth at low temperatures were due to these few true psychrophiles, probably this growth would develop on a more uniform curve. Since rapid growth of psychrophiles does not occur until after 4 days of storage, probably so-called facultative types may supplement the true psychrophilic population in the final rapid deterioration of the milk.

The development of the coliform organisms followed a pattern similar to that of the mesophiles and psychrophiles. As previously observed by Weber (9), the initial coliform counts in summer milks exceeded those of winter milks. Only two samples were found to be free from coliform bacteria and seven samples showed less than one coliform organism per milliliter during the summer trials. All samples were coliform positive at the conclusion of this series or when 0.03 per cent acid developed. On the other hand, six samples were found to be coliform free and nine had less than one organism per milliliter at the beginning of the winter study. Fourteen samples showed no coliform bacteria at the termination of these trials. In the overall tabulation (fig. 1) the coliform count in winter milk was little changed during the first 7 days of storage.

In evaluating these experimental data in terms of the practical keeping problem in home refrigerators, the conclusion seems justified that good quality commercially processed and distributed milks should maintain desirable bacteriological qualities for at least 4 days when stored at 40° F. Under winter conditions bacterial changes are insignificant from a quality standpoint until after 6 or 7 days of storage. In determining the bacteriological quality of stored milk, the psychrophilic organisms must be taken into ac-

TABLE 1

The comparative micro-flora of freshly pasteurized milk and the same milk after storage and development of 0.03 per cent lactic acid (random samples)

No.	Season of year	Type of bottle	Treatment*	Bacteria counts				Total storage period (days)
				Mesophilic		Psychrophilic		
				Fresh	After storage	Fresh	After storage	
1	Summer	½ pint	A	290,000	38,000	13	177,500,000	21
2	"	½ pint	A	9,000	530,000	8	167,000,000	24
3	"	½ pint	A	61,000	83,000	21	131,400,000	25
4	"	Quart	B	13,600	5,800,000	9	270,000,000	13
5	"	Quart	C	12,200	3,500,000	8	190,000,000	13
6	"	Quart	C	292,000	162,000	26	189,000,000	14
7	"	Quart	D	315,000	221,000	23	178,000,000	13
8	Winter	½ pint	A	14,900	19,600	74	164,000,000	33
9	"	½ pint	A	94,000	213,000	4	248,000,000	35
10	"	Quart	B	18,400	42,000	24	328,000,000	23
11	"	Quart	B	5,000	165,000	1	277,000,000	24
12	"	Quart	C	18,600	21,900	31	315,000,000	23
13	"	Quart	C	67,000	1,730,000	270	150,000,000	12
14	"	Quart	D	128,000	1,650,000	5	260,000,000	20
15	"	Quart	D	39,000	450,000	< 1	201,000,000	17

* A = One-half pint—left in refrigerator continuously; B = Quart—removed from refrigerator each day for sampling; C = Quart—allowed to stand out for 1 hour prior to storage, also removed from refrigerator each day for sampling; D = Quart—allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.

count because of their relatively greater numbers after 5 or 6 days at storage temperatures.

Changes in Micro-Flora during Storage

In analyzing the data taken in both the summer and winter studies and attempting to correlate the initial and final bacterial counts with the relative keeping quality of a product, nothing could be established as an index for a potential trend in bacterial growth. The data presented in table 1 represent a random selection of samples from these studies. These data demonstrate the fact that the initial mesophilic count of the freshly pasteurized milk is not a dependable index of the keeping quality of the product from a bacteriological standpoint. The initial psychrophilic count does not serve as a basis upon which to predict the potential keeping quality of any type of milk, regardless of treatment.

By comparing the data for samples no. 1 and 2 in the summer study and 8 and 9 in the winter study, it can be seen that high count milks (nos. 1 and 9) can have keeping qualities as acceptable as similar milks (nos. 2 and 8) with relatively low initial counts. This should not be taken to imply that the standard plate count is not a useful test for evaluating general milk quality, but it does suggest that its use as an index of potential keeping quality of pasteurized milk is questionable and probably unsound.

Acid Development in Stored Milks

The length of the storage period which elapsed before milks developed 0.03 per cent acid (expressed as lactic acid) naturally varied among the different lots and treatments. One-half pint samples which remained undisturbed in storage did not develop 0.03 per cent lactic acid until 21.8 days (average) of storage in the summer study and 26.6 days (average) in the winter trials. Quart samples which were allowed to stand out at room temperature for 2 hours before storage and were further exposed at periodic sampling periods developed 0.03 per cent lactic acid in 12.4 days (average) in summer and 16.6 days (average) in winter trials.

Most samples examined in these studies showed evidence of lactic acid development while mesophilic counts (standard plate) were still quite low. Some samples developed 0.03 per cent lactic acid while exhibiting a mesophilic count lower than that of the original fresh milk. Nicholas and Anderson (8) have reported "sour" flavor in samples with mesophilic counts which were little different from those of the fresh products.

In the present study the psychrophilic organisms largely are responsible for the development of the lactic acid in milk held in refrigerator storage at 40° F. The data plotted in figure 2 are for three samples, chosen at random from the many which were plotted and compared. A close correlation exists between the growth of psychrophilic bacteria and the development of lactic acid. By comparison, the magnitude of change in the numbers of mesophilic

organisms is relatively insignificant. This would suggest that the acid developed, at least in the early stages of deterioration, is a product of the psychrophilic bacteria. Apparently consideration of psychrophiles is of paramount importance in studies of this kind, particularly since acid development is considered to be one of the primary factors in milk spoilage or deterioration.

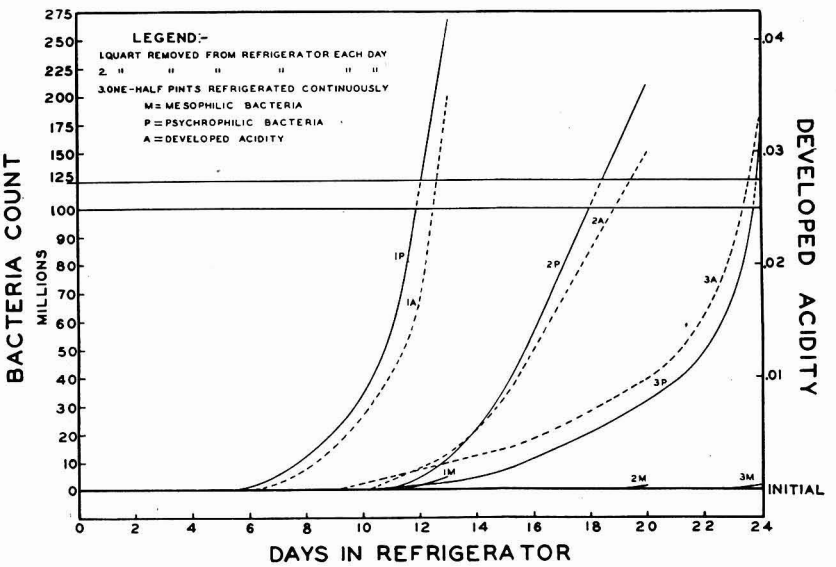

FIG. 2. The relationship between lactic acid development and bacterial growth in milks refrigerated for long periods of time (random samples from different periods during study).

The Effect of Refrigerator Storage on Riboflavin and Ascorbic Acid

Since riboflavin and ascorbic acid are subject to destruction by exposure to light and the latter is subject to natural oxidation on storage, a study of these two vitamins in stored milks appeared desirable.

The data shown in table 2 demonstrate the stability of riboflavin and ascorbic acid in milks stored under household refrigerator conditions. Because of the absence of light in the refrigerators, it is not surprising that riboflavin was stable in samples held continuously under these conditions. Even the quart samples B, C and D, which were exposed to the light of the room at the time of periodic samplings, still retained practically all of their riboflavin throughout the extended storage period. Apparently the normal daylight present in the laboratory was insufficient to cause any great degree of photolysis of this vitamin during these periodic exposures. These results confirmed our previous observations (6) where samples exposed in a bright kitchen for 2 hours lost only a small percentage of riboflavin.

TABLE 2

The effect of refrigerator storage on the stability of riboflavin and ascorbic acid (random samples)

No.	Season of year	Type of bottle	Treatment*	Ascorbic acid mg./l.			Riboflavin mg./l.		Total storage period (days)
				Fresh	After 1 day	After 4 days	Fresh	After storage	
1	Summer	½ pint	A†	7.1	1.6	0.0	1.51	1.52	28
2	"	½ pint	A	8.3	3.2	0.0	1.62	1.61	31
3	"	Quart	B	11.3	9.7	2.0	1.57	1.56	13
4	"	Quart	C†	14.0	13.5	12.1	1.61	1.61	14
5	"	Quart	D	10.1	8.1	2.8	1.55	1.52	12
6	Winter	½ pint	A†	9.1	2.8	0.0	1.62	1.63	25
7	"	½ pint	A†	15.1	12.7	12.0	1.73	1.71	24
8	"	Quart	B	9.1	0.0	0.0	1.73	1.71	15
9	"	Quart	C	6.7	2.0	0.0	1.39	1.37	20
10	"	Quart	D	6.6	1.9	0.0	1.40	1.39	20

* A = One-half pint—left in refrigerator continuously; B = Quart—removed from refrigerator each day for sampling; C = Quart—allowed to stand out for 1 hour prior to storage, also removed from refrigerator each day for sampling; D = Quart—allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.

† Homogenized milk.

The retention of ascorbic acid during storage was very poor in most samples. Even the one-half pint samples which were refrigerated continuously and not exposed to light retained very little ascorbic acid after 1 day in storage. The exposure of samples to room temperature and light conditions for 2 hours caused an initial loss of about 20 per cent of this vitamin during the summer studies and about 12 per cent in the winter trials.

The high level of ascorbic acid found in the homogenized milk from one plant (samples 4 and 7, table 2) was difficult to explain. This milk was pasteurized at 170–172° F. for 16 seconds. Additional samples procured over a period of weeks showed that this heat treatment produced some sulphydryl compounds which protected ascorbic acid. Whereas most samples (homogenized or unhomogenized) were completely depleted of ascorbic acid within 4 to 6 days, most of these homogenized samples retained significant quantities of this vitamin throughout the entire storage period. The regular unhomogenized milk from this plant was pasteurized at a conventional temperature and ascorbic acid losses conformed with those experienced with milk from other plants.

Flavor Changes during Storage

During the winter months the samples in these studies exhibited somewhat better keeping qualities than did those in summer months. However, off-flavor development was somewhat more prevalent in the winter. Oxidized flavor was more frequently found during the winter trials than during the summer. Only one sample developed a typical oxidized flavor during the summer trials.

On the whole, the milks retained their initial fine flavor until very near the end of the storage period (0.03 per cent developed acid), when stale, unclean, acid or bitter flavor defects usually developed. With the exception of the occasionally observed oxidized flavor previously mentioned, practically all samples retained their initial flavors for at least 7 days. In several cases samples exhibited excellent flavor for more than 20 days of storage. Apparently the problem of flavor development during storage is relatively no more important when milk is consumed within a week after pasteurization than if consumed within 2 days.

DISCUSSION

One of the most interesting observations made in the course of these investigations is the apparent rôle of the psychrophilic bacteria in the deterioration of milk stored under refrigerator conditions. The initial mesophilic count (standard plate) appears to have little bearing on the potential keeping qualities of milks stored under the conditions of these experiments. Mesophilic counts at the termination of the storage period (0.03 per cent developed acid) frequently were little different from those exhibited by the

fresh milk before storage. The suggestion that some mesophiles become adapted to storage conditions and eventually grow at these low temperatures seems justified, since mesophilic counts usually go down during the first few days of storage. This is further supported by the fact that rapid psychrophilic growth does not start until after 4 or 5 days of storage. The initial psychrophilic count does not appear to be a dependable index for predicting the potential keeping qualities of milk. The number of psychrophilic organisms in fresh pasteurized milk usually was found to be low (0–30 per ml.), but a considerably higher count never was found to prevent the product from exhibiting excellent keeping qualities.

