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Vol. XXXII, No. 3, March, 1949

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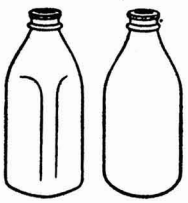

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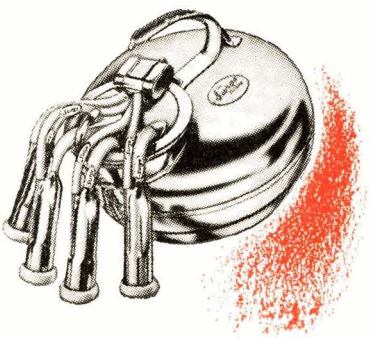

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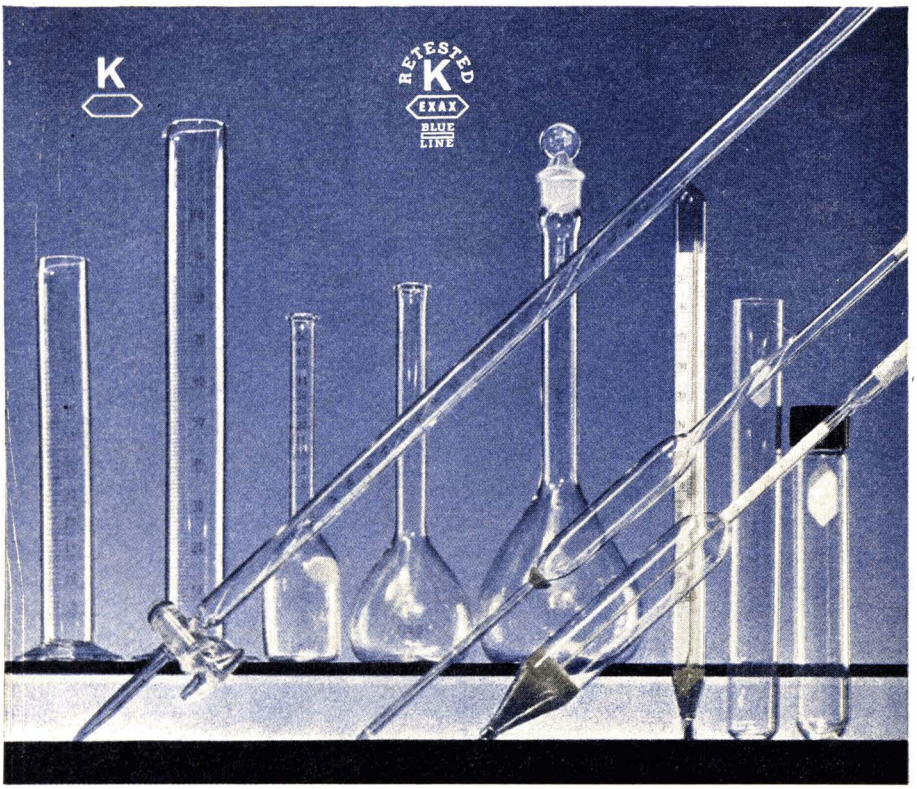

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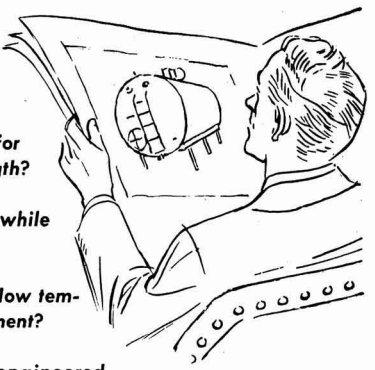

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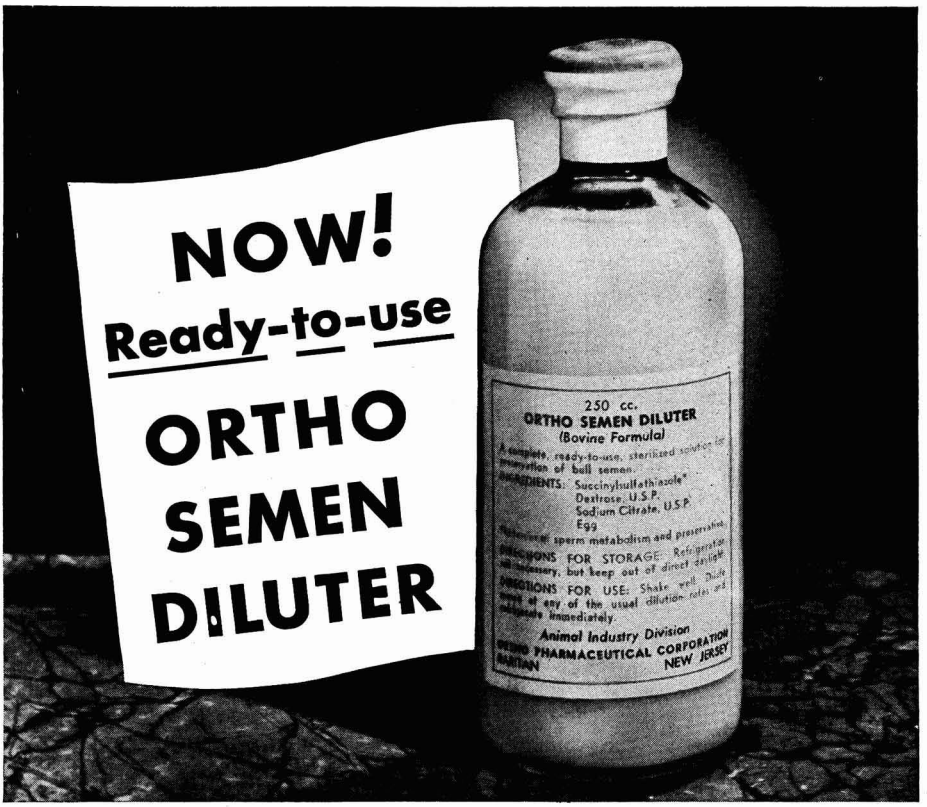

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JOURNAL OF DAIRY SCIENCE

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ASH ALKALINITY OF DRY BUTTERMILK

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This study was undertaken to determine what standard, if any, could be established to differentiate dry buttermilk manufactured from cream to which a neutralizer had been added from dry buttermilk manufactured from cream to which no neutralizer had been added.

METHODS

The ash alkalinity of the dry buttermilk was determined according to the method of Hillig (1). The source of all samples was cream from milk produced by the University herd. One-gallon samples of 35 per cent cream to be ripened were placed in 5-gallon milk cans, inoculated with 1 per cent butter culture and incubated at 70 to 72° F. in a water bath until the desired acidity was developed. The acidity was raised approximately that percentage which the neutralizer would lower it on a theoretical basis, calculating all acidity as lactic acid. When the desired acidity was developed, the calculated amount of neutralizer was added and the sample was pasteurized at 180° F. for 30 minutes. Some of the samples in which a starter was growing actively increased in acidity while they were being heated for pasteurization. Thus the developed acidity in some samples sometimes was higher than was intended.

Those samples which developed acidity and were not neutralized could not be pasteurized because the high concentration of acid would coagulate the protein. Therefore, the development of acid was checked in these samples by removing them from the water bath and placing them in a hardening room at about −15° F.

The data were analyzed statistically by the student "t" function method (3).

RESULTS

In this study, the ash alkalinity of 21 samples of dry sweet cream buttermilk to which no neutralizer had been added ranged from 6 to 92, with an arithmetic mean of 37 and a geometric mean of 35 (table 1). Fourteen samples gave values in the range from 0 to 49 while 7 were in the range from 50 to 99.

The ash alkalinity of 21 samples of unneutralized dry sour cream buttermilk ranged from 10 to 108 with an arithmetic mean of 49 and a geometric mean of 40. Twelve samples gave values in the range 0 to 49, 7 in the range from 50 to 99 and 2 in the range from 100 to 149 (table 1).

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พุทธมณฑล กรมวิทยาศาสตร์
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TABLE 1

Effect of developed acidity upon the ash alkalinity of dry buttermilk

Sample	A.A.V.[a] before acidity was developed	A.A.V.[a] after acidity was developed	% increase in acidity
1	54	50	0.51
2	50	105	0.47
3	25	52	0.51
4	32	35	0.54
5	18	62	0.62
6	30	40	0.65
7	27	48	0.58
8	23	38	0.59
9	36	34	0.63
10	21	22	0.56
11	6	38	0.52
12	27	26	0.57
13	32	10	0.45
14	15	28	0.43
15	20	12	0.46
16	23	10	0.39
17	58	58	0.57
18	68	72	0.60
19	52	98	0.61
20	92	108	0.61
21	78	88	0.50
Arithmetic mean	37	49	
Standard deviation	21	30	
Geometric mean	35	40	

[a] A.A.V. = Ash alkalinity value.

A statistical analysis of these data indicate 85 per cent certainty that samples containing developed lactic acid will possess higher ash alkalinity values than samples with no developed lactic acid. Therefore differences of this magnitude may be expected solely through errors of random sampling 15 times in 100.

Seven experimental trials were made in which each sample of cream was split into subsamples, ripened, neutralized the desired amount, pasteurized,

TABLE 2

Effect of neutralizing with varying amounts of sodium bicarbonate on ash alkalinity values of dry buttermilk

Sample	Per cent lactic acid neutralized					
	0.00	0.10	0.20	0.30	0.40	0.50
	Ash alkalinity values					
S0	41	.....	190	.....	371	.....
S2	54	124	142	231	296	356
S3	50	92	152	208	255	355
S4	25	70	120	172	262	390
S5	12	62	132	260	260	385
S6	18	92	162	212	292	348
S7	30	95	165	220	288	396
Arith. mean	33	89	152	217	289	371
Geom. mean	29	87	150	216	287	371

TABLE 3

Effect of neutralization with varying amounts of sodium bicarbonate on the frequency distribution of ash alkalinity values of dry buttermilk

Ash alkalinity values	No. of samples in each neutralization group (Neutralization expressed as per cent lactic acid)						
	0.00[a]	0.00[b]	0.10	0.20	0.30	0.40	0.50
0– 49	17	12					
50– 99	7	7	5				
100–149		2	1	6			
150–199				7	1		
200–249				1	4		
250–299					1	6	
300–349							1
350–399						1	5
Total samples	24	21	6	14	6	7	6
Arith. means[c]	36	49	89	158	217	289	372
Geom. means[c]	30	40	87	154	216	287	371

[a] No developed acidity.
[b] Developed acidity.
[c] Means of ash alkalinities.

cooled, held overnight, churned and dried. Sodium bicarbonate, the only neutralizer added, was used in quantities theoretically needed to neutralize 0.10, 0.20, 0.30, 0.40 and 0.50 per cent lactic acid. The data are presented in table 2. The arithmetic means of the ash alkalinities were 89, 152, 217, 289 and 371, respectively. None of the samples to which neutralizer had been added had ash alkalinity values falling in the range 0–49 (table 3). However, there were 12 such samples that had ash alkalinity values in the range 50–149. All of the ash alkalinity values of samples to which no neutralizer had been added fell within the range 0–149. All samples to which 0.10 per cent neutralizer had been added

TABLE 4

Effect of addition of various neutralizers on ash alkalinity values of dry buttermilk[a]

Sample No.	Cream acidity		Ash alkalinity values							
	Before ripening	Before churning	No neutralizer		MgO	$MgCO_3$	CaO	$CaCO_3$	K_2CO_3	$NaHCO_3$
			Unripened	Ripened						
	(%)	(%)								
N0	0.13	.....	21	.....	200	164	173	194	144	120
N1	0.10	.....	40	.....	125	132	119	98	114	128
N2	0.13	0.50	58	58	148	168	170	175	115	148
N3	0.13	0.45	68	72	185	162	195	162	158	182
N4	0.13	0.60	52	98	132	172	175	185	142	178
N5	0.13	0.60	92	108	195	190	190	232	188	200
N6	0.11	0.39	78	88	185	178	182	140	145	158
Arithmetic mean			58	85	167	167	172	169	144	159
Geometric mean			53	83	164	166	170	164	142	157
Standard deviation			24	20	31	18	27	42	25	29

[a] The acid of each cream was reduced 0.20 per cent after ripening and before pasteurization by the addition of the neutralizer indicated.

and six of the samples to which 0.20 per cent neutralizer had been added also fell within this range.