The development of acidity during storage was found to correlate quite definitely with psychrophilic growth. The mesophilic populations present were comparatively small and probably contributed very little to the development of lactic acid under the conditions of these experiments.

The general growth curves for mesophilic, psychrophilic, and coliform organisms all followed like patterns. The magnitude of population change was much greater in the case of the psychrophiles and was by far the least for the coliforms. In the summer studies little change was observed in any of the bacterial counts until after 4 days of storage, while in winter important changes were not noted until after 7 days.

The flavor of the milks changed very little until near the end of the storage period (0.03 per cent developed acid), when stale, unclean, acid or bitter flavors usually developed. In several cases during the winter studies and in one case during the summer trials, the oxidized flavor developed soon after the milk went into storage. With these exceptions the flavor of the milks was exceptionally good until measureable lactic acid was produced. In very few cases was it possible to taste any evidence of acid until the concentration was in excess of 0.03 per cent.

The storage of milk in the dark at 40° F. did not change the riboflavin content of the product, and the periodic exposure of milk to the daylight in the laboratory had little or no effect upon this vitamin. Ascorbic acid was depleted very rapidly both before and after storage, only insignificant amounts of this vitamin usually remaining after 1 day of storage. One exception was found in the case of homogenized milk which had been flash pasteurized at 170–172° F. The protective effect of sulphydryl compounds produced by this pasteurization temperature accounted for the high levels of ascorbic acid observed in and retained by these samples in storage.

Good quality pasteurized market milks will maintain good bacteriological and flavor qualities for at least 4 days in summer and 6 to 7 days in winter, if refrigerator temperatures are maintained near 40° F. Exposure of milk to room temperatures naturally will impair the keeping qualities of the product, but with good distribution practices and prompt removal of the milk from the doorstep, milk should retain excellent quality for a considerable period of time.

SUMMARY

The psychrophilic bacteria which develop in milk during refrigerator storage are primarily responsible for the deterioration of the product. These organisms apparently are responsible for the development of the acid which is produced in milk during storage at 40° F.

The initial mesophilic (standard plate) and psychrophilic counts do not serve as an index of the potential keeping quality of milk being stored at about 40° F. Mesophilic counts frequently are little changed during the entire storage period.

Riboflavin in milk is not affected by refrigerator storage, and periodic exposure to room daylight has no noticeable effect.

The ascorbic acid content of milk is depleted rapidly both before and during storage. After 1 day of storage, only insignificant quantities remain in milks processed by conventional procedures.

Milk of good quality can be expected to retain excellent bacteriological and flavor qualities for at least 4 days during the summer months and 6 to 7 days during winter months if refrigerator temperatures are maintained near 40° F.

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COMPOSITION OF MARES' MILK AS COMPARED WITH THAT OF OTHER SPECIES[1]

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The skeleton of mammals calcifies very rapidly during the period immediately following birth when there is a rapid progressive penetration of calcium salts into the cartilaginous areas of the skeletal tissues. Furthermore, from an extensive review of the literature dealing with the requirement for calcium during growth of the human infant, Holmes (33) showed that the most rapid skeletal growth occurred during the initial period of the life cycle and that this probably is true for other mammals. Nearly 50 years ago Abderhalden (1) reported that the average foal doubles its birth weight in 60 days. During that period the foal subsists very largely on its mother's milk, and obviously all the mineral elements needed by the foal for bone and tissue building must be derived primarily from the mare's milk. However, very few data are available regarding the mineral content of mares' milk. A number of investigators have reported the amount of ash found in mares' milk: *i.e.*, Linton (39) found 0.28–0.95 per cent, with an average of 0.51 per cent; Vieth (67) found 0.26–0.36 per cent, with an average of 0.30 per cent in the milk from 15 milk mares; Hildebrant (24), 0.32–0.74 per cent; Papp (51), summer milk 0.3 per cent and winter milk 0.5 per cent; Dittrich (13), 0.29–0.60 per cent; and Masek (42), 0.35 per cent in the milk of the wild mare Przewalski kept in the zoological gardens in Prague. However, these data supply little or no information concerning the amount of various mineral constituents such as calcium, magnesium, phosphorus, and potassium in mares' milk, and the present study was undertaken to accumulate data regarding these elements.

EXPERIMENTAL

The mares' milk used in this study was produced by one Palomino and four Percheron mares. All the mares were mature, well-developed, normal animals. Their ages varied from 4 to 10 years and, numbered 1 to 5, consecutively, they were in their second, fifth, first, fifth and first lactations, respectively. The study was made in the spring. The mares' daily ration consisted of 3 quarts of crushed oats, five large ears of thoroughly matured dent corn, all the good-quality hay they desired, and as much rapidly-growing grass as they could eat in 3 or 4 hours. The Palomino mare, no. 1, weighed 1,100 lbs. and the Percherons from 1,600 to 1,900 lbs. The stage of lactation varied from the ninth day for the first sample of milk from

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mare no. 1 to the 129th day for the last sample from mare no. 5. While the stage of lactation was not the same for all the animals, the days on which the milk was collected were identical for all the mares. The samples of milk were collected as described by Holmes and Lindquist (28) and Holmes and associates (31). Samples were taken directly to the laboratory, thoroughly mixed, and assayed for water, protein, reduced ascorbic acid, phosphorus, potassium, magnesium, and calcium by the official methods of the Association of Official Agricultural Chemists (3). In order to minimize possible decomposition, the ascorbic acid assays were begun within an hour after the milk was obtained from the mares.

COMPARISON OF MARES' MILK WITH THAT OF OTHER SPECIES

The results of the assays of the 26 samples of mares' milk collected during the early lactation period are reported in table 1. Data concerning the composition of milk produced by several mammalian species are included in table 2. These data do not constitute all that are available in this field, but they should be sufficient to permit a comparison of the composition of mares' milk with that of other species.

The composition of the milk from each of the five mares varied somewhat from day to day. Since the values obtained for the Palomino mare's milk were not in close agreement with those obtained for the other mares, the average values for milk from the four Percheron mares have been used in the discussion that follows.

Water. The average values for the water content of the milk examined in this study ranged from 88.7 per cent for mare no. 2 to 90.2 per cent for mare no. 4. These values are in good agreement with the results obtained by the English investigators and are greater than that reported for cow, goat, ewe, buffalo, camel, or human milk. However, the difference in water content between mares' milk and that of other species is not sufficiently large to preclude a satisfactory comparison of the other constituents of the milks of the various species.

Protein. The amount of protein in the milk was quite uniform for the individual mares, but the average values for the different animals varied from 2.0 to 2.7 per cent. The average value of 2.3 per cent obtained in this study is higher than that reported by Vieth (67) and Morrison (44) but lower than the values reported by Linton (39) and Hildebrant (24). The protein content of the mares' milk was less than that of cow, goat, ewe, buffalo, camel, or sow milk, but it was similar to that of human milk, as reported by several investigators. In view of the similarity in protein and lactose content of mare and human milk, attention has been given to the possibility of using mares' milk for infant feeding. From his studies in this field, Frendenberg (20) found that because of its low fat content, mares'

TABLE 1

Composition of mares' milk

Mare no.	Day of lactation	Water	Protein*	Ascorbic acid	Phosphorus	Potassium	Magnesium	Calcium
		(%)	(%)	(*mg./l.*)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
	9	88.2	2.9	62	78	74	10.8	126
	10	89.2	3.3	83	79	87	10.8	122
1	11	89.2	3.3	88	85	88	11.8	131
	12	89.1	3.2	90	87	99	11.2	133
	13	.....	3.3	90	90	91	14.7	107
	14	89.8	3.0	73	79	84	10.6	127
Av.	.....	89.1	3.2	81	83	87	11.7	124
	12	88.6	2.9	95	76	63	11.2	142
	13	88.4	2.7	94	61	46	9.1	111
2	14	88.6	2.5	99	65	53	8.9	122
	15	88.9	2.6	101	71	58	9.7	135
	16	88.8	2.6	101	68	60	9.4	127
Av.	.....	88.7	2.7	98	68	56	9.7	127
	25	89.9	2.4	79	72	87	12.9	115
	26	89.6	2.5	83	74	86	12.9	112
3	27	90.1	2.4	70	70	77	12.0	106
	28	89.9	2.3	73	69	76	9.7	102
	29	89.1	2.2	75	69	71	8.6	97
Av.	.....	90.0	2.4	76	71	79	11.2	106
	38	90.3	2.1	88	60	65	9.0	98
	39	90.0	2.1	88	59	66	9.3	97
4	40	90.3	2.0	74	58	62	8.3	93
	41	90.2	2.1	70	58	56	8.9	94
	42	90.0	2.2	68	59	66	7.3	91
Av.	.....	90.2	2.1	78	59	63	8.6	95
	125	89.5	2.0	112	52	55	7.1	79
	126	90.1	2.0	103	50	50	6.4	80
5	127	89.9	2.0	100	52	55	6.6	82
	128	90.1	1.9	108	52	62	6.2	78
	129	89.6	2.0	104	54	59	5.0	80
Av.	.....	89.8	2.0	105	52	56	6.3	80
Av. Percherons (mares 2 to 5, inc.)		89.7	2.3	89	63	64	9.0	102

* Protein = N × 6.38.

TABLE 2

Comparison of mares' milk with that of other species

Source of milk	Water	Protein	Ascorbic acid	Ash	Phosphorus	Potassium	Magnesium	Calcium
	(%)	(%)	(*mg./l.*)	(%)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
Mare	89.04 (39) 90.13 (67) 90.70 (34)	1.65 (67) 2.06 (39) 2.55–3.13 (24) 3.00 (44)	27–115 (54) 87–197 (10) 95 (9)	0.51 (39) 0.30 (67) 0.32–0.74 (24) 0.30–0.50 (51) 0.29–0.60 (13) 0.35 (42)	50 (44)	80 (44)		80 (44)
Cow	86.21 (50) 87.90 (2) 87.10 (66)	3.20 (34) 3.13–3.77 (2) 3.50 (44) 3.30 (14) 3.20 (66)	21–22 (61) 18–20 (32) 25 (38) 20–25 (46) 18 (26) 16 (30) 12–15 (42) 16 (29)	0.69 (14) 0.70 (66) 0.72 (50) 0.68–0.74 (34) 0.70 (44)	68–92 (48, 49, 57, 58) 76–113 (11) 82 (37) 84–127 (63) 93 (61) 100 (36)	123–180 (12) 126–192 (52) 105–172 (2) 143 (60) 140 (44)	5–22 (48, 49, 57, 58) 10–12 (52) 11–17 (63) 7–12 (41) 10 (36)	90–155 (12) 94–150 (63) 96–161 (11) 102–148 (2) 111–132 (52) 118 (60) 120 (36) 155–171 (41)
Goat	87.14 (2) 85.71 (34)	4.29 (34) 3.40 (27) 3.70 (44) 2.99–3.71 (52) 3.99 (4)	5–20 (55) 20 (65) 17 (30) 13 (22) 85 (56) 9 (8) 45 (9)	0.31 (70) 0.94 (6) 0.78 (2) 0.81 (25) 0.81 (43) 0.78 (71) 0.79 (5) 0.80 (68)	98 (5) 124 (19) 96 (6) 104 (43) 112 (27) 100 (44)	193 (6) 171–228 (52) 106–242 (27) 150 (44)	14–20 (52) 13–22 (27)	114 (5) 131 (43) 138 (6) 141 (19) 137 (27) 130 (44) 117–150 (52)

TABLE 2 (*Continued*)

Source of milk	Water	Protein	Ascorbic acid	Ash	Phosphorus	Potassium	Magnesium	Calcium
	(%)	(%)	(*mg./l.*)	(%)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)	(*mg./100 g.*)
Ewe	82.90 (2) 80.82 (34)	6.50 (44) 6.52 (34) 5.44 (2)		0.85 (2) 0.89 (34) 0.90 (44)	120 (44)	190 (44)		210 (44)
Buffalo	76.80 (2) 82.09 (2) 82.69 (34)	5.88 (34) 4.16–6.04 (2)	10 (8)	0.78–0.86 (2) 0.76 (34)				
Camel	87.61 (2)	2.98 (2)		0.70 (2)				
Sow		5.90 (44)		1.00 (44)				
Elephant				0.40 (64)				
Human	87.43 (23) 87.68 (6) 87.41 (34)	2.29 (34) 1.05 (7) 1.63 (23) 1.10 (17) 0.96 (18)	35 (8) 27 (9) 40 (16) 21–90 (53) 5–22 (69) 52 (45) 60–80 (59) 37 (35) 18–46 (62)	0.20 (19) 0.31 (70) 0.31 (6) 0.20 (7) 0.21 (43) 0.18–0.21 (71) 0.31 (34)	13 (43) 20 (19) 16 (47)			23 (19) 30 (47) 35 (43)

Figures in parentheses refer to the number of the reference cited.

milk could not be used successfully for infant feeding unless 1.0 to 1.5 per cent of cows' milk fat was added.