Seven lots of cream were used in the study of dry sour cream buttermilk in which the acidity was standardized with various neutralizers. Each lot of cream was divided into two parts. One sub-sample of each was churned and the buttermilk dried. The other part was ripened to give approximately 0.20 per cent additional acidity, after which that amount of neutralizer was added which was theoretically sufficient to neutralize 0.20 per cent lactic acid. The cream was churned and the buttermilk dried. The neutralizers used were magnesium oxide, magnesium carbonate, calcium oxide, calcium carbonate, potassium carbonate and sodium bicarbonate. The results of the ash alkalinity determinations on the dry buttermilk are shown in table 4.

A statistical comparison of ash alkalinity values of samples containing 0.20 per cent lactic acid neutralized with magnesium oxide and samples containing 0.20 per cent lactic acid neutralized with sodium bicarbonate indicates that in less than 55 cases out of 100 will the former have higher ash alkalinities than the latter. A similar comparison of samples neutralized 0.20 per cent with equivalent amounts of calcium oxide and sodium bicarbonate indicates that in 97 cases out of 100 the former will have higher ash alkalinities than the latter.

DISCUSSION

Kunkel and Combs (2) found that the range of ash alkalinity values for 16 samples of dry sweet cream buttermilk to which no neutralizer had been added was from 55 to 112, with an arithmetic mean of 78. In this study, the ash alkalinity values of 21 such samples ranged from 6 to 92 with an arithmetic mean of 37.

A statistical analysis indicated it was only 85 per cent certain that samples containing developed lactic acid will possess a higher ash alkalinity value than samples containing no developed lactic acid.

It also was indicated statistically that it was only 87 per cent certain that samples containing 0.10 per cent neutralized lactic acid would have higher ash alkalinity values than samples containing developed acid but no neutralizer.

Whether this degree of reliability is satisfactory or not depends on how it is used. If used merely as an indicator of quality with an awareness of its limitations, it is satisfactory; if used for regulatory work, a test which fails to identify properly 13 samples out of every 100 hardly could be called satisfactory.

The addition to liquid buttermilk of magnesium oxide, magnesium carbonate, calcium oxide, calcium carbonate, potassium carbonate and sodium bicarbonate sufficient to neutralize 0.20 per cent lactic acid increased the ash alkalinities of the dry buttermilks.

The degree of reliability is such that 99 times in 100 a sample which has had 0.20 per cent lactic acid neutralized by any one of these six compounds will have a higher ash alkalinity value than the same sample containing developed lactic acid but no neutralizer would have had.

In all cases where an ash alkalinity value of 150 or higher was obtained, a neutralizer had been added to the buttermilk before drying.

An ash alkalinity value of 149 or less would include all the samples to which no neutralizer had been added, all samples to which 0.10 per cent neutralizer had been added and approximately one-half of the samples to which 0.20 per cent neutralizer had been added. All samples neutralized more than 0.20 per cent would be excluded from this group.

CONCLUSIONS

From the data obtained on dry buttermilks secured from cream separated from University milk of high quality, it is evident that it is not possible to select a definite ash alkalinity value which will exclude all samples containing added neutralizers and which will include only those samples containing no added neutralizers. This is to be expected because the ash alkalinity values of non-neutralized dry sweet cream buttermilks cover a rather wide range.

Statistical analyses on the samples reported indicate that it would not be possible to distinguish samples of dry buttermilk containing 0.10 per cent neutralized lactic acid from non-neutralized samples of dry buttermilk by means of this test in over 90 cases out of 100.

ACKNOWLEDGMENT

Grateful acknowledgment is made to the American Dry Milk Institute, Inc., of Chicago, Illinois, for the financial aid which made this study possible.

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THE INFLUENCE OF TOCOPHEROLS AND COD LIVER OIL ON THE STABILITY OF MILK[1]

VLADIMIR N. KRUKOVSKY, J. K. LOOSLI, AND FRANK WHITING

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In the course of studies relating to the possible cause of the effect of cod liver oil in depressing the fat percentage of milk (8) samples of milk were obtained to test interrelationships between the fat constants and lipolysis of the milk fat. It was observed that the depressing effect of cod liver oil on fat production and the resulting high iodine numbers might not necessarily be permanent. In other words, the cow may adjust to this condition of feeding, and the fat production and the iodine numbers[2] may return to normal within a cod liver oil feeding period.

Since these observations also bear a relationship to the susceptibility of milk to the development of the oxidized flavors, it was thought of interest to present them together with more recent data on the influence of tocopherol and cod liver oil on the milk and fat production (12) and the stability of milk.

EXPERIMENTAL

The original data on the effects of the addition of cod liver oil to the feed of cows and of subsequent drenching on the iodine numbers and the susceptibility of milk to the development of oxidized flavors are presented in figure 1. Two cows were used in this experiment. From November 6 to 30 (period A) both cows were fed 0.5 ml. of a commercial grade cod liver oil per kilogram body weight by mixing it in the feed (dry beet pulp) (8). At the end of this period, the same amount of cod liver oil was administered to cow no. 1 by drench; this was continued until December 16 (period B), while cow no. 2 received no cod liver oil.

The data present in figure 1 show that from November 6 to 30 (period A) both the iodine numbers and, to some extent, the fat production returned to normal after an ecliptic rise or drop in their values for both cows. The following drench feeding of cod liver oil, from December 1 to 16 (period B), apparently destroyed the ability of the cow no. 1 to readjust physiologically to this feeding condition. The resulting rise in iodine values of the fat was, however, much more gradual than at the beginning of the experiment. Although these data are not sufficient to warrant a definite conclusion, they show a possibility that the conditions of feeding and the length of the feeding period both are responsible for the physiological behavior of the cow. It should be noted, how-

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[1] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned no. *212* in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

[2] Hanus method (1).

ever, that the samples of milk were collected and analyzed daily, and that the foregoing effect would be masked if composite samples of milk were collected and analyzed.

It further was noticed that the development of the oxidized flavors in fresh pasteurized milk[3] obtained from the cows on the cod liver oil feeding trial varied directly with the iodine numbers of the fat. The data in figure 1 show that the drop in the iodine numbers of the fat resulted in the retardation of the development of oxidized flavors, whereas the rise in the iodine numbers during the drench period stimulated the development of oxidized flavors.

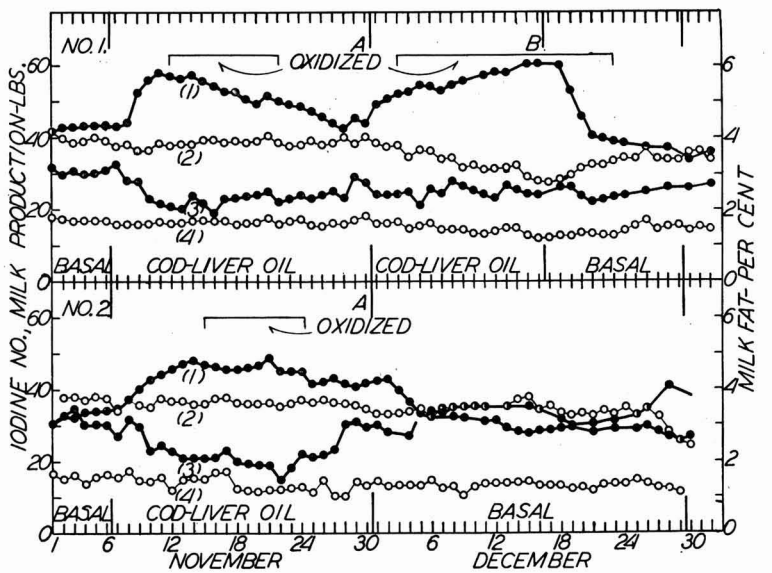

FIG. 1. The effects of the addition of cod liver oil to the feed of the cow (No. 1-A and No. 2) and of the subsequent drenching (No. 1-B) on iodine number, fat content and the susceptibility of milk to development of the oxidized flavors. The symbols indicate: (1), iodine numbers; (2), total milk production; (3), milk fat—per cent; (4), morning milk production.

Since our observations have indicated that there might be a relationship between the vitamin E content of the fat, as affected by seasonal variations of the feed (primarily pasture and hay feeding) (6, 7), and the susceptibility of fresh milk to the development of oxidized flavors, it was thought of importance to learn if the depressing influence of cod liver oil upon the tocopherol content of fat was partially responsible for the inability of milk to resist oxidation.

Consequently, the milk samples obtained from 16 dairy cows of Holstein, Brown Swiss and Guernsey breeds were used for the studies of the influence of tocopherols and cod liver oil on milk fat production and the natural ability of

[3] Milk was pasteurized at 61.6° C. for 30 minutes and then held at 0 to 5° C. for 7 days.

milk to resist the oxidative deterioration. One gram of mixed natural tocopherol and 28.35 g. of veterinary grade cod liver oil were added, either alone or together, to a control ration. The detailed procedure for this study is described in the preceding paper (12).

The feeding of cod liver oil alone caused a decrease in average tocopherol content of the fat (from 2900 μg. to 2529 μg./100 g. fat), and when the tocopherol and cod liver oil were fed together the average tocopherol content of the milk fat (3590 μg./100 g. fat) remained essentially the same as when the tocopherol was fed alone (3569 μg./100 g. fat).[4]

The data presented in figure 2 show the per cent distribution of tocopherols in the samples of stable and unstable milks as affected by tocopherol and cod

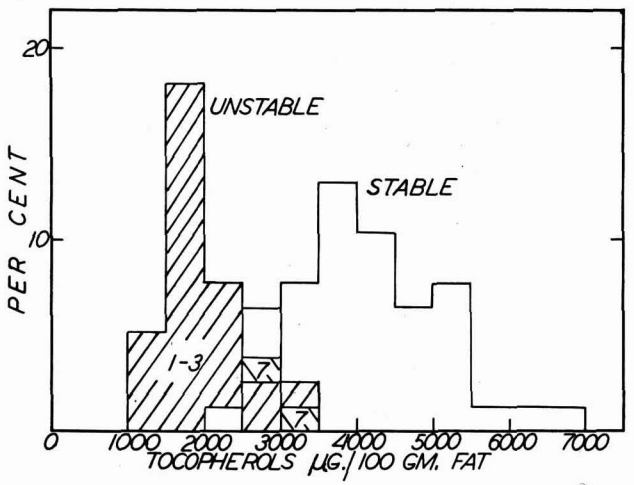

FIG. 2. The distribution of tocopherols in 77 samples of stable and unstable milk as affected by tocopherol and cod liver oil supplements. (The numbers (1–3 and 7) indicate the days required for oxidized flavor development in unstable milk.)

liver oil supplements. The stability of milk was determined on the basis of its ability to resist the reaction which produces oxidized flavors during a storage period of 7 days at 0 to 5° C.

A highly significant correlation (+0.51) was found between the tocopherol content of the milk fat and the ability of milk to resist development of oxidized flavors. The data also reveal that by varying the tocopherol content of the milk fat through feeding the stability of the fresh pasteurized milk was improved when its tocopherol content was increased to 3000 μg. per 100 g. of fat and above. All such samples were stable during the 7-day experiment. On the other hand, the milk containing less than 2000 μg. tocopherols per 100 g. of fat showed poor keeping qualities with respect to the development of oxidized

[4] Vitamin A and E were determined using Koehen and Sherman (2) and Quaife (9) methods, respectively.

flavors. This milk could be improved, however, by destroying its total vitamin C.

Furthermore, a relationship also was indicated between the tocopherol content of milk fat, as influenced by various hays, and the stability of milk (7), and between the tocopherol and carotenoid content of milk fat during pasture and winter feeding (6).

In a preliminary study concerning the palatability of hay, the deposition of fat-soluble vitamins in the milk fat and the stability of milk, 15 Holstein cows were fed six types of hay in an incomplete block design experiment. It was found that ladino clover hay and late-cut timothy hay in the rations were responsible for a decrease in the vitamin content of the fat (average values per 100 g. of fat: carotene, 347 μg.; vitamin A, 438 μg.; tocopherols, 1,873 μg.). On the other hand, the feeding of birds-foot trefoil hay, which appealed to the cows more than any other hay, resulted in an appreciable increase in the content of vitamins A and E in the fat (average values per 100 g. of fat: carotene, 723 μg.; vitamin A, 703 μg.; tocopherols, 2,952 μg.), possibly bringing it up to the level observed during pasture feeding.

Milk of poor keeping quality resulted during the ladino clover feeding and could be correlated with the low content of tocopherol, suggesting a possibility that the development of the oxidized flavors in the milk is caused by the type of hay or other roughages fed. This study, however, will be repeated to obtain more information concerning the influence of hay upon the vitamin content and the keeping quality of milk.

Thus, it would appear that the increase of the reducing power of fat, as determined by the tocopherol method, might result not only in the stabilization of market milk, but also in better nutritional properties with respect to vitamins E and A.

DISCUSSION

There apparently are two systems in the milk which are antagonistic to each other: one involves the oxidation of ascorbic acid and of the unstable lipid fraction of the milk (3), and the other is represented by the antioxidant activity of the fat itself. The authors believe that the antioxidant activity of the fat, as determined by the vitamin E method (9) (reducing power), is an important factor with respect to the stabilization of both the fat and the unstable lipid fraction of the milk. The latter is a part of the fat-globule-stabilizing membrane. It is known that buttermilk containing the materials adsorbed on the surface of the fat globules undergoes oxidative deterioration in the presence of ascorbic acid at a much faster rate and to a greater extent than does fresh milk (3, 4). It would be logical to assume, therefore, that the antioxidant activity centered in the fat phase of the milk might exert a protective influence not only to the fat itself but also to the materials which are adsorbed on the surface of the fat globules.

A private communication from Betty M. Watts (11) supports our observations concerning the role played by ascorbic acid and tocopherols in stimulation

or inhibition of oxidative rancidity in edible fats. Their study was concerned with the acceleration by ascorbic acid of oxidative rancidity in rendered pork fat. They found that the acceleration changed to inhibition as the level of tocopherols was raised.

In this connection it should be noted that the rate of ascorbic acid oxidation is a primary factor responsible for the development of oxidized flavors in fresh milk (4, 5). This oxidation of ascorbic acid often is accompanied by a gradual increase in Eh factor up to the point when all of the ascorbic acid is oxidized to dehydroascorbic acid, and then the Eh declines, finally attaining approximately its original value. This process could be repeated by the readdition of ascorbic acid. For example, the photo-oxidation of ascorbic acid to dehydroascorbic acid was found to be accompanied by a rise in Eh factor (10).

The removal of the catalyst (light) at any point along the line, but prior to completion of ascorbic acid oxidation, results invariably in a temporary stability in the Eh factor. The following changes in Eh would depend primarily upon the rate of ascorbic acid oxidation. Finally, when all of the ascorbic acid is oxidized, the Eh value might stabilize itself at the level approaching that of the original system.

It has been shown that the partial photo-chemical oxidation of ascorbic acid to dehydroascorbic acid stimulates the reaction which produces oxidized flavors in milk, whereas its complete oxidation either retards or prevents the reaction (4, 5). It would appear, therefore, that the Eh should be considered only as the by-product of the reaction involving ascorbic acid oxidation. It could not be considered, under any circumstances, as a criterion of the ability of fresh milk to resist the reaction which produces oxidized flavors.

SUMMARY

A significant correlation was found between the tocopherol content of milk fat and the ability of milk to resist the reaction, involving ascorbic acid oxidation, which produces oxidized flavors. This might explain the differences between the stabilities of winter and summer milks.

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THE EFFECT OF HANDLING AND PROCESSING OF MILK ON ITS OXYGEN CONTENT

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The effect of dissolved oxygen on the flavor and keeping quality of milk has been shown by a number of investigators. The purpose of this study was to determine the effect of various handling and processing operations on the oxygen content of market milk.

REVIEW OF LITERATURE

The oxygen content of anaerobically-drawn milk has been reported in volumes per cent as 4.29 by Hoppe (5), as 1.93 and 4.79 by Stechnow (12), as 1.19 and 1.06 by Pfluger (8), as 2.88, 3.09, 2.69, 2.14, 1.60 and 2.12 by Marshall (6), and as 1.17 by Frayer (2). On the other hand, Guthrie (3), Guthrie *et al.* (4) and Sharp *et al.* (9) reported that milk in the udder of the cow is practically devoid of oxygen. Hoppe's (5) oxygen values were obtained from goat's milk. It is presumed that the values reported by all the other investigators were obtained from cow's milk.

Guthrie *et al.* (4) reported that machine-milked milk contained 1 to 2 mg. of oxygen per liter less than that milked by hand. They found that milk dissolved 3.84 to 9.74 mg. of oxygen per liter during milking. Frayer (2) and Noll and Supplee (7) reported that the oxygen content of raw milk varied from 0.40 to 0.50 volumes per cent. Sharp *et al.* (9) found that 40 cans of raw milk received in a plant in New York City contained 4 to 7.1 mg. of oxygen per liter in samples which represented over half a million pounds of raw milk, and in another trial (10) they reported 9.3 to 11.7 mg. of oxygen.

EXPERIMENTAL PROCEDURE

Four plants, designated as *A, B, C* and *D,* were selected for this study. Plant *A* is the University of Illinois Creamery where milk is obtained from the University farm, while the other plants are located in the Champaign-Urbana area.

Plant A. In this plant ten trials of 1 day each were completed in order to determine how closely the results from each trial would agree and thus provide a basis for establishing the minimum number of trials that would be necessary in similar experiments in other plants.

Samples were collected from the same ten animals for 1 day at the farm. A different group of ten cows was used in each of the ten trials which were conducted during the period from January 15 to March 1. Samples were taken in the morning and in the evening from each of the cans as they were filled with milk from the surface cooler at 8 to 10° C., and they were held at the farm and

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delivered to the processing plant at or near these temperatures. When the milk arrived at the processing plant, samples were taken from the following points: weighing tank, preheater at 27 to 30° C., clarifier at the discharge side, pasteurizer at 38 to 39° C., milk pasteurized at 66° C. for 30 minutes and before it was cooled, milk immediately after it had passed through homogenizer at 55° F. and at 2,000 lb. pressure, milk at 50 to 53° C. from top of surface cooler, milk at 3 to 4° C. from bottom of surface cooler and before it reached the trough, bowl in the bottle filler at 5° C., bottles immediately after being filled and bottles after storage at 4 to 8° C. for 24 hours.

Samples were collected at plant *A* for Eh determinations which were made with a Model G Beckman potentiometer. Plate counts were made on the raw and pasteurized samples according to Standard Methods (1).

Plant B. Two trials were made in this plant during the fourth week of March. Two different trucks delivered milk to this plant from producers who averaged two to four 10-gallon cans daily which, in the majority of cases, were filled only partially. The average temperature of the milk at the time of delivery was 7° C.

Samples were taken from the following points: weighing tank from ten producers, filter by draining milk at 7 to 10° C. through valve, storage tank where filtered milk was held at 8 to 10° C., plate heater just before milk passed through, plate heater, after milk at 68 to 72° C. had passed through, clarifier, as milk at 71° C. was discharged, milk immediately after it passed through homogenizer at 69 to 70° C., at 1,500 lb. pressure, from three 200-gallon holding vats with milk at 65 to 67° C., plate cooler, where milk was cooled at 9 to 10° C., bottle filled with milk at 9 to 10° C., bottles immediately after being filled and bottles after storage at 4 to 8° C. for 24 hours.

Plant C. In this plant, two trials were conducted during the first week of April. This plant received one truck load of milk from patrons, each of whom delivered two to three cans of milk which were not completely full in all cases. The temperatures of the milk varied from 9 to 27° C.

Samples were taken from the following points: weighing tank, storage tank with milk at 17 to 20° C., preheater with milk at 43° C., clarifier as milk at 41° C. was discharged, pasteurizing vat with milk at 49 to 52° C., milk pasteurized at 65° C. for 30 minutes, milk immediately after it passed through homogenizer at 63 to 64° C. at 2,000 lb. pressure, plate cooler after milk had been cooled at 8° C. bottle filler of vacuum type with milk at 8° C., milk from bottles immediately after being filled and milk from bottles after storage at 4 to 8° C. for 24 hours.

Plant D. Two trials were conducted in this plant during the second week of April. The milk, at 10 to 15° C., was delivered to the plant about 11:30 a.m. and consisted of two to five cans from each producer.

The oxygen determinations of the milk were made at 21 to 25° C. by the method of Sharp *et al.* (11), the only variation being that the tubes in which the samples were collected and the oxygen determinations made were painted black.

Samples of milk were taken at easily accessible points in the processing operations with a 50 ml. pipette. Samples were taken from the closed milk lines through sanitary pipe and fittings to which stopcocks were attached. The milk flowed into the sampling tubes through glass tubes which were attached to the stopcocks with short pieces of rubber tubing. Immediately after the samples were taken at the different processing points, the tubes were stoppered tightly and the contents cooled to 3 to 5° C. in an iced bath. The samples were analyzed for oxygen about 2 to 4 hours after they were taken, except those from the farm of plant *A*, which were analyzed within 6 hours.