Reduced ascorbic acid. The average ascorbic acid content of the milk produced by the Percheron mares, 89 mg. per liter, was much higher than that previously reported by Holmes *et al.* (31), but was within the range reported by Cimmino (10) and by Rasmussen *et al.* (54), and was practically identical with the value reported by Cimmino (9). The reduced ascorbic acid content of fresh mares' milk was much greater than that of cow, goat, or buffalo milk. The reported ascorbic acid content of human milk varies over wide limits. Widenbaur and Kühner (69) found a minimum of 5 mg. per liter, and Quesada (53) reported as much as 90 mg. When Ingalls *et al.* (35) administered massive doses of ascorbic acid orally or intravenously to women, the milk subsequently secreted had an ascorbic acid content of 116 mg. per liter. A number of investigators (16, 35, 59) have increased the amount of ascorbic acid in human milk several-fold by adding ascorbic acid-rich foods to the diet. Furthermore, other workers found a marked seasonal variation. Sinkko (62) made observations on ten subjects in February and again in September and found 1.8 mg. and 4.6 mg. per liter, respectively. Other authors have found seven or eight times as much ascorbic acid in human milk in late summer as in the winter.

Ash. The reported assays for the amount of ash in mares' milk indicate that the average value is between 0.4 and 0.5 per cent. The mineral content of cow, goat, ewe, buffalo, and camel milk is 0.7 per cent. A number of investigators have reported upon the mineral content of human milk and the average value seems to be between 0.2 and 0.3 per cent. Thus mares' milk contains about two-thirds as much ash as the other species of animals cited and about twice as much as human milk.

Phosphorus. The phosphorus in the twenty samples of milk from the Percheron mares averaged 63 mg. per 100 g. This value is somewhat higher than the value reported by Morrison (44), but it is only about two-thirds as high as in cows' milk. Goats' milk has a slightly higher phosphorus content than cows' milk, but judged by the limited reports found in the literature, human milk contains only about one-fifth as much phosphorus as mares' milk.

Potassium. The average amount of potassium (64 mg. per 100 g.) found in the samples of Percheron mares' milk included in this study is somewhat less than the 80 mg. reported by Morrison (44). The potassium content of cows' milk, as indicated by the reports cited in table 2, is more than twice that of mares' milk. The amount of potassium in goats' and ewes' milk is appreciably higher than that of cows' milk and decidedly higher than that of mares' milk.

Magnesium. The magnesium content of mares' milk as obtained for the individual mares was fairly consistent, but the average values for the differ-

ent animals varied from 6.3 mg. to 11.2 mg. per 100 g. The available literature supplied no data regarding the amount of magnesium in mares' milk. The mean result obtained for the four Percheron mares was 9 mg. of magnesium per 100 g. of milk. This definitely is less than the amount of magnesium present in cows' milk and goats' milk. No data were found concerning the amount of magnesium in human milk.

Calcium. The calcium content of milk, particularly that of cows and goats, has received considerable attention, since these milks are used most frequently as substitutes for human milk in infant feeding. The mares' milk was found to contain an average of 102 mg. of calcium per 100 g. This amount is significantly less than the amount present in cows' or goats' milk. However, it is three or four times the amount reported by Forbes and Keith (19), Nims *et al.* (47), and Maynard (43) for human milk.

Calcium-phosphorus ratio. Since calcium and phosphorus are required in much larger amounts than other minerals for bone formation, the ratio of calcium to phosphorus is of interest to those concerned with animal and human nutrition. The ratio of calcium to phosphorus in cows' milk has been reported as 1.40 (37), 1.20 (36), and 1.27 (60); in goats' milk as 1.44 (6), 1.26 (43), 1.22 (27), 1.14 (19), and 1.16 (5). The values noted in the literature for the ratio of calcium to phosphorus in human milk were very limited and extremely variable: *i.e.*, 2.69 (43), 1.84 (47), and 1.25 (19). The ratio of calcium to phosphorus obtained for mares' milk was 1.62, a value which is definitely higher than that of cows' or goats' milk but possibly lower than the calcium-phosphorus ratio of human milk. It may be noted that Linton (40) has reported that the calcium and phosphorus content of mares' milk decreased linearly with the advance of lactation, but the calcium-phosphorus ratio remained quite constant throughout the lactation period.

SUMMARY

Twenty-six samples of milk produced by one Palomino and four Percheron mares were assayed for water, protein, ascorbic acid, phosphorus, potassium, magnesium, and calcium. The milk was produced in the early lactation period during late winter and early spring months when the mares were subsisting principally upon hay and grain. The milk from the Palomino mare contained more protein, phosphorus, potassium, and magnesium than the Percheron mares' milk. The average values for the milk of the Percheron mares were: water 89.7 per cent, protein 2.3 per cent, reduced ascorbic acid 89 mg. per liter, phosphorus 63 mg., potassium 64 mg., magnesium 9.0 mg., and calcium 102 mg. per 100 g. These values indicate that mares' milk contains more water than cow, goat, ewe, buffalo, camel, or human milk; less protein than cow, goat, ewe, buffalo, or camel milk, but more than human milk; more ascorbic acid than cow, goat, or human milk;

less phosphorus than cow or goat milk but more than human milk; only about one-third as much potassium as cow or goat milk; and less magnesium and calcium than cow or goat milk, but about four times as much calcium as human milk. The ratio of calcium and phosphorus is considerably higher in mares' milk than in cows' or goats' milk but possibly lower than in human milk.

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FLAVONES AND FLAVONE DERIVATIVES AS ANTIOXIDANTS

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The demands of war lent encouragement to the search for acceptable inhibitors of oxidative deterioration of food fats. This impetus resulted in the discovery of many effective processes and chemical agents, most of which have been patented but few of which have been acted upon by the Food and Drug officials.

The literature dealing with antioxidants has been covered by several reviews (6, 8, 9, 22, 26, 36, 37) and the characteristics and limitations of antioxidants and synergists have been discussed by Mattill (32), Golumbic (14), and others.[2] A partial list of antioxidants has been prepared (2).

The resistance of dry milk fat to oxidation has been shown to be due in part to its content of reducing substances (11) as determined by the Emmerie-Engel reagent. This reagent was found to be non-specific for α-tocopherol, a fact now universally recognized. It recently has been modified to determine the solubility in fats of several phenolic antioxidants (29). Experiments with this reagent suggested that reducing substances or components of oxidation-reduction systems that occur naturally in foods and that have food value in themselves should be ideal for incorporation into fats or fat-containing foods to serve as protectors against oxidation.

Reducing substances have been found among citrus juices, citrus peel, flower petals, rose hips and many other naturally occurring materials. Shrader and Johnson (44) recognized in orange juice distinct zones of oxidizing and reducing effects, the reducing effects being associated with the pigments. Svirbely and Szent-Györgyi (45) attributed part of the reducing value of orange juice to phenolic compounds later identified as flavonols. Hamburger and Joslyn (18) and Joslyn and Marsh (23), in studies related to browning of citrus juices, considered that among the reducing substances present in the juice were ascorbic acid and flavonols which might serve as a defense against browning. They believed that all of the reduced ascorbic acid first must be oxidized before the unknown reducing substance can itself be oxidized. This is in harmony with Golumbic's view (14). The flavones are known to oxidize ascorbic to dehydroascorbic acid (24).

Frankenthal (12), investigating the methylene blue reducing system of Palestine orange peels, found that the peel juice contained two factors not

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[2] B. F. Daubert and H. E. Longenecker (Food Technology, 1(1): 7–10, 1947) recently have given a comprehensive discussion of the rôle of antioxidants in flavor problems.

adsorbed on animal charcoal and also an iodine-reducing, heat-stable factor which is adsorbed on animal charcoal and which is not ascorbic acid. The author recognized an oxidizing system in peel juice which acted upon ascorbic acid. Supporting evidence that flavone or flavone-like pigments contribute to the total reducing power of orange and raisin juices was contributed by Gatet (13), who studied the reducing power of quercetin, quercitrin, and their oxidation products. The impurity accompanying the flavones was thought to be a contributing factor. The reducing activity of these juices and of the flavone-type solutions, as measured by the 2-6,dichlorophenolindophenol method, was increased by first oxidizing in air at pH 8, followed by reduction by cysteine at pH 4. This may help to explain the beneficial results obtained by Shrader and Johnson (44) when oxygen was bubbled through certain batches of orange juice prior to packaging.

The belief that the flavone and flavanone pigments participate in oxidation-reduction reactions is strengthened by the work of Wawra and Webb (48) with citrin in which evidence was presented to show that citrin consists of hesperidin and the chalcone of hesperidin existing in equilibrium in an aqueous medium. In nature they probably exist as protein complexes and serve as an oxidation-reduction enzyme. The chalcone was shown to be capable of being reversibly oxidized and reduced. A small amount of quercitrin is believed to accompany the citrin, especially in the citrus fruit (41).

A review of the literature indicates that no attempt has been made to utilize the flavones or other phenyl-benzopyrone derivatives as antioxidants for animal fats. Greenbank and Holm (16) reported that quercetin seemed to possess antioxidant properties for cottonseed oil; Bradway and Mattill (5) found quercetin from quercitrin to be antioxygenic for a mixture of lard and cod-liver oil.

From the physiological, pharmacological, and nutritional aspects, the addition of flavones and their derivatives to foods appears acceptable and beneficial. They have been reported as vitamin P in a wide variety of foods (3, 38, 43, 47) following the postulation of Szent-Györgyi (46) that citrin, a mixture of flavones, possessed vitamin P activity. Many workers have used the biological technique to determine the vitamin P potency of fruits and vegetables (3, 41, 42); others have employed the chemical method of Lorenz and Arnold (28). Weatherby and Cheng (49) utilized the boric-citric acid reagent and reported the flavone or quercetin-like values of food products in terms of quercetin equivalents. Lemon peel ranked highest among the materials tested.

The occurrence, chemical nature, and vitamin P activity of several members of the flavone group have been reviewed (20, 27, 43). Direct evidence that the flavonol quercetin enhances the nutritive value of butter oil has been reported by El-Rafey *et al.* (10). This is in harmony with the growing appreciation of the role of antioxidants in fat, vitamin A, and carotene utilization.

Regardless of whether the flavone-type compounds complement the physiological activity of ascorbic acid, there seems to be accepted evidence that most of the representative compounds studied have beneficial pharmacological effects. The authors feel incompetent to analyze the extensive medical literature dealing with use of these compounds as therapeutic agents. They appear to have little or no toxicity, to be non-accumulative (17), and to have nutritional and medical significance (1, 31).