EXPERIMENTAL RESULTS

Plant A. The results of the ten trials showing the dissolved oxygen contents of the milk at each of the processing points are averaged in table 1. Assuming

TABLE 1

The effect of handling and of processing on the oxygen content of milk in plant A

Source of milk	Oxygen content mg./l.	standard error
Pails, P.M.[a]	4.50	± 0.11
Pails, A.M.[a]	4.25	± 0.10
Cans, P.M.[a]	6.76	± 0.20
Cans, A.M.[a]	6.80	± 0.21
Weigh tank[b]	6.83	± 0.22
Preheater[b]	6.86	± 0.22
Clarifier	7.15	± 0.26
Before pasteurization[b]	6.20	± 0.18
After pasteurization[b]	5.06	± 0.11
After homogenization[b]	5.72	± 0.23
Top of cooler[b]	5.70	± 0.24
Bottom of cooler[b]	6.79	± 0.22
Filler[b]	6.73	± 0.15
Bottled[c]	6.59	± 0.20
Bottled, after 24 hours[c]	6.76	± 0.19

[a] Average of 10 samples in each trial.
[b] Average of 3 samples in each trial.
[c] Average of 5 samples in each trial.

there is little, if any, free oxygen in milk in the udder (3, 4, 9), the greatest solution of oxygen occurred at the time of milking. The oxygen content of the cooled milk in the cans increased over that in the pails but remained about the same for both the evening and morning milk. The oxygen contents of the milk in the cans, in the weigh tank and in the preheater do not show any significant differences as indicated by the means and standard error of the means; in fact, the oxygen content increased only slightly at the clarifier. As the milk was heated in the vat before pasteurization, the oxygen content decreased, and it decreased further during pasteurization. The oxygen content increased to 5.72 mg. immediately after homogenization and remained near this level at the top of the cooler. The oxygen contents of the milk samples from the bottom of the cooler, from the filler, from milk bottled immediately, and from that bottled for 24 hours, do not show any significant differences.

Plate counts were made of the raw and pasteurized milk in each of the ten trials and they varied from 4,200 to 48,000 and 300 to 1,300 per ml., respectively, and are insufficient to affect the oxygen content of raw and pasteurized milk measurably.

Oxidation-reduction potential (Eh) determinations were made of the samples of milk taken at the different processing points in all ten trials. The Eh values increased from 301.3 mv. at the time of milking to 311.3 mv. in the freshly-bottled milk, to 315.3 mv. for milk held in the bottles for 24 hours.

Since the standard errors of the average oxygen determinations made at each of the processing points in the ten trials conducted in plant *A* were fairly consistent and of about the same magnitude (table 1), it was decided that two experimental trials of 1 day each in plants *B*, *C* and *D* would be sufficient.

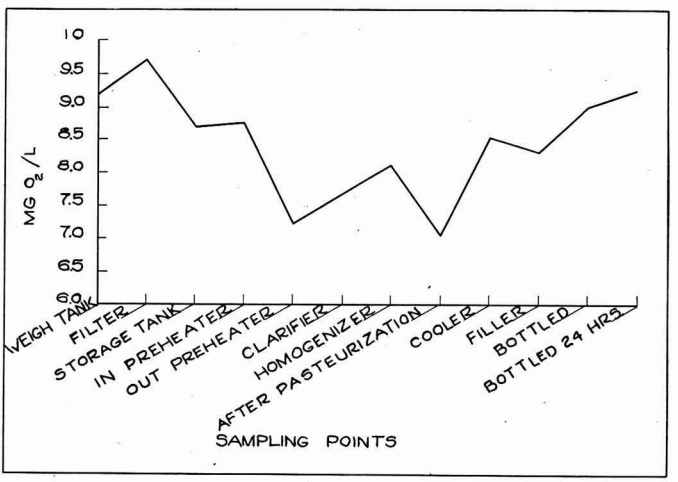

FIG. 1. Average oxygen content of milk at various steps during processing operations in plant *B*.

Plant B. The raw milk as it arrived at the plant had a relatively high oxygen content which probably was due to agitation of the partially-filled cans of cold milk (figure 1). The oxygen content of the milk increased as it passed through the filter from the weigh can, but at the storage tank it decreased 1 mg. From the storage tank to the preheater, the oxygen content remained about the same, followed by a decrease of 1.5 mg. after preheating, but increased again after clarification. At the homogenizer the oxygen content increased, followed by a substantial decrease in the holding vat after pasteurization. It increased about 1.7 mg. as it left the cooler, dropped slightly at the filler but increased slightly after bottling and after 24 hours storage.

The plate counts of the raw milk in the two trials were 2,200,000 and 2,400,000 per ml. and of the pasteurized milk 27,000 and 41,000 per ml., respectively.

Plant C. The milk delivered to this plant had a lower oxygen content at

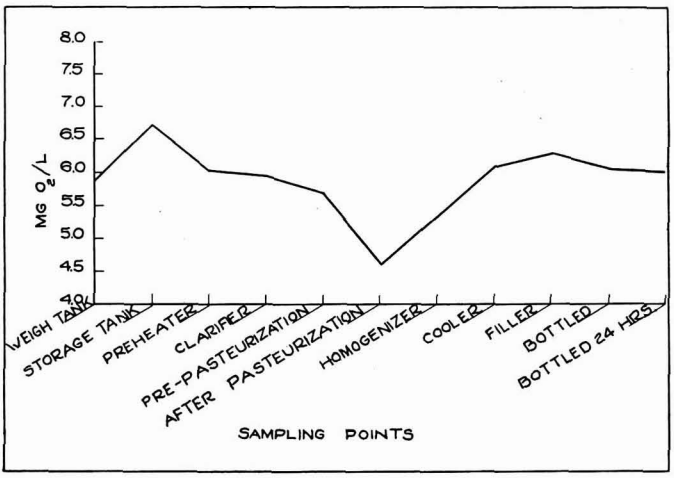

FIG. 2. Average oxygen content of milk at various steps during processing operations in plant *C*.

the weigh tank than that found at any of the other plants (figure 2). This may be due to the greater counts of bacteria and to the higher temperatures of the milk at the time of delivery.

At the weigh tank the milk had an average oxygen content of 5.84 mg./l. which increased to 6.72 mg. in the storage tank but decreased at the preheater and slightly decreased at the clarifier; the lowest oxygen level occurred during

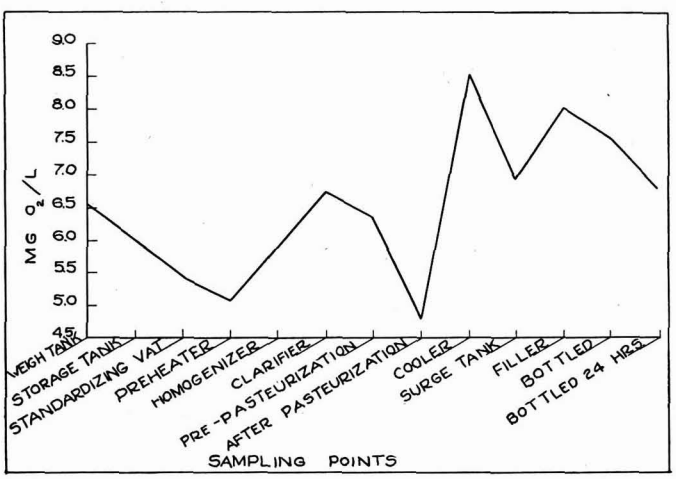

FIG. 3. Average oxygen content of milk at various steps during processing operations in plant *D*.

pasteurization. The oxygen content of the pasteurized milk increased 0.7 mg. at the homogenizer and increased further after cooling, but remained about the same at the bottle filler and in the bottles.

The plate counts of the raw milk in these trials were 6,500,000 and 6,200,000 per ml., and the pasteurized milk, 130,000 and 67,000 per ml., respectively.

Plant D. The milk at the weigh tank had an average oxygen content of 6.54 mg./l. (figure 3). After being cooled and held in the storage tank, there was a decrease of about 0.5 mg., and a further decrease in the standardizing vat and at the preheater. The oxygen content of the milk increased at the homogenizer, increased further at the clarifier and reached its lowest level during pasturization. As the milk flowed from the cooler, the oxygen content increased nearly 4 mg. but decreased about 2 mg./l. in the surge tank. Milk from the filler bowl showed an increase in oxygen which was followed by decreases immediately after bottling and after storage for 24 hours.

The plate counts of the raw milk in both trials were 500,000 and 470,000 per ml., and the pasteurized milk 9,700 and 9,200 per ml., respectively.

DISCUSSION

Assuming only small amounts of oxygen in milk in the udder, the greatest oxygen absorption occurred at the time of milking. The oxygen content was higher when milk was subjected to agitation in partially-filled cans enroute to the plant.

The same general trend in oxygen content of the milk at similar processing points was observed in all four plants. There was a close relationship in all plants between the oxygen content of the raw milk at the time of delivery and its oxygen content at the various points of processing; however, the percentage decrease or increase at any of these points was approximately the same when calculated on the basis of the oxygen content of the raw milk in the weigh tank.

As expected, the lowest oxygen level occurred in milk during pasteurization, being 21.2 to 27.3 per cent less than the raw milk in the weigh tank, and it increased again when the milk was cooled. In plant *D*, where a high-temperature short-time pasteurizer was used, a reduction in oxygen occurred during pasteurization in this closed system, with a subsequent increase in cooling. The effect of heat on the solution of oxygen in milk conforms to the solubility of gases in liquids as affected by temperature, a fact pointed out by Noll and Supplee (7).

The lower oxygen level in the raw milk in plant *C* probably was due to the higher bacterial count and to the higher temperatures at which most of the raw milk was delivered. Furthermore, it is interesting to note that the oxygen content of the milk at comparable processing points was lower in plant *C* than in any of the other plants.

In all plants, the amount of oxygen in the bottled milk was approximately the same as that in the weigh tank.

CONCLUSIONS

1. The greatest amount of oxygen absorption occurred during milking.

2. The amount of dissolved oxygen in raw milk presumably was influenced by its temperature and bacterial content and by the amount of milk in the cans at the time of delivery.

3. The oxygen content of the milk at various processing points was related to its oxygen content in the weigh tank.

4. The oxygen content in the bottled milk attained an equilibrium comparable to that of the raw milk in the weigh tank.

5. The oxygen content of the milk showed the same general trend at comparable processing points in all four plants.

6. The solubility of oxygen in milk conforms to the solubility of gases in solutions as affected by temperature.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. III. SEVERAL FACTORS AFFECTING VITAMIN A AND CAROTENOID CONTENT[1, 2]

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Vitamin A and carotenoids in colostrum from dairy cows have been the subject of numerous investigations. It has been observed that levels of these constituents generally are high in the initial colostrum and colostric fat but decrease rapidly as the mammary secretions change to normal milk (4, 6, 8, 11, 14, 20, 21, 22, 25, 26, 27, 28, 29). Similar changes were noted in colostrum from sheep (1, 23, 31) and from women (5, 15, 16). During the early stages of the transition, decreases in concentration of fat-soluble pigments of bovine colostrum have been reported to follow a logarithmic trend (22), but an expression of the rate of change of vitamin A apparently has not been published.

In one study (27), supplementation of the ration of preparturient cows with vitamin A and carotene (carrots) did not appear to increase levels of these constituents in colostrum; however, others found that pasture increased the carotene (11) and the total vitamin A potency (14, 21). Recent investigations have shown that prepartal vitamin A supplementation augmented the vitamin A content of colostrum from cows (7, 25) and from does and sows (30).

Much of the previous work has emphasized the variability in concentrations of vitamin A and carotenoids of colostrum, even from animals maintained under similar conditions. Vitamin A levels of the first postpartum mammary secretions from first-lactation cows were found to be approximately double those from cows in later lactations, but differences have not been reported for carotenoids (4, 10, 11). The effect of breed upon vitamin A and carotenoids of colostrum either has been investigated with too few cows to warrant general conclusions (9, 24) or results have been complicated by inclusion of both first- and later-lactation animals in the same experimental groups (28, 29).

Since it seemed desirable to obtain additional information on effects of various factors on vitamin A and carotenoid levels in colostrum and early milk, the present study was undertaken. Factors investigated were individuality of cows, breed, lactation number, type of prepartal ration and stage in the transition period.

EXPERIMENTAL

Feeding and management of experimental animals. In the major trials, comparisons were made of vitamin A and of carotenoid concentrations in co-

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[2] Portion of a thesis presented by D. B. Parrish in partial fulfillment of the requirements for the degree Doctor of Philosophy in chemistry at Kansas State College.

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lostrum from groups of cows receiving either typical unsupplemented barn feeds or similar rations supplemented with seasonal pasture or vitamin A concentrates. The 86 experimental subjects, which included both first- and later-lactation cows of Holstein, Ayrshire, Jersey, and Guernsey breeds, were divided into three groups. The reference group, designated as the "barn-fed," received a typical barn ration consisting of a concentrate mixture, Atlas sorgo silage and hay, fed according to conventional methods. The "pasture-supplemented" and the "vitamin A-supplemented" groups received, respectively, pasture grasses and vitamin A concentrates[4] in addition to the barn rations.

The hay fed consisted principally of alfalfa, but some prairie hay also was used. Pasture grazing was supplied by rye, a combination of alfalfa and brome grass, native grass or Sudan grass, depending on availability at different periods.

The trials were designed to restrict all the cows to experimental rations during the major portion of the 6- to 8-week prepartal conditioning period. Duration of the experimental feeding, however, varied considerably, depending upon seasonal conditions and upon deviations from expected date of parturition. In only six cases was the length of the prepartal supplemental-feeding period less than 2 weeks. Cows in barn-fed groups were off pasture 2 to 6 months prepartal. Supplementation with either pasture or vitamin A was discontinued at parturition, except as noted later.

Two major feeding trials were conducted, no. I in 1945 and no. II in 1946. In the former trial, the vitamin-A-supplemented cows received 1,000,000 I.U. of vitamin A daily during the 2 weeks preceding expected parturition date; in the latter, a 2-week period of supplementation (28–14 days prepartal) was added, during which time the cows received 500,000 I. U. of vitamin A daily. In pasture-supplemented groups, less concentrate was fed in trial II than in trial I in order to conserve grains. This was accomplished by shortening the period of prepartal concentrate feeding of these cows and by returning them to pasture on the fifth day postpartum instead of the fourteenth. In tables that follow, results for the eighth day, therefore, are not included for pasture groups of trial II.

Collection of samples. In order to avoid sampling difficulties, calves were not allowed to nurse. Each sample represented aliquots of well-mixed mammary products obtained by as thorough evacuation of the udder (10, 19) as possible by standard milking procedures, either hand or machine. The first milking generally was completed within 4 hours after parturition, and subsequent samples were collected at the regular morning and evening milking periods. Immediately after collection, samples were stored in the dark at 4° C. until removed for vitamin A and for carotenoid analyses, usually within 4 days.

Method of analysis. Vitamin A and carotenoids were determined by an adaptation of the Boyer *et al.* method (3). Since colostrum normally is more viscous than milk and is higher in vitamin A and carotenoids, larger volumes of ether and wash solutions were employed than prescribed in the original method.

[4] A concentrate of natural vitamin A ester in a special soybean flour base; however, two cows received vitamin A in a liver meal base and one cow received vitamin A in the form of a fish oil concentrate.

Modifications of the procedure were as follows: For extraction, 50 and 30 ml. quantities of ether were used, respectively, in the first and the second separatory funnels. The solutions containing the non-saponifiable matter were washed by gentle mixing with 100 ml. of water, followed by shaking with 40 ml. and then 25 ml. of acidified alcoholic wash solution. The two ether solutions were combined in one separatory funnel and 15 ml. of Skellysolve B were added to decrease the water content. The combined solutions were given a final washing by shaking with 25 ml. of cold water. An all-glass assembly of about 125 ml. capacity was used for evaporation of solvents. The residue was dissolved in Skellysolve B and diluted to volumes of 25 to 50 ml., the quantity employed depending upon the concentrations of vitamin A and carotenoids. The resulting solution was used for measuring total carotenoids.[5] A Coleman spectrophotometer was employed in the colorimetric measurements. Calibration data and a modification of the instrument for vitamin A analysis have been published (17). The antimony trichloride reagent was prepared essentially as outlined by Koehn and Sherman (13).

It was necessary to modify the procedure for colostrums of a high specific gravity since apparently either saponification or extraction was incomplete. Vitamin A and carotenoid values of colostrum samples having a specific gravity greater than 1.060 sometimes were increased as much as 25 per cent when saponification by boiling under reflux for 20 minutes was substituted for the original 3-hour saponification at room temperature.

Expression of results. The results are presented primarily as concentrations of vitamin A and of carotenoids per unit of fluid, since this yields information on both nutritive value and effects of dietary vitamin A and carotenoids on changes of each of these constituents in the mammary products. Total vitamin A and total carotenoid outputs during the first eight or nine milkings of the colostric period also are presented. Exceptions are made to the foregoing methods of stating results and other values are discussed only when expression in different units alters interpretations.

RESULTS AND DISCUSSION

Variations among cows of the same groups. Marked individual differences were found in colostrum from cows of the same breed, lactation and dietary groups. In comparisons of concentrations of either vitamin A or carotenoids in the first samples of colostrum from cows within each of the individual experimental groups (designated in tables 2 and 3), one-third of the ratios of the highest concentration to the lowest was more than 3:1, but similar comparisons of milk near the end of the transition period revealed that only one-tenth of the corresponding ratios was more than 3:1. In colostrum of the first milking, the widest ratio (12:1) for carotenoids was found in samples from first-lactation Jerseys receiving vitamin A supplements; the widest ratio (52:1) for vitamin A was in samples from first-lactation Jerseys fed the winter barn ration. The lowest

[5] A β-carotene calibration curve was used. Approximately 90 to 95 per cent of the total carotenoids may be considered as carotene (4, 18, 24).

value in the latter group was obtained from a heifer, J-367, that secreted an atypical colostrum that somewhat resembled whey (table 1). The extremely low vitamin A level associated with colostrum of low specific gravity was observed in secretions from only two other animals, also first-lactation heifers. Vitamin A and carotenoid levels in the milk at the end of the transition period were in the normal range.

It is difficult to account for the individual variations in colostrum, which phenomenon also has been noted by others (4, 8, 10, 11, 26, 28). Among the factors that might be involved are heredity, endocrine activity, appetite and dietary history.

Effect of breed. Although considerable variation within breeds was observed, the data from trials I and II (tables 2 and 3), especially the latter, show that con-

TABLE 1

Comparison of vitamin A and carotenoid contents of typical and atypical colostrum from Jersey heifers fed winter barn rations

Mammary secretion	Constituents	Number of milking								
		1	2	3	4	5	6+7[c]	8+9	12+13	16+17
		($\mu g./100$ *ml.*)								
"Normal"[a] (Av. of 3 heifers)	Carotenoids	147	189	107	59	41	35	27	16	17
	Vitamin A	319	398	218	125	72	75	67	30	25
"Low specific gravity"[b] (Heifer J-367)	Carotenoids	19	32	32	23	31	28	23	20	22
	Vitamin A	10	26	37	40	53	54	48	36	26

[a] In first colostrum, specific gravity = 1.060, total solids = 23.8 per cent and fat = 5.45 per cent; in composite of 16th and 17th milkings, corresponding values were 1.033, 13.9 and 4.27.

[b] In first colostrum, specific gravity = 1.027, total solids = 9.0 per cent and fat = 1.15 per cent; in composite of 16th and 17th milkings, corresponding values were 1.033, 13.4 and 4.1.

[c] Composite sample.

centrations of carotenoids in colostrum and in early milk from Jerseys and Guernseys tended to be higher than in similar secretions from Holsteins and Ayrshires. Highest values were noted most frequently in colostrum from Guernseys. The relationship of breed to vitamin A concentrations was variable and indefinite. Data reported by Sutton *et al.* (28, 29) also indicated that carotene was higher in colostrum from Guernseys than from other breeds, but the results from first- and later-lactation cows were not segregated.

Average total output of carotenoids during the transition period (first eight or nine milkings, tables 2 and 3) by Jerseys and Guernseys were higher than those from Holsteins and Ayrshires of the same experimental groups; however, in trial II there was a tendency for the latter breeds to secrete more vitamin A.

Effect of number of lactations. Vitamin A levels were higher in nine-tenths of the colostrum samples (first five milkings) from first-lactation cows than in those from cows in later lactations receiving the same treatment (tables 2 and 3). In more than one-half of the samples from the former groups, averages were at

TABLE 2

Effect of type of ration, number of lactation and breed of cows on vitamin A and on carotenoid contents of colostrum and early milk (Trial I)

Ration[a]	Breeds of cows	No. of cows of each breed	Lactation no.	Number of milking										Total output first 9 milkings
				1	2	3	4	5	6+7[b]	8+9	12+13	16+17	24+25	
				Carotenoids, μg./100 ml.										mg. carotenoids
Barn	Hol.-Ayr.	1–2	First	92	157	81	47	30	32	19	23	9	8	29
	Jersey	4	First	115	150	88	50	38	33	26	17	19	23	31
	Jersey	1	Later[c]	68	38	39	42	33	38	38	33	21	21	22
Pasture	Hol.-Ayr.	2–2	First	191	147	80	61	48	45	29[e]	17[e]	18	23[e]	40[e]
	Holstein	1	Later	560	110	58	.....	.....	.....	.....	.....	.....	.....	.....
	Jersey	4	First	434	351	207[d]	152[e]	138[e]	96[e]	69[d]	39[e]	29[e]	36[e]	78[e]
	Jersey	1	Later	173	151	125	100	104	101	80	62	55	44	50
Vitamin A supplement	Holstein	1	First	160	85	32	25	21	13	13	5	5	6	22
	Ayrshire	2	Later	95	69	59	32	24	26	14	19	12	16	27
	Jersey	1	Later	82	331	153	43	21	25	17	13	18	10	35
				Vitamin A, μg./100 ml.										mg. vitamin A
Barn	Hol.-Ayr.	1–2	First	324	460	183	104	71	61	32	28	24	17	72
	Jersey	4	First	242	305	173	104	68	70	62	32	26	27	64
	Jersey	1	Later[c]	45	28	25	42	29	30	43	13	10	18	19
Pasture	Hol.-Ayr.	2–2	First	416	353	171	115	54	54	43[e]	19[e]	18	20[e]	83[e]
	Holstein	1	Later	563	101	67	.....	.....	.....	.....	.....	.....	.....	.....
	Jersey	4	First	436	379	200[d]	187[e]	157[e]	97[e]	65[d]	37[e]	31[e]	36[e]	87[e]
	Jersey	1	Later	52	62	60	73	79	76	89	60	54	33	36
Vitamin A supplement	Holstein	1	First	1260	725	338	249	199	99	42	42	54	34	177
	Ayrshire	2	Later	359	382	275	149	126	129	72	56	52	45	124
	Jersey	1	Later	279	1480	700	220	100	130	76	49	42	44	165

[a] See text for description of rations.
[b] Composite sample.
[c] Two to four lactations.
[d] Mean calculated from one less than number of animals indicated.
[e] Mean calculated from two less than number of animals indicated.