EXPERIMETAL

Substrates. Dry milk fat or butter oil was prepared by churning fresh pasteurized cream, melting the butter at 60° C., and decanting and filtering the resulting fat at 60° C. This fat was divided into small portions and refrigerated until required. The lard was prepared by melting at 60° C. fresh pure lard, obtained in the local market, filtering and refrigerating. For the experiments with milk, 2 p.p.m. of copper as copper sulfate were added to pasteurized winter milk, susceptible to copper-induced oxidation.

Antioxidants. These contained the phenyl-benzo-gamma-pyrone nucleus, usually with hydroxyl, glyco, or methoxy groups or the chalcone isomer. Quercetin, quercitrin, rutin, hesperidin, methylated hesperidin, hesperidin-chalcone, and others of the flavone group were studied.[3] Attempts to isolate and purify the individual components of Szent-Györgyi's citrin (46) were unsuccessful. Difficulty was experienced in separating and eluting the fractions in the Mager method (30). Recent methods should prove more fruitful (21, 39).

Incorporation of antioxidants. Most of the antioxidants studied were more soluble in alcohol than in water or fat and hence were dissolved in either hot ethanol or glycerol before being incorporated into either a small quantity of fat in a relatively high concentration or directly into the fat in the concentration desired. The alcohol then was evaporated off in partial vacuum at from 90 to 96° C. In the experiments with milk, 20 mg. of the antioxidants dissolved or suspended in 5 ml. of hot water were added to 200 ml. of milk. The excess of the material settled out during the storage period.

For the experiments in which quercetin was compared with antioxidants native to milk fat, stock solutions were prepared as follows:

A. *Soya bean phospholipid.* Commercial soya bean lecithin[4] was purified by dissolving twice in ether and precipitating with acetone. Eight grams, dissolved in 5 ml. of chloroform, were added to 400 ml. of milk fat to give a 2 per cent solution.

[3] Quercetin, Eastman Kodak Co. 1635; quercitrin, Eastman Kodak Co. T1629, purified (34); rutin, courtesy of Dr. J. F. Crouch, Eastern Regional Res. Lab., Philadelphia; hesperidin-chalcone, methylated chalcone, and hesperidin, courtesy of California Fruit Growers Exchange.

[4] Courtesy of Dr. Eichberg, American Lecithin Company, Elmhurst, New York.

B. *Tocopherol.* 0.1 g. α-tocopherol, dissolved in 1 ml. of chloroform was added to 100 ml. of milk fat to give a 0.1 per cent solution.

C. *Quercetin.* 0.1 g. quercetin, dissolved in 2 ml. hot absolute alcohol was added to 100 ml. of milk fat to give a 0.1 per cent solution.

The solvent was removed as described.

Incubation. Fat stabilities were determined by the open jar method, in which a uniform amount of the fat is placed in similar containers and incubated uncovered. The temperature conditions were secured by a controlled oil bath, electric oven, or refrigerated cabinet. The cabinet was maintained at approximately 10° C. for the milk samples.

Tests for stability. Peroxide values were determined by the Henderson and Young modification (19) of the Wheeler method (50). Carotene losses were measured as decreases in optical density (10% solution of the fat in gasoline, 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.) using the Coleman, Model 11, spectrophotometer. The milk samples were scored for flavor as unknowns.

RESULTS

Effect of concentration of antioxidant. Preliminary trials having shown that quercetin is antioxygenic for milk fat and for lard, experiments were made to determine an effective concentration for its use. Figures 1 and 2 show that a concentration of 3 mg. per 100 g., while affording protection, is not as adequate as a concentration of 15 or 30 mg. per 100 g. at an incubation temperature of 82° C. The figures also show that the incorporation of 15 mg. quercetin in 100 g. fat extended the times required to reach a peroxide value of 5 for milk fat or 10 for lard (respective arbitrarily chosen ends of the induction periods) from 30 to over 144 hours for milk fat and from 26 hours to over 96 hours for lard. Competent judges were unable to detect by taste or color the presence of at least 30 mg. per cent of quercetin in butter oil, lard, or butter-like preparations made from them.

Protection of milk fat in storage. It was shown previously (11) that soya bean phospholipids and α-tocopherol, either alone or in synergistical combination were antioxygenic for butter oil held at 79.5° C. In order to determine the effectiveness of these compounds at a lower temperature and to compare their effectiveness with that of quercetin, a milk fat was isolated in the manner described except that melting and filtration were carried out at 41–47° C. rather than at 60° C.

Using the stock solutions A, B, or C, or combinations of A and B, samples of this fat were prepared to contain from 0.01 to 0.1 per cent added phospholipid (mostly lecithin), from 0.003 to 0.015 per cent added α-tocopherol, or from 0.003 to 0.015 per cent quercetin. Synergistic combinations were prepared containing 0.01 per cent phospholipid and 0.003 per cent α-tocopherol; 0.1 per cent phospholipids and 0.003 per cent α-tocopherol; or 0.1 per cent

phospholipid and 0.015 per cent α-tocopherol, in addition to those naturally present.

With the exception of samples 1 and 4 which were stored in test tubes

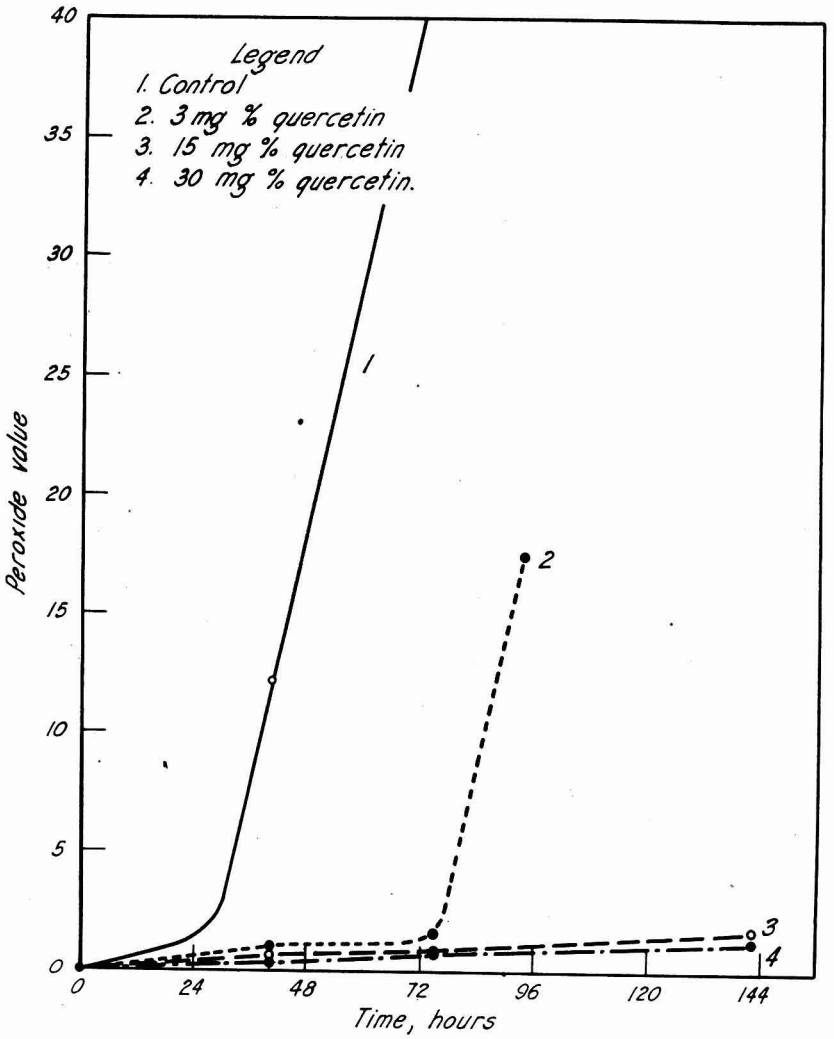


FIG. 1. The effect of adding quercetin to butter oil on its resistance to oxidation at 82° C.

at 4.5–10° C. (40–50° F.), approximately 100 ml. aliquots of each sample, contained in one-quarter pint milk bottles, with loosely-fitting caps, were stored at 40–50° F. from July 27, 1943, until about October 1, 1943. They

then were placed in a wooden cabinet at room temperature. At intervals the fats were melted and samples were taken for peroxide and color determinations. The results are shown in table 1. The experiment does not lend

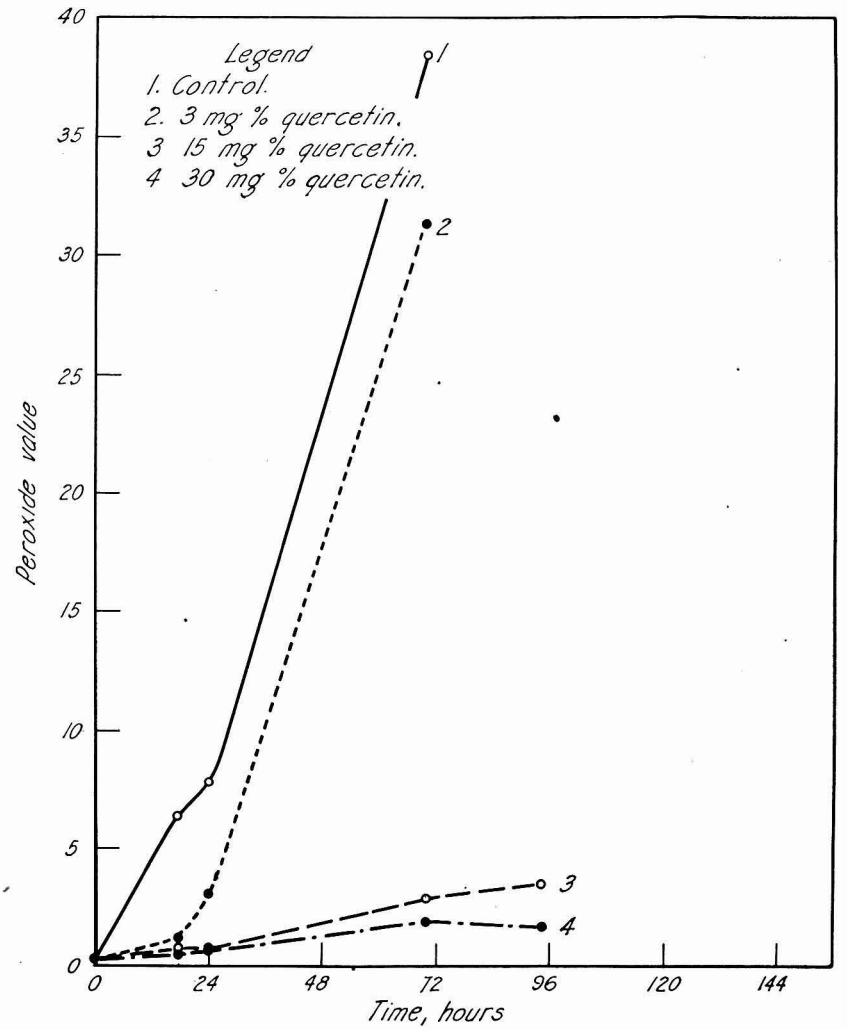


FIG. 2. The effect of adding quercetin to lard on its resistance to oxidation at 82° C.

itself to an evaluation of absolute protection factors of the antioxidants, but the data suggest that α-tocopherol added to milk fat, normally containing tocopherol (2–3 mg./100 g.), has no protective value under these conditions

TABLE 1

Effect of antioxidants on the storage life of milk fat

(Samples were prepared in July, 1943, held cold until Oct., 1943, and subsequently stored at room temperatures)

Sample no.	Description	Peroxide no.* and optical densities† (in parentheses)					
		Feb. 5, 1944	June 2, 1944	Sept. 26, 1944	May 7, 1945	Sept. 8, 1945	July 22, 1946
1	Control, milk fat held at 40–50° F.	.....	0.75 (0.255)	2.5	70 (white)	.....	.....
2	Milk fat plus 0.01% P‡	0.96	1.91 (0.243)	3.45 (0.21)	(white) (0.075)	.....	.....
3	Milk fat plus 0.05% P	0.63	0.73 (0.26)	2.35 (0.24)	18.4 (0.05)	.....	.....
4	Milk fat plus 0.1% P held at 40–50° F.	.....	0.0 (0.26)	0.0 (0.26)	1.52	.....	.....
5	Milk fat plus 3 mg.% T‡	1.1	3.71 (0.20)	8.16 (0.15)	(white)	.....	.....
6	Milk fat plus 9 mg.% T	1.7	6.39 (0.23)	12.15 (0.13)	(white- 0.08)	.....	.....
7	Milk fat plus 15 mg.% T	2.94	14.5 (0.255)	16.26 (0.115)	(white)	.....	.....
8	Milk fat plus 0.01% P and 3 mg.% T	0.37	1.3 (0.27)	3.56 (0.22)	(white- 0.075)	.....	.....
9	Milk fat plus 0.1% P and 3 mg.% T	0.24	0.92 - (0.264)	2.83 (0.23)	Almost white	.....	.....
10	Milk fat plus 0.1% P and 15 mg.% T	0.48	1.98 (0.256)	7.1 (0.23)	..... (0.07)	.....	.....
11	Milk fat plus 3 mg.% Q‡	0.55	0.83 (0.275)	1.33 (0.25)	3.88 (0.205)	6.43	.....
12	Milk fat plus 15 mg.% Q	0.24	0.53 (0.31)	0.89 (0.29)	1.96 (0.30)	2.46	3.18
A	Milk fat plus 2% P	.....	.....	0.0 (0.27)	0.84 (0.25)	2.02	3.83
B	Milk fat plus 0.1% T	.....	.....	35.1 (0.21)	..... (0.06)	.....	.....
C	Milk fat plus 0.1% Q	.....	.....	0.0 (0.29)	0.82 (0.265)	1.44	1.35

* Millequivalents per kg. of fat.

† Optical density (10% solution of fat in gasoline: 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.)

‡ P—phospholipid; T—α-tocopherol; Q—quercetin.

of storage. It even appears prooxygenic. Soya bean phospholipid (mainly lecithin), of itself and in combination with α-tocopherol, is definitely antioxygenic for milk fat. The proportion of 0.003 per cent tocopherol to 0.1 per cent phospholipid (sample 9) appears a desirable addition.

The phospholipid content of the control fat was 0.0214 per cent (calculated as lecithin). Assuming that milk fat contains 0.0025 per cent tocopherol, sample 9 had a tocopherol: phospholipid ratio of approximately 1:22, whereas the ratio for the control fat was approximately 1:8.6.

The effectiveness of quercetin, especially in the 0.015 per cent concentration, is outstanding. The 0.1 per cent concentration, while effective, is in considerable excess of the solubility of quercetin in fat. The protective action of quercetin for carotene will be the subject of a later paper.

The relative effectiveness of quercetin and commercial quercitrin. Quercetin and quercitrin (unpurified), Eastman Kodak Co., were added to milk fat and to filtered lard colored with carotene by the addition of 0.4 ml. of a concentrate[5] to 200 ml. lard. After removal of the solvent, ethanol, 50 ml. samples, in 200-ml. straight-walled bottles, were incubated in the dark at 47–50° C. Table 2 shows that quercetin is more effective than quercitrin on the weight basis. The latter is a quercetin 3-rhamnoside and has a quercetin equivalent of 354 mg. per gram as determined by the method of Weatherby and Cheng (49). On this basis, then, the quercitrin was present only in a quercetin-equivalent concentration of approximately 7 and 10.5 mg. per cent in the milk fat and lard, respectively.

Other flavone-type compounds as antioxidants. Following the precedure suggested by Wawra and Webb (48), crude preparations of hesperidin and its chalcone were made from air-dried orange and lemon peels. Their identity and purity were not established. The compounds, dissolved in ethanol, were incorporated by the described procedure, but the samples were subjected to a fluctuating temperature of 72–75° C. for the first 72 hours, except for about 6 hours at 90° C. They then were incubated at 48–50° C. The results in table 3 show that rutin from tobacco (quercetin-equivalent approximately 300 mg. per gram) has lower antioxygenic properties than quercitrin. Sample 4, containing rutin and a crude mixture of hesperidin and its chalcone, had a pronounced fruity odor after the first heating. This largely disappeared as incubation progressed. The sample showed remarkable resistance to the accumulation of peroxides. Sample 6 indicates the potential antioxygenic properties in the chalcone. This is demonstrated more conclusively in table 4. Partially purified flavanones (hesperidin, hesperidin-chalcone, methylated hesperidin-chalcone) and a lemon peel infusion concentrate prepared by the method of Szent-Györgyi for vitamin P studies were kindly furnished by California Fruit Growers Exchange. These were incorporated into milk fat in the manner already described, and

[5] Courtesy of H. M. Barnett, Barnett Labs., Long Beach, California.

TABLE 2

Effect of quercetin and quercitrin on the keeping quality at 47–50° C. of milk fat and of lard containing carotene
(Approximately 9,000 I.U. vitamin A per lb.)

Sample no.	Description	Peroxide values and optical densities* (in parentheses)									
		Dec. 17, 1943	Dec. 23, 1943	Dec. 27, 1943	Dec. 31, 1943	Jan. 11, 1944	Feb. 2, 1944	Mar. 2, 1944	Mar. 25, 1944	Mar. 28, 1944	Apr. 7, 1944
B	Milk fat untreated	..... (0.30)	0.26	.....	.....	.....	.....	.....	.....	.....	.....
L	Lard plus carotene untreated	..... (0.41)	0.65	.....	.....	.....	.....	.....	.....	.....	.....
1‡	B-control	..... (0.28)	0.48 (0.28)	3.7 (0.22)	22.8 (0.06)	110.7 (0.02)	217 (0.02)	.....	.....	.....	.....
3	B plus 20 mg.% QT†	..... (0.30)	0.86 (0.32)	1.94 (0.34)	1.91 (0.29)	2.61 (0.27)	35.4 (0)	.....	.....	.....	.....
4	B plus 20 mg.% Q	..... (0.31)	0.36 (0.33)	..... (0.35)	1.03 (0.30)	1.9 (0.33)	3.44 (0.16)	3.72 (0.11)	6.47 (0.1)	25.4 (0.05)	113 (0.01)
5	L-control	..... (0.39)	1.04 (0.38)	14.9 (0.09)	43.3 (0.03)	97.4 (0.09)	150 (0.02)	.....	.....	.....	.....
7	L plus 30 mg.% Q	..... (0.50)	0.88 (0.52)	..... (0.40)	1.41 (0.47)	2.61	3.54 (0.31)	4.98 (0.21)	7.68 (0.19)	9.49 (0.11)	15.3 (0.08)
8	L plus 30 mg.% QT	..... (0.50)	2.02 (0.51)	2.07 (0.50)	3.67 (0.43)	25.36 (0.16)	136 (0.04)	.....	.....	.....	.....

* Optical density (10 per cent solution of fat in gasoline; 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.).
† Q—Quercetin; QT—quercitrin (not purified).
‡ Samples 1 and 5 received the same pre-incubation treatment as samples 3, 4, 7, and 8.

TABLE 3

Effect of quercitrin, rutin, and hesperidin chalcone on the keeping quality of milk fat at 40–50° C.
(1944)

Sample no.	Description*	Peroxide values (me/kg. fat)										
		May 12	May 15	May 17	May 23	May 26	June 9	June 15	June 23	June 30	July 24	Oct. 4
1	QT 30 mg.%	1.37	1.58	1.60	1.93	2.11	2.66	3.0	3.41	3.49	6.72	.....
2	R 30 mg.%	1.71	2.35	2.46	3.4	12.94	.....	.....	.....	.....	.....	.....
4	R 30 mg.% plus HC	0.0	0.0	0.0	0.0	0.0	.....	.....	.....	.....	0.8	3.36
5	R 20 mg.% (dry)	1.87	2.26	2.64	15.4	42.2	.....	.....	.....	.....	.....	.....
6	Control	2.03	9.39	23.5	.....	.....	.....	.....	.....	.....	.....	.....
7	HC conc. not determined	1.33	1.93	1.93	2.29	2.52	18.5	.....	.....	.....	.....	.....

* QT—quercitrin from lemon flavine (34); R—rutin from tobacco; HC—hesperidin chalcone, a purified extract of orange and lemon peels.

TABLE 4

Hesperidin and hesperidin chalcone as antioxidants for milk fat
(1945)

	Peroxide values (me/kg. fat)										
	Initial Oct. 2 3 p.m.	Oct. 3		Oct. 4		Oct. 5		Oct. 6 11 a.m.	Oct. 10	Oct. 12	Oct. 16
		1:20 p.m.	4:15 p.m.	9 a.m.	3:45 p.m.	11 a.m.	4 p.m.				
Experiment 1											
Control	1.7	2.32	2.41	7.04	.....	.....	.....	.....	.....	.....	.....
HC, 10 mg./30 g. fat	.....	.....	.....	4.81	9.8	11.95	.....	.....	.....	.....	.....
HC, 20 mg./30 g. fat	.....	.....	.....	2.62	2.48	3.98	6.24	13.75	.....	.....	.....
Quercetin 10 mg./30 g. fat	.....	.....	.....	2.08	1.96	2.11	1.91	2.82	2.87	3.52	4.88

	Initial Oct. 4 3 p.m.	Oct. 5		Oct. 6 11 a.m.	Oct. 8 4 p.m.	Oct. 9 4 p.m.	Oct. 10 4 p.m.
		10 a.m.	3:45 p.m.				
Experiment 2							
Control	1.31	2.5	3.96	6.9	8.32	13.25	.....
Crude H ca. 50 mg./30 g. fat	.....	2.45	2.29	4.54	7.58	9.54	13.15
Lemon peel infusion conc. ca. 40 mg./30 g. fat	.....	2.23	2.41	2.73	2.76	6.35	10.11

HC—Hesperidin chalcone—Courtesy California Fruit Growers Exchange.
H—Hesperidin, crude—Courtesy California Fruit Growers Exchange.
Lemon peel infusion for vitamin P studies—Courtesy California Fruit Growers Exchange.

the fat samples were incubated at from 60 to 80° C. during the day and chilled during the night or over the weekend. The results indicate that hesperidin has little or no antioxidant value. Other experiments, not reported, showed that the methylated chalcone probably is too stable to serve as an antioxidant. The data, however, show that the natural chalcone protects milk fat against oxidation.

Inhibiting oxidation in susceptible milk. Twenty mg. of the compounds listed in table 5 were added to 200 ml. of warm pasteurized susceptible milk

TABLE 5

Flavone-type compounds as antioxidants for milk susceptible to copper-induced oxidation

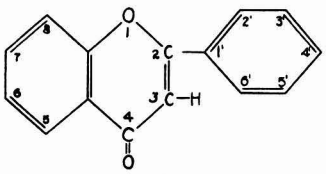
Sample no.	Antioxidant	Numerical score*	Comments
1	Key, no copper	21	Old, very sl. oxidized
2	Hesperidin	18	Oxidized
3	Hesperidin-chalcone-protein complex†	21	Sl. foreign
4	Rutin	21	Old
5	Quercitrin	22	Lacks freshness
6	Control, with copper	18	Oxidized
7	Lemon peel infusion concentrate (vitamin P)	20	Old, sl. oxidized
8	Quercetin	22	Old, lacks freshness
9	Same as 7, except one-half concentration	19	Oxidized
11	Hesperidin chalcone	20	Foreign flavor

* Flavor and odor; 25, no criticism.
† Prepared by method of Wawra and Webb (48).