TABLE 3

Effect of type of ration, number of lactation and breed of cows on vitamin A and on carotenoid contents of colostrum and early milk (Trial II)

Ration[a]	Breeds of cows	No. of cows of each breed	Lactation no.	Number of milking								Total output first 8 milkings
				1	2	3	4	5	6	7+8[b]	15+16	
				Carotenoids, μg./100 ml.								mg. carotenoids
Barn	Hol.-Ayr.	2–2	First	91	65	50	41	35	26	16	13	15
	Hol.-Ayr.	4–4	Later[c]	78	75	39	29	26	22	18	8	20
	Jer.-Guer.	2–4	First	230	196	100	73	59	41[d]	39	25	31[d]
	Jersey	2	Later	199	155	51	46	36	45	40	9[d]	27
Pasture	Hol.-Ayr.	2–2	First	185	198	99	90	60	59	42	.....	32
	Hol.-Ayr.	4–3	Later	222	186	121	93	79[d]	72[d]	45	.....	61[d]
	Jer.-Guer.	2–1	First	298	328	262	146	85	97	86	.....	58
	Jer.-Guer.	5–2	Later	342	341	207	179[d]	127[e]	138[e]	83[e]	.....	92[f]
Vitamin A Supplement	Hol.-Ayr.	1–2	First	64	61	38	28	20	15	15	9	13
	Hol.-Ayr.	3–1	Later	100	46	23	32	23	21[d]	19	11	23[d]
	Jer.-Guer.	2–1	First	162	121	73	82	52	62	37	16	26
	Jer.-Guer.	1–1	Later	270	64[d]	55	37	38	26	29	25	30
				Vitamin A, μg./100 ml.								mg. vitamin A
Barn	Hol.-Ayr.	2–2	First	409	316	195	142	95	53	35	20	50
	Hol.-Ayr.	4–4	Later[c]	148	157	79	54	48	40	34	22	41
	Jer.-Guer.	2–4	First	333	278	158	99	78	45[d]	36	18	38[d]
	Jersey	2	Later	276	209	43	45	45	55	42	23[d]	33
Pasture	Hol.-Ayr.	2–2	First	443	400	182	151	86	83	65	.....	62
	Hol.-Ayr.	4–3	Later	190	168	98	79	80[d]	61[d]	38	.....	51[d]
	Jer.-Guer.	2–1	First	294	317	222	125	69	73	62	.....	49
	Jer.-Guer.	5–2	Later	211	214	117	99[d]	73[e]	79[e]	49[e]	.....	48[f]
Vitamin A Supplement	Hol.-Ayr.	1–2	First	670	725	391	227	162	103	100	43	122
	Hol.-Ayr.	3–1	Later	482	266	159	141	127	87[d]	69	27	97[d]
	Jer.-Guer.	2–1	First	548	387	242	270	163	180	106	40	96
	Jer.-Guer.	1–1	Later	588	169[d]	145	100	119	83	61	45	75

[a] See text for description of rations.
[b] Composite sample.
[c] Two to seven lactations.
[d] Mean calculated from one less than number of animals indicated.
[e] Mean calculated from two less than number of animals indicated.
[f] Only three cows represented in mean value.

least twice as high as were those of samples from the latter groups. On the other hand, only about two-thirds of the colostrum samples from first-lactation cows had a higher average carotenoid concentration than did those from later-lactation cows, and in many cases there was not a consistent trend. Although calculations of carotenoids per unit of fat revealed higher values for first-lactation cows more frequently than did calculations per unit of secretion, differences were not marked enough to justify definite conclusions.

A further comparison of carotenoid and vitamin A contents of early mammary secretions from two different lactation groups is shown in table 4. As also noted

TABLE 4

Effect of the number of lactation on the carotenoid and the vitamin A contents of colostrum and early milk. (Same cows on similar dietary regimens used for comparison)

Vitamin A constituents	No. of cows	Lactation no.	Number of milking							
			1	2	3	4	5	6	7+8[a]	15+16
			Winter barn ration							
			μg./100 ml.							
Carotenoids	4	1st	121	179	88	49	35	35	23	10[b]
		2nd	136	111	44	39	30	33	27	10[b]
Vitamin A	4	1st	416	551	233	125	80	69	37	20[b]
		2nd	196	160	56	46	48	49	35	25[b]
			Pasture							
			μg./100 ml.							
Carotenoids	3	1st	221	188	109	80	82[b]	42[b]	36[b]	29[c]
		2nd	184	185	157	116	73[b]	74[b]	42[b]	8[c]
Vitamin A	3	1st	316	253	152	108	66[b]	42[b]	47[b]	10[c]
		2nd	127	134	107	81	63[b]	51[b]	43[b]	30[c]

[a] Composite sample.
[b] Average calculated from one less than number of animals indicated.
[c] Figure represents value from one cow only.

by others (4, 10, 11), carotenoid concentrations of colostrum were not related definitely to number of lactation, but vitamin A values were consistently higher in samples from the cows during their first lactation. These differences in vitamin A possibly are related to length of the non-lactating period before calving (26).

Relative effects of type of ration fed during latter stages of gestation. a. Roughages (carotenoids). It is shown by data of tables 2 and 3 that carotenoid contents of colostrum and early milk from pasture-supplemented cows of the same breed and lactation groups were from two to four times higher than corresponding mammary secretions from cows maintained on typical barn rations. An exception, however, was noted for the first-lactation Holstein-Ayrshire group of trial I in which such marked differences were not found in samples from the second through the thirteenth milkings.

Comparisons of the effects of pasture and of barn rations on vitamin A levels of the mammary secretions (tables 2 and 3) showed that differences in the averages generally were less marked than noted for carotenoids, vitamin A concentrations being higher for the pasture groups in only two-thirds of the samples compared. Supplementary calculations[6] indicated that vitamin A concentrations per unit of fat in colostrum and early milk from Jerseys and Guernseys of the pasture groups were no higher than those found in samples from cows of the same breeds fed only barn rations. Total output of Vitamin A, however, was consistently higher in colostrum from cows on pasture than from those receiving only barn rations.

If data on vitamin A and carotenoids were combined as total vitamin A potency, average values for pasture cows would be appreciably higher than those for similar groups of barn-ration cows in more than three-fourths of the samples.

Additional information on the comparative effects of dry rations and of pasture upon vitamin A and carotenoids of colostrum and early milk was obtained from a study involving five cows (Holsteins and Ayrshires) that were fed a carotenoid-low basal ration in which beet pulp replaced a part of the roughages. Three of the cows were allowed supplemental pasture both pre- and postpartally. Sampling and treatment of colostrum and milk were as previously described, except only secretions from the left half of the udder were used. Some samples were frozen and stored, which probably had little or no effect on vitamin A and carotenoid values (10). Concentrations of both vitamin A and carotenoids were more than two times higher in colostrum and early milk from cows receiving pasture than from those receiving only low-carotenoid rations.

These results are in accord with earlier studies (14, 21) in which increases of total vitamin A potency were observed, but are somewhat discordant with those of others (25) in which carotene supplements increased only carotene of colostric fat. The increases in carotenoids resulting from pasture grazing are in harmony with observations of Henry *et al.* (11), but elevation of the levels of vitamin A, as reported herein, were not indicated by the latter authors. Failure of Stewart and McCallum (27) to detect increases of carotene in colostrum from cows receiving prepartal supplements of carrots might have been due either to amounts ingested and/or to sampling methods employed.

b. Vitamin A supplements. Colostrum and early milk from cows receiving vitamin A supplements prepartally contained appreciably more vitamin A than did the secretions from cows fed only barn rations (tables 2 and 3). In more than half of the samples representing each of the first six milkings, average vitamin A concentrations in the secretions from cows receiving supplemental vitamin A were at least twice as high as were those from similar cows fed only barn rations. Unexpectedly, some of the higher vitamin A levels were found in colostrum from cows fed vitamin A supplement for the shorter periods of time. Also, cows receiving vitamin A produced colostrum of a higher vitamin A content than did cows receiving pasture, but, in general, differences were less marked than those found when vitamin A-supplemented and barn-ration cows were compared.

[6] Space does not permit presentation of these data as well as some other in later sections; data are available to anyone interested.

Increases in vitamin A levels of colostrum following supplementation of the ration with this vitamin also have been noted by others (7, 25, 30). Stewart and McCallum (27), however, did not find cod-liver oil supplements providing 70,000 I. U. vitamin A per cow daily effective in increasing the vitamin A levels of colostrum. This low level of supplementation, the sampling methods and the possible reduction of milk fat by cod-liver oil (2) might have affected their results.

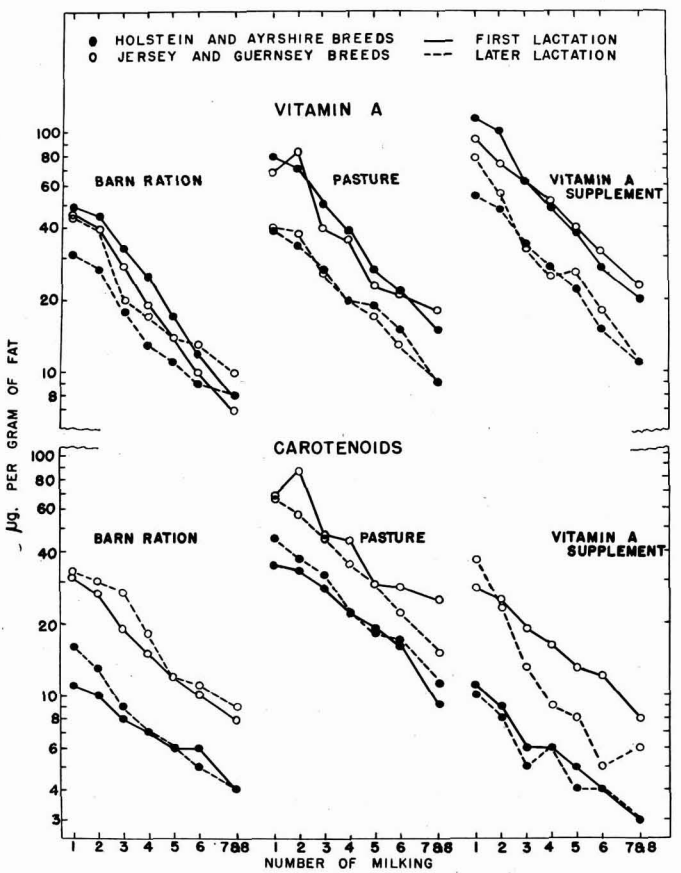

FIG. 1. Changes in concentration of vitamin A and of carotenoids in colostric fat during the transition period.

Several reports previously reviewed (32) indicated that a large intake of vitamin A decreases the cartenoid content of milk. When the diet of cows that had various degrees of mastitis was supplemented with 1,250,000 I. U. of vitamin A daily, both pre- and postpartally, suppression of carotenoids in colostrum and early milk seemed to occur, as also noted by Esh *et al.* (7). On the other hand,

when supplementation with 1,000,000 I. U. of vitamin A daily was discontinued at parturition, the effect on carotenoid contents of the secretions was variable (tables 2 and 3). In the present study, suppression of carotenoids was observed somewhat more frequently in the colostric fat than in the complete mammary secretion; explanation for this difference is obscure.

Effect of stage in the transition period. Vitamin A and carotenoid contents of the mammary secretions normally decreased rapidly during the first few milkings following parturition (tables 2, 3, and 4), as also observed by other investigators. Characteristic transitional changes of vitamin A and carotenoids in colostric fat (trial II) are presented on a semi-logarithmic scale in figure 1. In spite of irregularities observed, especially in secretions from some of the smaller groups of cows, decreases of both vitamin A and carotenoids tended to follow a logarithmic course. This trend is more pronounced when results are expressed as content per unit of fat instead of per unit of fluid secretion. Rates of change resemble those found for tocopherols (19), and a similar trend also has been reported for carotene (22). Observations over extended periods disclosed that the rates of change in concentrations of vitamin A and carotenoids generally had undergone a marked decrease by the eighth to the tenth milking postpartum. The rates of change of vitamin A and carotenoids of colostric fat apparently were not affected markedly by breed, number of lactation and type of prepartal ration.