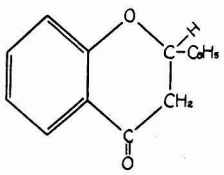
along with 2 p.p.m. of copper. A control was prepared omitting the compound; a key sample, containing no added copper, was included. All were cooled and placed in a refrigerated cabinet (10° C.) and scored as unknowns on the third day. The scores and comments of one of the judges, concurred in by the other judges, are shown in the table. All of the flavones and flavanones studied inhibited the development of the oxidized flavor common to winter milk. Experiments are contemplated in which the antioxidant will be incorporated prior to pasteurization.

DISCUSSION

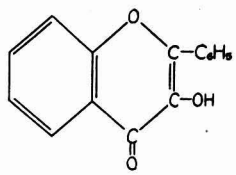
The flavones and flavone derivatives are distributed widely in the plant kingdom and occur as glycosides, in the free state, and in association with proteins and tannins. They all have the basic phenyl-benzo-gamma-pyrone structure, the pyrone nucleus apparently being responsible for their characteristic chemical activity. The key structures, according to Mayer and Cook (33), are as follows:



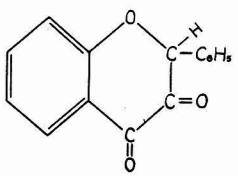
Flavone



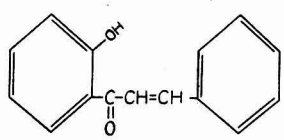
Flavanone



Flavonol
(enol form)

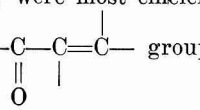


Flavonol
(keto form)



Chalcone

Mayer and Cook state that a flavanone may be dehydrogenated to a flavone and a chalcone may be converted to the flavonol by treatment with hydrogen peroxide. The chalcone in acid medium is believed to isomerize into the flavanone, the reverse taking place in the alkaline medium (48).

Of the flavone-type compounds studied, those giving a positive boro-citric test (51) were most efficient as antioxidants. This test apparently involves the  group. Quercetin (3,5,7,3′,4′, pentahydroxy-flavone), quercitrin (3-rhamnoside of quercetin), rutin (3-rutinoside), and possibly the chalcone of hesperidin (48) all contain this grouping and all have antioxidant activity. It would seem logical, therefore, to infer that the labile pyrone confers antioxidant properties. The closed, saturated ring of hesperidin apparently does not satisfy this condition and it has very little, if any, antioxidant value, at least in the absence of a synergist of lower oxidation potential.

A theoretical discussion of the manner in which flavones and their derivatives protect a fat against oxidation is somewhat premature. Milk fat as usually isolated is afforded some protection by the phosphatides, tocopherols, and other reducing substances which it contains. These are difficult to remove without effecting changes in the fat itself. Carotene cannot be ignored. In milk itself the natural oxidation-reduction system or systems is not a simple one, even if ascorbic acid is excluded. Furthermore, individual flavones and flavanones are not easily isolated from their isomers and other naturally-occurring pigments.

The data are interpreted as supporting the modern concept of antioxidants. Very little seems to be known of the oxidation-reduction potentials of the flavones in a fat medium. The potential of a fat peroxide is not known, but that of a fresh fat may be about 1.0 volt (15). Gatet (13), by oxidizing quercetin in air at pH 8, followed by reduction with cysteine at pH 4, increased its apparent concentration toward 2,6-dichlorophenolindophenol about four-fold. It would seem that the potential of this activated quercetin in a water-alcohol medium at pH 7 is somewhat less than 0.22 volt. If this is true, sulfhydryl compounds and possibly ascorbic acid might well be expected to act synergistically with these flavones. Sample 4, table 3, suggests that a combination of a flavone and a chalcone may be very effective in inhibiting the accumulation of peroxides. This aspect is being studied.

It is not known if ingested flavones or flavanones will be deposited in the body fat, carried in the blood stream, or secreted in the milk of animals, as appears to occur under certain conditions with tocopherol (7). None was found in the livers of rabbits (51) or in milk (35), and Robeznicks (40) was unable to establish either the presence or absence of flavones in the liver, kidney, or milk of animals. Feeding citrus molasses or dried citrus pulp to dairy cows has proved satisfactory and no ill effects on the milk flavor have been found (4, 25). If ingested flavones are found to be deposited in the body fat and secreted in milk, the authors are of the opinion that these ingested compounds would result in enhanced stability of meat products and improved keeping quality of winter milk and of whole milk products.

The lack of specific tests for flavones and flavanones, especially in the presence of interfering substances, coupled with the difficulty of securing foodstuffs free from flavones, makes conclusive results hard to obtain.

CONCLUSIONS

1. Flavones, as illustrated by quercetin, quercitrin, and rutin, have been shown to be effective antioxidants for milk fat and lard.

2. The flavanone glycoside hesperidin appears to have little or no antioxidant values; its chalcone is active.

3. It is suggested that the —C(=O)—C=C— group in the pyrone ring or in the open chalcone is responsible for the antioxidant activity.

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Cornell Veterinarian

Dairy Industries
Dairy World

Endocrinology

Food in Canada
Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
Ice Cream Field
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Medical Association
Journal of the American Oil Chemists Society
Journal of American Veterinary Medical Association
Journal of Animal Science
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Endocrinology
Journal of Experimental Medicine
Journal of General Physiology
Journal of Genetics
Journal of Heredity
Journal of Infectious Diseases
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology
Journal of Veterinary Research

Lancet
Le Lait

Milk Dealer
Milk Industry
Milk Plant Monthly
Molkeritidende

National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Nordisk Mejeri-Tidsskrift

Pacific Dairy Review
Proceedings of Society of Experimental Biology and Medicine

Refrigerating Engineering

Scientific Agriculture
Southern Dairy Products Journal

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
Milk Industry Foundation

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BUTTER

169. Is Mold Mycelia Count a Satisfactory Index of Butter Quality? P. R. ELLIKER, Purdue University, Lafayette, Ind. Natl. Butter and Cheese Jour., *38,* 3: 38. March, 1947.

The greatest faults of application of the mold mycelia count to butter have been: (1) Its value is only seasonal. (2) Even in the warm months it operates successfully only in certain parts of the country. (3) In Indiana and surrounding regions it does not eliminate the quality inferiority represented by more than 50% of all cooking grade butter and declares about one-third of the 90 score butter illegal during the warm months. Better approaches to quality control might be reached by careful organoleptic grading and sorting and by using mold mycelia counts on finished butter only as a supporting test to grading and testing for extraneous matter. W.V.P.

170. What We Can Do to Increase Butter Consumption. H. SCHUMACHER, Swift & Co., Chicago, Ill. Natl. Butter and Cheese Jour., *38,* 4: 40. April, 1947.

A five-point program is given: (1) Quality is the only safe basis for increasing sales and building repeat business. (2) The product must be properly protected, preferably by a wrapper or coverings more effective than a single sheet of parchment. (3) The package must have eye appeal. (4) Adequate advertising is needed. (5) Displays of butter in modern cases will increase sales. W.V.P.

CHEESE

171. Standards of Identity for Cheese. A. B. EREKSON, Plymouth, Wis. Natl. Butter and Cheese Jour., *38,* 2: 42. Feb., 1947.

Proper standards should protect health, values, quality, and investments of established manufacturers. Pasteurization of milk, suitable aging of raw-milk cheese, and elimination of preservatives are health protection measures. Labeling of skim milk cheese is not adequate protection of values. Specifications should provide maximum moisture and minimum fat contents for each recognized variety or class of cheese. W.V.P.

172. Manufacture of Granulated Type American Cheddar Cheese. E. C. DAMROW, Damrow Bros. Co., Fond du Lac, Wis. Natl. Butter and Cheese Jour., *38,* 4: 44. April, 1947.

Granular type Cheddar now can be made with mechanical equipment

and a minimum of hand labor. The regular milled curd process is followed until the time of draining, although a slightly lower cooking temperature may be used to increase the moisture content of the finished cheese. When the curd is firmed, special rake paddles are substituted for the stirring paddles of the usual mechanical agitator. A half-round strainer is used to hold the curd away from the gate end of the vat. The whey is drained with practically continuous mechanical raking of the curd so that matting is prevented. Removing the whey requires about 30 min. by this process. Salt is applied from 30 to 45 min. after dipping ends and when the whey acidity approximates 0.30%. The whole process requires from 3 to 4 hr. from the time of renneting to hooping. W.V.P.

173. Grade and Composition of Swiss Cheese in Northwestern Wisconsin. C. B. LANE, Blue Moon Foods, Thorp, Wis. Natl. Butter and Cheese Jour., *38*, 3: 34. March, 1947.

Data on the composition of 19,642 wheels of Swiss (grade A, 4,046; grade B, 5,393; grade C, 4,899; grade open standard, 2,639; and grade standard, 2,665) obtained from 13 factories for a year beginning September, 1945, showed no practical correlation between composition (moisture and fat) and grade. W.V.P.

174. Factors Influencing Acid Production by Cheese Cultures. F. J. BABEL, Iowa Agr. Expt. Sta., Ames, Iowa. Natl. Butter and Cheese Jour., *38*, 2: 38. Feb., 1947.

This is an abridged discussion of the paper in the Journal of Dairy Science, *29*, 9: 597. 1946.

CHEMISTRY

175. The State of Vitamin A in Colostrum and in Milk. D. B. PARRISH, G. H. WISE, AND J. S. HUGHES, Kansas Agr. Expt. Sta., Manhattan, Kans. Jour. Biol. Chem., *167:* 673–678. 1947.

Vitamin A alcohol was separated from the vitamin A ester by the use of an alumina adsorption column, the ester passing through the column. Colostrum samples were taken within 4 hr. postpartum and milk samples from the same cows 3 to 8 weeks later. One Guernsey, 6 Jerseys, 7 Ayrshires, and 4 Holsteins were used in the study.

The authors conclude that: "Practically all of the Vitamin A in both colostrum and milk was found to be in the form of the ester." Preliminary studies indicated most of the vitamin A in the blood of these cows was in the form of alcohol. "Most of the fat-soluble yellow pigment in colostrum and in milk was found to be carotene." A.O.C.

176. Application of Sendroy's Iodometric Chloride Titration to Protein-Containing Fluids. D. D. VAN SLYKE AND ALMA HILLER, Hosp. Rockefeller Inst. for Med. Res., New York. Jour. Biol. Chem., *167:* 107–124. 1947.

The Sendroy iodometric method for chlorides is based upon the liberation and measurement of the IO_3 radical according to the following equation: $AgIO_3 + Cl^- = AgCl + IO_3^-$. The IO_3^- is measured by titrating against a standard thiosulfate solution.

"In the present application, the titrimetric procedure for protein-containing fluids is simplified by carrying out the reaction with $AgIO_3$ and precipitation of proteins simultaneously in a single operation, so that an entire analysis, including removal of the mixed precipitate and titration of the filtrate, can be carried through in about 6 minutes."

A table comparing this method and the nitric acid digestion method in the analysis of cows' milk is given. A.O.C.

177. The Determination of Iron in Biological Material. A. J. WOIWOD, Wellcome Physiological Research Laboratories, Beckenham, Kent (England). Biochem. Jour., *41,* 1: 39–41. 1947.

"A method for determining iron in biological materials with a high P : Fe ration is described. It is applicable over the range 0.5–10 μg. Fe/ml. All analytical manipulations, except the final centrifuging before colour reading, are performed in the crucible in which the sample has been ashed. Blanks are therefore kept at a minimum.

"The method has proved satisfactory with protein hydrolysates and cows' milk and may be suitable for other materials where phosphate interference is met."

A value of 4.7 μg. Fe/10 ml. is reported for raw milk. Twelve references are given. A.O.C.

178. Constant Pressure Oxygen Absorption Fat Stability Test. G. GILMONT, H. S. LEVENSON, AND L. W. ELDER. General Foods Corp., Hoboken, N. J. Oil and Soap, *23,* 8: 248. Aug., 1946.

Details are given for the equipment and operation of the General Foods Method of determining fat stability. The method, essentially a modification of the Barcroft-Warburg procedure of Johnson and Frey, differs from the latter method in two respects: (a) The oxygen is absorbed under constant pressure and can be recorded volumetrically on a macro scale. (b) The induction periods can be evaluated graphically from the direct plot of the experimental data without further calculation. With a 2-ml. sample the induction periods were reproducible with a precision of 1 to 2% in most cases or a maximum variation of 5% in the most unfavorable cases.

J.L.H.

179. Interfacial Tension of Oil-Water Systems Containing Technical Mono- and Diglycerides. R. O. FENGE, So. Regional Res. Lab., New Orleans, La. Jour. Amer. Oil Chemists Soc., *24,* 2: 49. 1947.

Technical mono- and diglycerides have wide industrial use as oil-soluble emulsifying agents in the manufacture of superglycerinated shortenings and margarine. Commercially available products are composed of mixtures of mono-, di-, and triglycerides. Known mixtures of mono-, di-, and triglycerides have been studied but little with respect to their power of lowering the interfacial tension at vegetable oil-water interfaces. Technical mono- and diglycerides were prepared for study. When both mono- and diglycerides are present in the oil phase, the interfacial tension is substantially a function of the monoglyceride content. A constant weight of a given monoglyceride preparation had practically an equal effect in lowering the interfacial tensions against water for peanut, cottonseed, and soybean oils. A concentration of 1% of monoglyceride in the oil phase was found to lower the interfacial tension at the oil-water interface by approximately one-half, and 6% lowered the interfacial tension to practically zero.

J.L.H.

FEEDS AND FEEDING

180. Effect of Dehydration on Enzymic Destruction of Carotene in Alfalfa. H. L. MITCHELL AND H. H. KING, Dept. of Chem., Kansas State College, Manhattan, Kans. Jour. Biol. Chem., *166:* 477–480. 1946.

As alfalfa cures in the field the enzyme lipoxidase is responsible for the destruction of a considerable part of the carotene. Peroxidase, another oxidative enzyme present in alfalfa, is inactivated by blanching prior to dehydration (mechanical drying). By blanching and dehydration both enzymes are inactivated and neither is regenerated during storage of alfalfa meal for 2 months.

"Blanching of alfalfa prior to dehydration did not increase the retention of carotene during storage. Carotene destruction during storage does not appear to be enzymic in nature." A.O.C.

FOOD VALUE OF DAIRY PRODUCTS

181. The Effect of Fat on the Absorption and Utilization of Galactose by the Rat. MARIE L. NIEFT AND H. J. DEUEL, JR., Dept. of Biochem. and Nutr., Univ. of Southern California, School of Medicine, Los Angeles, Calif. Jour. Biol. Chem., *167:* 521–525. 1947.

The present work confirms, to a large degree, that reported by Geyer *et al.* (See Abs. 358, Jour. Dairy Sci., *29,* 10: A164. 1946.) The investi-

gators conclude: "The percentage of ingested galactose which is excreted in the urine varies inversely with the percentage of fat in the diet."

"The fat effect appears to be independent of the type of fat at a 20 per cent level, since butter fat, corn oil, and cottonseed oil give essentially the same results. Howevér, if the fat level is cut to 10 per cent, cotton-seed oil gives significantly lower urinary excretion values than either butter fat or corn oil." A.O.C.

ICE CREAM

182. Shrinkage in Ice Cream. C. D. DAHLE, D. J. HANKINSON, AND J. A. MEISER, JR. Ice Cream Rev., *30,* 6: 41. Jan., 1947.

The least amount of shrinkage was observed with ice cream stored in glass containers, followed in order by metal, paper paraffined inside and outside, paper paraffined on the inside only, paper paraffined on the outside only, and untreated paper. Shrinkage was greatly reduced when a cabinet temperature of + 5° F. was used as compared with + 10° F., irrespective of the type of container used. No shrinkage was observed in ice cream samples stored at – 15 to – 20° F. Substitution of 30% of the cane sugar on a dry weight basis with sweetose, cerelose, Frodex, or honey increased the amount of shrinkage in the order named. When corn sirup was substituted for 30% of the cane sugar, less shrinkage was observed than when cane sugar alone was used. Pasteurization of the mix at 160° F. for 20 min. or 160° F. for 45 min. resulted in slightly less shrinkage than when pasteurization temperatures of 145° or 175° F. were used. Wet (incompletely frozen) ice cream was found to shrink more than that frozen enough to yield a dry appearing ice cream when drawn from the freezer. High homogenization pressures were found to increase shrinkage, with little or no relationship being observed between the homogenization pressures used and the dryness of the ice cream at the freezer.

Four different stabilizers showed little difference in the amount of shrinkage. Certain emulsifiers and egg yolk increased the dryness of the ice cream and increased shrinkage somewhat. No relationship was observed between the source of gelatin nor its Bloom strength and shrinkage. The use of superheated condensed milk resulted in less shrinkage in ice cream than when plain condensed milk was used. Butterfat from different sources gave variable and inconsistent results as related to shrinkage. The mix acidity and protein stability could not be correlated definitely with shrinkage. The addition of sodium salts to ice cream mix had little or no effect on the amount of shrinkage observed. The addition of calcium salts did accentuate shrinkage slightly. Presence of 0.4% free fatty acids in the mix increased markedly the amount of shrinkage observed in the ice cream. The use of dry ice was found to accentuate shrinkage, particularly with ice cream frozen in a continuous freezer. W.J.C.

183. Manufacture of Powdered Ice Cream Mix. HARRY PYENSON. Ice Cream Rev., *30,* 8: 54. 1947.

The composition, advantages, markets, and results of experimental studies dealing with the manufacture, storage, and use of powdered ice cream mix are discussed. Stabilizers of vegetable origin gave more satisfactory results than those of animal origin and improved the whipping properties of the mix when used in conjunction with an emulsifying agent, such as glycerol monostearate S. Addition of all of the sugar to the mix prior to condensing resulted in a caramel flavor, reduced the capacity of the drying equipment, and interfered with the drying process. The addition of only 25% of the sugar prior to condensing is recommended. High quality dairy products processed in stainless steel equipment, high pre-heating temperatures, presence of antioxidants and low moisture content (below 1.5%) were essential to the production of powdered ice cream mix of good keeping quality. Such a product showed very little change in flavor for 8 to 12 months or longer.

A suggested procedure for the production of powdered ice cream mix is as follows: (1) Combine fluid milk and cream to give the desired ratio of fat to solids-not-fat, condense, and add 25% of the sugar and stabilizer, or add the sugar and stabilizer to the milk and cream prior to condensing. (2) Preheat to not less than 170° F. for 20 min., 180° F. for 10 min., or 190° F. for 5 min. (3) Condense to 30–36% solids, depending on whether stabilizer is added before or after condensing. (4) Homogenize the condensed mix at 150° F., using a pressure of 2,500 lbs. for the first stage and 500 lbs. for the second stage. (5) Preheat the homogenized mix to 150° F. before spray drying. (6) Spray-dry the mix to yield a product to contain not over 1.5% moisture. (7) Cool the powdered mix immediately. This will minimize cooked flavor and retard the development of stale flavor. (8) Add the remainder of the sugar. (9) Gas pack the powder in tins.

Powdered mix usually analyzes: Butterfat, 27–30%; M.S.N.F., 27–28%; sugar, 39.5–44%; stabilizer, 0.6–1.0%; and unspecified material, 2.75%. The powder may be reconstituted by adding it to cold water in the freezer and freezing immediately, but better quality ice cream resulted when the reconstituted mix was allowed to age for 24 hr. prior to freezing. W.J.C.

184. Improving Package Ice Cream. J. H. ERB. Ice Cream Rev., *30,* 8: 44. March, 1947.

The challenge confronting the ice cream industry is to produce in factory-filled packages the same desirable characteristics the consumer associates with hand-dipped ice cream. Key factors in attaining this objective are: (1) Total solids content of the mix should be between 38.5–40%. (2) Use of high quality raw ingredients. (3) Thorough homogenization. (4)

Aging of mix for not less than 5 hr., irrespective of the stabilizer used. (5) Maintaining proper weight in all packaged ice cream. (6) Adoption of overrun standards well below those used for bulk ice cream. Freezers in battery operation should be checked to see that ice cream of the proper overrun is being delivered by each freezer. (7) Freezing the ice cream to a stiff dry consistency at the freezer and avoiding the use of long, small diameter lines in conducting the ice cream from the freezer to the filler head. (8) Hardening time not to exceed 6 to 8 hr. W.J.C.

185. "Sanitary Spoon Rest." ANONYMOUS. Ice Cream Rev., *30,* 8: 43. March, 1947.

Sales resistance to tall fountain drinks on the part of the customers who do not like to put wet spoons on the counter when not in use may be overcome through the use of an ingenious paper spoon holder known as "Sanitary Spoon Rest". This device, manufactured and introduced in the Los Angeles area by Coast Curries Ice Cream Co., helps keep the counters clean, prevents sloppy serving of the spoon, and also is an effective medium for advertising. W.J.C.

186. Lower Costs in the Ice Cream Industry. CARL H. ZANZOW, JR. Ice Cream Rev., *30,* 8: 48. March, 1947.

The objective of every ice cream plant to increase productivity and, simultaneously, reduce costs can best be achieved by the establishment of a complete production and engineering department, responsible only to top management. Functions of such a department are: supervisory training, plant layout and process improvement, work simplification and methods improvement, work measurement, employee training, planning and scheduling of operations, job evaluation, establishment of sound wage incentives, and cost control. A common understanding of the entire program is essential by all levels of management, the employees and their union, and can be achieved best by a series of group-participating conferences.

Results to be expected from a properly functioning production engineering program are: (1) proper plant layout and process flow, (2) simplified and standardized working methods, (3) establishment of fair and equitable work standards, (4) establishment of a fair and equitable base wage rate structure built upon a sound job evaluation procedure, (5) improved production scheduling throughout the plant, (6) increased employee earnings through the use of wage incentives, (7) reduction in unit costs by a greater output per man hour, (8) the control and elimination of material waste, (9) decreased labor turnover, (10) the establishment of standard costs, and (11) effective cost control reports to measure progress and point out weaknesses that can be remedied. W.J.C.

187. Trends in Ice Cream Merchandising. R. A. PERRY. Ice Cream Rev., *30,* 8: 45. March, 1947.

A program designed to surround ice cream with an atmosphere which will intensify its appeal and remove any barriers which might serve as obstacles to consumer acceptance must start with the manufacturer and carry through to the ultimate consumer.

The manufacturer has the responsibility of seeing that a uniformly high quality product, attractively packaged, is supplied at all times. The plant and delivery equipment must meet the most exacting demands of sanitation, orderliness, and attractiveness. The ice cream manufacturer must realize that he must follow his product through to the ultimate consumer. This involves closer cooperation between supplier and dealer. Sanitation at the point of sale is stressed as the phase of activity most deserving of immediate attention. The appearance of the store, its personnel, odors, cleanliness of the counter, seats, glasses, silverware, etc., all are factors which influence customer satisfaction.

Education of the dealer through actual visual demonstrations is presented as the most effective means of dealer education on the part of the ice cream manufacturer. Eleven concrete suggestions for making the dealer into an effective merchandiser for ice cream are presented. W.J.C.

188. Selling Ice Cream through Vending Machines. E. THOM. Ice Cream Rev., *30,* 7: 41. Feb., 1947.

The use of vending machines for the distribution of ice cream opens up a new and virtually untouched market. Experience gained since 1940 by Miller Bros. in New York City should prove extremely helpful to any ice cream company contemplating entering this field.