However, some exceptions to the general trend were noted. These occurred especially in the group averages of samples from first-lactation, pasture-supplemented Jerseys and Guernseys of trial II (fig. 1) and in those from the individual Jersey cows of the barn and the pasture groups of trial I (table 2). These apparently anomalous tendencies might have been due to changes that were observed in the fat content of the secretions. It is not known to what extent results were affected by inability to evacuate the gland completely at each milking (29).

In transitional mammary secretions from three cows receiving rations fortified with vitamin A, 1,250,000 I. U. daily (32), both pre- and postpartally the concentrations of this vitamin remained high over a longer period than did the levels in corresponding secretions from comparable cows (trials I and II) receiving 1,000,000 I. U. of supplemental vitamin A daily only to the time of parturition. The differences were marked after the third milking. At the end of the transition, the values of vitamin A in the milk of the postpartally supplemented cows were eight times higher than in milk from cows that were not supplemented after parturition; the concentrations in the fat were three times higher.

SUMMARY

A study was made of the effects of individuality, breed, lactation number and prepartal diets on vitamin A and carotenoid contents of colostrum and transitional milk from 86 cows representing four dairy breeds.

Marked individual differences were found in the vitamin A potency of colostrum from cows of the same breed, lactation (first or later) and dietary group. A greater degree of variability was observed in early colostrum than in the milk

from the same cows at the end of the transition period. Occasionally, first-lactation cows secreted an atypical colostrum in which vitamin A and carotenoids were abnormally low.

The carotenoid content of colostrum and early milk from Jerseys and Guernseys was higher than in the corresponding secretions from Holsteins and Ayrshires, but differences with respect to Vitamin A were not marked.

Concentrations of vitamin A in the mammary secretions from first-lactation cows generally were higher than in those from cows in later lactations, but consistent differences in carotenoids were not observed.

Access to pasture during the terminal weeks of gestation produced higher levels of carotenoids in colostrum and transitional milk than did typical barn rations. Although levels of vitamin A generally were increased by pasture grazing, the increase was not so great as observed for carotenoids.

High intakes of vitamin A concentrates (500,000 and 1,000,000 I. U. daily, respectively, 4–2 and 2–0 weeks prepartal increased the vitamin A content of colostrum and transitional milk to levels higher than those in corresponding mammary products from cows fed either typical barn rations or pasture.

Supplementation of the ration with 1,250,000 I. U. of vitamin A daily both pre- and postpartally, tended to decrease the concentration of carotenoids in the mammary secretions; but supplementation during the prepartal period only (4–2 and 2–0 weeks, at levels of 500,000 and 1,000,000 I. U. daily, respectively) had no consistent effect on the carotenoids.

Concentrations of vitamin A and carotenoids in first colostrum generally were several times higher than in milk at the end of the transition period. Even when daily supplementation with high levels of vitamin A was continued postpartally, as well as during the prepartal period, the first colostrum averaged almost 3 times higher in vitamin A content than did milk from the same cows 14 days later.

The transition in vitamin A and in carotenoid contents of colostric fat was rapid during the first eight milkings, both constituents following a similar logarithmic trend. Neither number of lactation (first or later), breed, nor prepartal rations seemed to influence appreciably the rate of transition, but in all cases definitely smaller rates of change were evident by the eighth to the tenth milking. Since vitamin A and carotenoids of colostrum are concentrated primarily in the fat, the logarithmic trend usually was manifested more clearly when results were expressed as concentrations per unit of colostric fat instead of as concentrations per unit of total secretion.

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THE ISOLATION OF FURFURYL ALCOHOL FROM HEATED SKIMMILK

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The identification of some of the end products resulting from the heating of skimmilk should present a workable approach to the problems of browning and caramelized flavor development in milk and other dairy products. The constituents native to milk are well known and with some knowledge of the end products, formulation and comprehension of the underlying chemical mechanisms would be facilitated greatly. Very little work has been done in this field of dairy research. Most of the information pertaining to the subject concerns the formation of volatile acids (formic in particular) and lactic acid. Acid production in milk as a result of heating has been observed by many workers; the findings of Gould (5, 6) and Gould and Frantz (7) are most significant on this point. Hankinson *et al.* (8) have attempted to identify a dialyzable substance from raw milk which when heated gives rise to a heated milk flavor and odor. Even though their efforts to isolate this compound were unsuccessful, they did succeed in establishing some of the characteristics of the compound.

Of late, considerable interest has attached to the possible role of furan compounds in the browning of many stored foods. Although it might be expected that such compounds are generated in milk under the influence of heat, there seems to be no direct evidence in the literature supporting this theory.

In view of the scarcity of information on this subject and the obvious value which further findings would have, it seemed worthwhile to attempt isolation and characterization of compounds formed in milk by heat.

EXPERIMENTAL

Removal of ether-soluble substances from heated skimmilk. Skimmilk pasteurized at 62.2° C. for 30 minutes was placed in three 2-gallon milk cans and autoclaved, 5 gallons at a time, for 90 minutes at 126.6° C. The autoclaved milk was allowed to cool over night in a refrigerator at 4° C. The following day the milk was extracted with an equal volume of redistilled ethyl ether.

The extraction was done by hand using either a 2-liter or 4-liter aspirator bottle with outlets at the bottom and top for separating the milk and ether layers. One- or 2-quart quantities of milk were extracted at a time, and the extraction was accomplished by vigorous shaking of the ether-milk mixture for a period of 1 to two minutes. The ether-soluble substances were concentrated by distilling off the excess ether. This ether subsequently was reused for further extraction. All stoppers were lined with tinfoil to prevent the accumulation of impurities in the ether. Two separate large scale experiments were carried out involving the extraction of 22 gallons of heated milk in the first and 25 gallons in the second.

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Purification of the ether-soluble substances. When the ether extract was concentrated to a volume of approximately 200 ml., the balance of the ether was removed by vacum, using a water pump. Following removal of the ether, the residue immediately was taken up in 300 ml. of water. The water solution, which also contained insoluble matter, was extracted with two 50 ml. portions of petroleum ether (35–65° C. boiling fraction) to remove the true petroleum ether-soluble substances. The water fraction then was extracted with three 600 ml. volumes of redistilled ethyl ether. The ether solution was dried by shaking with anhydrous sodium sulfate and concentrated again in the manner outlined previously for the crude ether extract. In this way, it was possible to separate the true ether-soluble substances from those predominantly water-soluble or petroleum ether-soluble.

Distillation of the ether-soluble residue. The dry ether-soluble residue (approximately 10 ml. of material) was transferred to a 25 ml. distilling flask which then was fitted with a capillary and a 100° C. thermometer. This flask delivered into a 10 ml. distilling flask. The side arm of the receiving flask was connected to a vacuum system which operated at 1 to 2 mm. pressure in the first experiment and at less than 1 mm. in the second. A warm water bath was used as the heating medium and the receiving flask was submerged in an ice bath.

In the case of the first experiment, 1 ml. of material distilled between 22 and 57° C., but at 57° C. the temperature held quite steadily. A new receiving flask was inserted and an additional 2 to 3 ml. distilled between 57 and 59° C. At this point, the water bath temperature was 65° C. Raising the temperature of the bath to 100° C. accomplished no further distillation and merely darkened the residue in the distilling flask so the distillation was halted at this point. The fraction boiling between 57 and 59° C. was redistilled under vacuum, the first few drops being discarded. The same procedure was used in the second experiment and essentially the same results were obtained, except that the major portion of the residue distilled between 46 and 48° C., no doubt due to the higher vacuum. The procedure used in the isolation of this distillate from the autoclaved skimmilk is illustrated schematically in figure 1.

Characteristics of the compound constituting the vacuum distillate. Since the vacuum distillate showed some indication of purity by its constant boiling characteristic, it was thought advisable to attempt identification of the major compound present. The first experiment revealed the following properties of the distillate: Boiling point—740 mm., 165° C.; refractive index—(n_D^{25}), 1.485; density—(D_4^{25}), 1.1+; freezing point—, $< -30°$ C.; water soluble, ether soluble; containing no nitrogen, sulfur or halogen; average carbon 60.15 per cent; average hydrogen 6.07 per cent.

The carbon-hydrogen analysis of the distillate corresponded well to an empirical formula of $C_5H_6O_2$. It was observed further that the distillate gave typical reactions for certain furan compounds. It turned a pine splint moistened with concentrated hydrochloric acid blue-green (9). It reddened aniline-hydrochloric acid reagent (10). It also produced a black resin with concentrated sulphuric acid.

At this point it was suspected that the distillate might be mainly furfuryl alcohol and a number of tests were performed with the distillate and a control sample of furfuryl alcohol. The results indicated great similarity in respect to

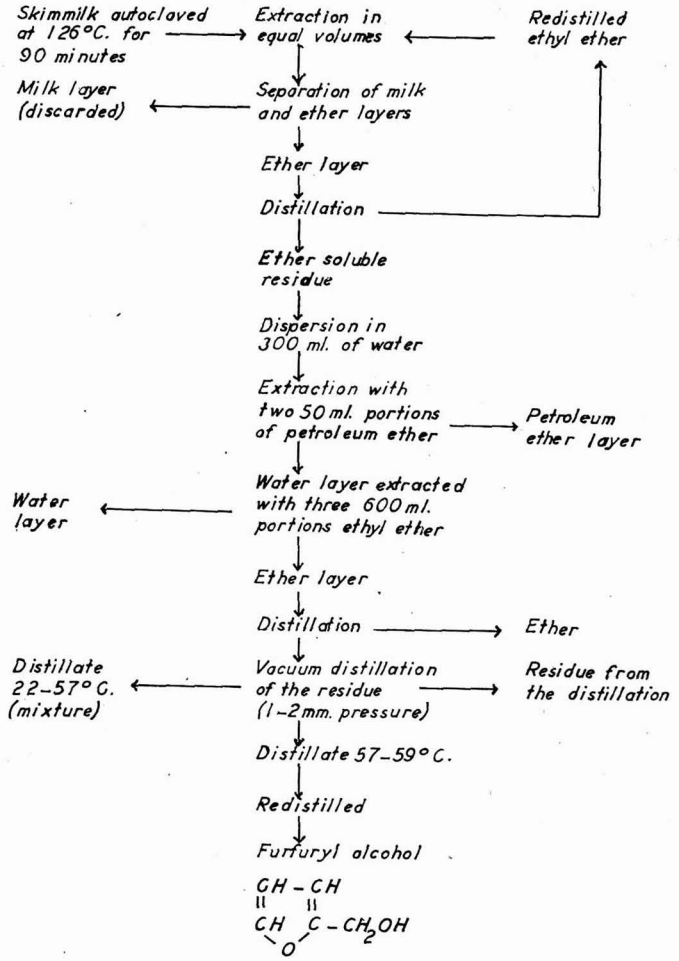

Fig. 1. Flow diagram for the isolation of furfuryl alcohol from heated skimmilk.

bromine uptake, reduction of hot ammoniacal silver nitrate and alkaline permanganate, inertness toward Fehling's reagent and toward 2,4-dinitrophenylhydrazine reagent.

Preparation of derivatives. In order to obtain more material for the prepa-

ration of suitable derivatives and to verify the findings of the first experiment, it was necessary to conduct a second experiment. Investigation of the distillate from the second trial revealed it to have the same physical and chemical properties as indicated for that of the first.