The operation of vending machines demands the full-time attention of a separate department or a separate company. It cannot be handled successfully as a part-time job on the part of some individual within the organization. The ice cream served must be of unvarying high quality which will merit repeat sales, and flavors should be confined primarily to vanilla and chocolate. The nickel ice cream cup is the item which probably can be sold most successfully through vending machines, although the possible vending of ten-cent cups and other novelty items is being tried out in a limited way. Vending machines with a capacity of not less than 200 cups should be installed and these must be kept supplied with ice cream at all times. Service men should be paid in part on a commission basis to increase their interest in keeping the machines filled and in operating order. They should not be expected to make repairs on machines except for minor adjustments requiring 10 min. or less. Vending machines should be located with firms primarily interested in providing a service for their employees rather than with

firms interested in the commission which may be received as rental for the space and electricity provided. This method of distributing ice cream has the advantage of being a strictly cash business and serves to bolster sales during the winter months. W.J.C.

189. The Need for Cooperation. C. J. PALMER. Ice Cream Rev., *30,* 7: 48. Feb., 1947.

Cooperation between manufacturers of ice cream and of soda fountain equipment can do much to make soda fountain operators appreciate more fully the importance of good equipment and the importance of providing outstanding service to their customers. Such cooperation will result in increased sales of both ice cream and soda fountain equipment, thereby working to the mutual advantage of both groups. The two groups should cooperate to the end that: (1) Soda fountain operation will be above any possible criticism from a sanitary standpoint. (2) Proper facilities will be provided for the cleaning and sterilization of all multi-use utensils. (3) The possibilities of expanded food service at the fountain will be brought to the attention of the operators. (Surveys have shown that offering both food and fountain service will result in greatly increased ice cream and fountain sales as well as a more profitable over-all operation.) (4) Fountain drinks of superior quality will be served as a result of use of proper equipment and education of the operator in the essential steps of correct carbonation. W.J.C.

190. The Importance of Ice Cream in the Dairy Industry. R. C. SMELLIE. Canad. Dairy and Ice Cream Jour., *26,* 3: 37. March, 1947.

The value of ice cream can be illustrated best by its continued manufacture during the war and at a volume in excess of the 1939 production. An increase in population and a decline in cow and heifer population are making less milk available for ice cream in Canada today. The dairy producers have to be convinced that all the milk they produce will have a profitable outlet. Ice cream is officially recognized as a food in Canada and more publicity will be given to it when more ice cream is available. H.P.

MILK

191. Milk Production Trends in the U.S.A. and Possible Competition from Canada. T. M. ADAMS. Canad. Dairy and Ice Cream Jour., *26,* 3: 59. March, 1947.

The sound position of dairying in the northeastern region of U.S.A. lies in the adaptability of the area to the economical production of an abundant supply of high quality roughage and pasture and its nearness to large populations of consumers of fluid milk. Cost of transportation and the quality

factors associated with the movement of milk over long distances have made for an economic barrier excluding midwestern fluid milk from New England markets. Competition from Canada may increase if the barriers which limit milk imports are removed or lowered. H.P.

192. Operation of Six-Day-Week Milk Delivery. A. GIGANAC. Canad. Dairy and Ice Cream Jour., *26*, 3: 66. March, 1947.

Windsor, Ontario, has successfully operated on a 6-day-week milk delivery for the past 4 years. Milk is not delivered on Sunday or on a holiday that occurs on Wednesday or Thursday. The management, employees, and the public find 6-day delivery more economical and more pleasant, and it simplifies delivery operations. Six-day delivery and plant work are not difficult providing good storage facilities and excellent facilities for raw milk are available. H.P.

MISCELLANEOUS

193. Pasteurization by Ultra-Violet Rays. E. CAPSTICK, H. HALL, AND F. K. NEAVE. Milk Indus., *27:* 8. Feb., 1947.

The German Prof. Lembke and Dr. Bayha have developed a process for pasteurization by ultra-violet rays. The optimum wave length for a bactericidal effect is 2537 Å. The effective penetration is restricted to approximately 1 mm. A sheet metal cabinet houses 28 quartz-tube mercury vapor lamps. Milk flows through quartz tubes on a three-pass system to form a continuous path 328 feet long. The milk in each bank of 50 tubes is irradiated by 12 mercury vapor lamps. Air temperature in the cabinet is maintained at 20–30° C. The unit was designed to operate at 1,200 l. per hour (260 gallons) but operates at the present time at about 140 gallons per hour. The equipment is easily cleaned by circulating cold water, followed by a warm alkaline detergent solution, then water acidified slightly with HCl, and finally clear cold water. The bacteriological tests made show that an exposure for 1.75 min. with the milk at 30° C. gave better results than milk held for 30 min. at 63° C. With poor quality milk coliform organisms survived in the irradiated milk. Many more investigations have to be made to determine the proper amount of radiation, temperature of milk and lamps, turbulence, the importance of such factors as aeration and deaeration of the milk, and the effect of the process upon flavor, keeping quality, pathogens, and vitamin A. H.P.

194. Safeguarding Your Water Supply. N. P. NUPSON, Pennsylvania Salt Mfg. Co., Philadelphia, Pa. Natl. Butter and Cheese Jour., *38*, 3: 42. March, 1947.

Bacterial contamination of the water supply may constitute a problem

of health or quality control or both, depending upon the types of organisms present. Get rid of improperly constructed wells, keep water supply tanks clean, and eliminate dead-end piping. If contamination exists, then the water coming in contact with the food can be pasteurized, the well itself may be chlorinated, the water supply tank can be chlorinated (usually 5 to 10 p.p.m. is adequate dosage), or a device can be used to inject hypochlorite solution continuously into the water line. W.V.P.

195. Saving and Disposal of Creamery Waste. O. W. SANDBORG, Armour & Co., AND A. J. STEFFEN, Wilson & Co., Chicago, Ill. Natl. Butter and Cheese Jour., *38*, 2: 34. Feb., 1947.

Improper disposal of dairy wastes can cause justified complaints and law suits. Dairy wastes are caused by inefficient equipment, methods, or operations. They may consist of buttermilk, skim milk, other by-products, rinsings, and wash water. The wastes can be reduced by using drippings from emptied cans for animal feed, using automatic controls where milk may overflow, preventing leaks, educating personnel to avoid spilling and splashing of products, and using all by-products. The system of waste disposal to be employed can be determined only by careful study of waste flow, waste concentration, proximity to a flowing stream, and relation to city sewage disposal. Frequent waste sampling and testing plus the determination of the causes of excessive losses are essential for loss reduction. W.V.P.

196. Booster Compressors. BERNARD SAVEY. Ice Cream Rev., *30*, 8: 52. March, 1947.

Booster compressors are suggested as the solution in adapting present ice cream refrigeration systems to fit the needs of the modern ice cream factory. Boosters are described as compressors which pump the gas from freezers, hardening rooms, and other low temperature rooms through water cooled and gas cooled coolers into the conventional or second stage compressors, which, in turn, compress and discharge the gas into condensers.

Advantages claimed for such a system are lower initial cost, maintenance of constant low temperatures unaffected by other plant loads, lower operating cost due to precooling of liquid and intercooling of low pressure gases, and less ammonia condenser cooling water required due to less total heat to be removed from the high pressure gas.

Although conventional type compressors may be used as boosters, boosters designed for this purpose are more economical. They are lighter in weight and have less bearing surface between the pistons and cylinder to contribute to friction losses. W.J.C.

197. Refrigerating Oil Carryover at High Temperatures. J. M. LEBEAUX, AND LUIS H. BARTLETT, Univ. of Texas. Refrig. Engin., *53,* 3: 203–207. March, 1947.

Experimental results on oil carryover were obtained for 15 oil samples by means of a specially designed test flask. It was proved conclusively that refrigeration oils vaporize at the discharge temperatures of the compressor. The amount of oil carryover increased considerably with an increase in temperature, the pressure being held approximately constant. The greatest carryover occurred with most of the lighter oils. However, the heavier oils with lower carryover are unsuited to small high-speed installations. On the other hand, if the oil can be excluded from the evaporator, the pour test is a matter of little consequence. The oil also should have either a negligible solubility or complete solubility in the refrigerant. The most suitable refrigerating lubricants are petroleum oils of paraffinic and napthenic types; the latter, due to their inherent freedom from wax and resultant low pour test, are in general use throughout the refrigerating industry. The napthenic oils are mandatory where sulfur dioxide is used as a refrigerant but are equally satisfactory with the other common refrigerants. Heavier oils are called for in large machines for the heavier duty requirements or for refrigerants that have a high miscibility or mutual solubility with oil. Rotary type compressors require heavier oils than reciprocating or piston type.

The precise selection of the best lubricating oil will depend upon the design, capacity, operating conditions, and the type of the refrigerant used, and should be governed also by the vapor pressure of the oil to insure a minimum carryover and hence a maximum efficiency of the system.

L.M.D.

198. Air Blast Quick Freezing. REGIS GUBSER, California Consumers Corp. Refrig. Engin., *53,* 1: 23. Jan., 1947.

The essential features of air blast freezing systems are presented briefly. Air movement at the rate of 1500 c.f.m. per ton of refrigeration application is required. The maximum performance of a freezing tunnel is obtained when the air passages are kept small around the product and around the coil surface. The higher the velocity the greater the K factor. In no case should the velocity be less than 500 ft./min., and it should be as much above this rate as will be permitted by the friction loss and the static pressure developed by the fan. L.M.D.

199. Frozen Cooked Foods. FAITH FENTON, State College of Home Economics and School of Nutrition, Cornell University. Refrig. Engin., *53,* 2: 107–111. Feb., 1947.

Details of carefully controlled laboratory procedures for freezing certain

foods, including custard-base ice creams, are reported. Results favored the freezing of raw yeast rolls and unbaked pies and their subsequent baking after removal from freezer storage. Rolls made with milk scored higher in flavor and moisture than did those made with water. Ice creams frozen in a hand freezer and hardened in the various parts of the freezer cabinet were superior in smoothness. Ice cream frozen in the ice cube compartment, which ranged from 13 to 15° F., was smoother than that frozen in the freezer compartment, −20° F., or in the storage space, −5° F., the latter producing the coarser ice crystallization. The quicker freezing in the ice cube section, with the bottom of the tray containing the ice cream being in direct contact with the freezing plate, indicated that the rate of freezing was more important than the environmental temperature, even with forced convection in the freezer compartment. Ice creams developing coarse ice crystallization while freezing became increasingly coarse with prolonged storage. L.M.D.

200. A Qualitative Method for Detecting Surface Active Agents. L. F. HOYT, National Aniline Div., Allred Chemical and Dye Corp., Buffalo, N. Y. Jour. Amer. Oil Chemists Soc., *24,* 2: 54. Feb., 1947.

A new method, consisting of the solubilizing of a Brilliant Oil Blue B.M.A. solution to produce a blue solution, is described for the qualitative detection of small amounts of surface active agents. The method is applicable to all types of surface active agents (*i.e.*, anionic, cationic, and nonionic) and to dry, liquid, or paste surfaces. Fifty agents have been tested. J.L.H.

201. World Food Outlook and the Dairy Industry. GOVE HAMBIDGE, Canad. Dairy and Ice Cream Jour., *26,* 3: 25. March, 1947.

The work of the Food and Agricultural Organization of the United Nations is solving the widespread food shortage on a health standard for all people. The article discusses the dangers of over-production, the world food board, the Bruce commission to study proposals and make recommendations to the Food and Agricultural Organization, the high increase in production, and markets in backward lands. H.P.

202. Public Relations. J. W. LAWRENCE. Canad. Dairy and Ice Cream Jour., *26,* 3: 33–36. March, 1947.

Public relations begin at home, and your own staff must be sold on the company before you can sell the public on it. The employees are partners as well as consumers in the industry. The producers' viewpoints must be recognized. Individuals and companies should work with their competitors

on the same major problems. The dairy, in general, works for the interests of the public, and consumer education should not be neglected. Profits and wages are the lubricant of industry. Publicity is only one of the tools of public relations. H.P.

203. **The Need for Apprentice Training in the Dairy Industry.** W. H. SPROULE. Canad. Dairy and Ice Cream Jour., *26*, 2: 28–30. Feb., 1947.

A well-formulated apprenticeship scheme can aid greatly in developing trained workers to provide leadership to the industry in the years to come. H.P.