The naphthyl and phenyl urethanes and the 3,5-dinitrobenzoate derivatives of the distillate were prepared in accordance with the procedures outlined by McElvain (11) and Shriner and Fuson (14). Table 1 gives the melting points of the derivatives obtained together with those given by Huntress and Mulliken (9) for furfuryl alcohol. These data indicate quite conclusively that the distillate is mainly furfuryl alcohol.

Procedure and observation in connection with the control experiment. In order to establish the fact that in this investigation furfuryl alcohol was produced in skimmilk by heating and was not a constituent native to the milk or an accumulated impurity resulting from the method or reagents used, it seemed advisable to conduct a control experiment. Five gallons of fresh raw skimmilk were extracted with ether and the ether extract concentrated in the same manner as described for the heated skimmilk. Qualitative tests for furan compounds were performed on the ether extract residue. The pine splint and aniline-hydrochloric acid tests were negative. The residue did not darken sulfuric acid appreciably. The results of these tests are sufficient evidence that furfuryl alcohol is not a normal constituent of raw milk but is produced by heat treatment.

TABLE 1

Melting points of derivatives prepared from the vacuum distillate obtained by ether extraction of heated skimmilk as compared with those for the same known derivatives of furfuryl alcohol.

Derivatives	Observed melting point	Melting point of furfuryl alcohol derivative[a]
	(°C.)	(°C.)
Naphthyl urethane	129–130	129–130
Phenyl urethane	43–45	45
3,5-dinitrobenzoate	79–81	80–81

[a] Huntress and Mulliken (9).

DISCUSSION

It has been known for some time that furfural may be produced by heating pentosans in the presence of concentrated sulfuric acid. The general character of the reaction appears to be the removal of three molecules of water from the pentose molecule, although the reaction may be stepwise and somewhat more complicated (3). This method serves as the basis for the commercial production of furfural, which is a very useful intermediate in the synthesis of many other furan derivatives (1, 13). Furan compounds also have been synthesized in the laboratory by ring closure of certain diketones and other suitable compounds (3). Whereas pentoses yield furfural when heated in an acid medium, hexoses yield hydroxy-methyl furfural (15).

The chemistry of furan compounds has been reviewed extensively by Gilman and Wright (4) with respect to syntheses, reactions, aromatic character of the ring, ring substitution and scission. Further review of the subject is not within the scope of this investigation, but it should be pointed out that the mechanism by which furfural may be oxidized to the acid or reduced to the alcohol (3) may have some bearing on the formation of furfuryl alcohol in heated milk.

The isolation of furfuryl alcohol was somewhat incidental in the present investigation, since the major objective was the isolation of heat-generated flavor compounds in milk. However, its importance as an associated substance and an end product of the chemical reactions initiated in milk by heat should not be overlooked. At the very least, its presence is an indication that furan compounds are involved in the chemical changes induced in milk by heat, and the possibility exists that furfuryl alcohol is produced from furfural under the influence of the strong reducing conditions existing in heated milk. Sulfhydryl groups which are strong reducing substances and which have been observed to disappear during the processes of browning and caramelized flavor development in milk may be implicated in these changes. For example, furfuryl mercaptan in low concentrations has an odor of roasted coffee, and several observers have described the odor of the ether extract residue in this investigation as resembling that of coffee. Although such thinking is quite speculative, the possibility that furan compounds are involved in the caramelized flavor mechanism should not be overlooked.

It would be logical to assume that furfuryl alcohol is a heat degradation product of lactose, although proof is lacking on this point. Following this assumption a step further, it is evident that the chemistry must be somewhat devious, since hexoses give rise to six carbon furans. A decarboxylation mechanism might account for this inconsistency. Another potential source of furan compounds in milk is ascorbic acid. Cranston (2) recently has shown that ascorbic acid may yield furfural under certain conditions. Patton and Josephson (12) have observed that the addition of ascorbic acid to raw milk at the rate of 1 g. per liter will bring about the development of caramelized flavor at time-temperatures much lower than those noted under normal conditions (90° C.-flash). The role of ascorbic acid under these conditions is not clear, but its possible relationship to caramelized flavor and furan compounds is a point of interest.

SUMMARY AND CONCLUSIONS

A method for removing and purifying the ethyl ether-soluble substances of heated skimmilk is presented. Using this method in combination with a vacuum distillation technique, it was possible to isolate furfuryl alcohol in a fairly high state of purity from heated skimmilk. Confirmatory evidence of the presence of furfuryl alcohol is given by the results of qualitative tests and the preparation of suitable derivatives. The control experiment demonstrated that furfuryl alcohol is a compound generated in milk by heat and is not a normal constituent of unheated milk.

Although the significance of furfuryl alcohol as an end product of the heat

induced chemical reactions in skimmilk is not entirely evident at this time, the knowledge of its presence promises to be valuable in further clarifying the nature of browning and caramelized flavor development in milk and milk products.

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FUSED TRICALCIUM PHOSPHATE AS A LOW-FLUORINE PHOSPHORUS SUPPLEMENT FOR DAIRY CATTLE[1]

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Added impetus was given during the recent world war to the search for a phosphorus supplement for cattle which safely would replace the bone meal in the ration, due to the shortage of feeding-grade bone meal. The Tennessee Valley Authority developed a process for defluorinating rock phosphate which lowered the fluorine considerably and lowered the phosphorus only slightly (7). This process involves fusion of the rock phosphate in a shaft furnace and quenching in high-velocity water jets when the fluorine has been driven off. From an original material containing 3.5 to 4.0 per cent of fluorine, a fused tricalcium phosphate containing less than 0.4 per cent of fluorine can be produced.

REVIEW OF LITERATURE

Early attempts in the use of raw rock phosphate as a calcium and phosphorus supplement usually met with disaster, although the material seemed almost ideal because the proportions of calcium and phosphorus in the phosphate were in the same ratio as in the bones of animals. The toxic effect of this material was traced to its fluorine content.

The literature relating to fluorine in the ration of various animals has been reviewed by Mitchell (9). Work with dairy cattle has been reported by Reed and Huffman (12) and by Phillips *et al.* (11). Reed and Huffman showed that when raw rock phosphate was added to the ration at the rate of 1.5 per cent of the grain mixture, poor health, reduced appetite, reduced milk production and badly worn, cold-sensitive teeth resulted. The metatarsal bones and maxillae were exostotic and there was some evidence of ankylosis. However, reproduction did not seem to be affected. A complex mineral mixture produced similar results, probably due to the use of raw rock phosphate in the mixture.

Phillips *et al.* (11) supplied six groups of three animals each with varying amounts of minerals; three of the grain rations contained 0.022, 0.044 and 0.088 per cent fluorine from raw rock phosphate. The rate of growth to 2 years was

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reduced slightly on the high fluorine rations but weight differences increased with each lactation; milk production also was markedly reduced. In addition to causing exostoses of the bones and excessive abrasion of the teeth, high fluorine intake interfered with tissue respiration through its effect on enzyme systems. The critical level of fluorine tolerance was considered to be 2–3 mg. per kg. of body weight.

The value of fused tricalcium phosphate as a phosphorus supplement has been studied with rats (1, 3, 4, 14) and with chicks (2, 5, 8). In general, the phosphorus of the fused tricalcium phosphate has been found slightly less available than in tricalcium phosphate, secondary calcium phosphate or bone meal, when the amounts fed were at the borderline in regard to sufficiency of phosphorus. In some cases, with larger amounts of phosphorus available, differences were less marked. Gullickson and Olson (6) studied a defluorinated rock phosphate produced by calcination and acid treatment with less than 0.1 per cent of fluorine. Differences in weight gains of heifers were slightly in favor of the bone meal group, but the difference was not significant.

On reviewing published work, Mitchell (9) stated: "One could not be far wrong in assuming, on the basis of available data, that a level of 0.01 per cent of fluorine in the total dry rations of these animals is approximately borderline between safe and unsafe concentrations." He referred to pigs, sheep and cattle. This statement was the basis for the levels of fluorine used in this experiment, with one group at approximately one-fourth of this amount which was considered as a safe level, one group at this 0.01 per cent level as borderline, and one group at twice this level to produce readily-observed symptoms.

EXPERIMENTAL

Six Holstein bull calves were divided into three groups of two each, at random. They were castrated between the ages of 4 and 6 weeks and were placed on experiment March 24, 1944, at which time they ranged in age from 3 to 10 weeks. Whole milk was fed up to the age of 6 weeks, when it was replaced gradually by calf starter. At the age of 5 months, the starter was replaced gradually by a simple concentrate mixture. Hay was fed *ad libitum* from the time the calves would eat it. Silage was fed during the winter months after the calves were approximately 9 months of age. The steers were not pastured at any time.

The calf starter was a commercial mixture from which the steamed bone meal and ground limestone had been omitted. The simple concentrate mixture was made up of two parts ground yellow corn, two parts ground oats, two parts wheat bran and one part linseed meal. This mixture was modified slightly from time to time, as the availability of certain ingredients was limited. To both the basic calf starter and the simple concentrate mixture, the experimental phosphates were added as follows:

Group 1 (steers 49 and 50) 2 per cent of fused tricalcium phosphate containing 0.24 per cent of fluorine.

Group 2 (steers 51 and 52) 1 per cent of mixed phosphate containing 2.0 per cent of fluorine.

Group 3 (steers 53 and 47) 2 per cent of mixed phosphate containing 2.0 per cent of fluorine.

The mixed phosphate was prepared by mixing raw rock phosphate containing 3.51 per cent of fluorine and fused tricalcium phosphate containing 0.02 per cent of fluorine. Thus, the three grain mixtures contained 0.0048, 0.02 and 0.04 per cent of fluorine, respectively. The amount of grain fed was increased periodically to meet the increased maintenance and growth requirements. At the age of 3.5 years the steers were receiving 8 lb. of grain daily. Periodic examinations of the incisor teeth, the legs and the ribs of the steers were made and the results recorded. Weight and height-at-withers measurements were taken monthly at the beginning of the experiment, with the frequency decreasing slightly toward the end of the period.

The steers were slaughtered in October, 1947, when they were between 42 and 44 months of age. The liver, heart, spleen, kidneys and suprarenal glands were examined macroscopically at the time of slaughter. The left foreleg and the upper and lower jaws were cleaned of all flesh and outer connective tissue, the metacarpal being cut longitudinally, and the parts photographed. Before cutting, the smallest circumference of the diaphysis and the circumference in the region of the epiphyseal-diaphyseal junction were determined. A sample of bone was taken from a cross section of the left radius, and the lower left first molar and the lower left third molar were prepared for analysis. The bone samples were analyzed for fluorine, calcium and phosphorus, and the tooth samples for fluorine only. Blood plasma calcium and inorganic phosphorus were determined on samples of blood collected at the end of the experiment.

The growth data were analyzed by analysis of covariance, using simple linear regression for the weight-age relationship. In the case of height at withers, a curvilinear relationship was apparent, and height was plotted against the logarithm of age. The compositions of the bone and tooth samples were treated by analysis of variance with individual comparisons between groups made by separating the individual degrees of freedom for treatment.

RESULTS

Growth. The regression coefficients for weight on age for each individual steer, the average regression coefficient for each group of two, and the average coefficient for all steers are presented in table 1, together with the regression of height on the logarithm of age. The correlation coefficients for both height and weight, also presented in table 1, indicate quite clearly the close fit of these regression curves to the actual data. The analysis of covariance of height-at-withers indicated highly significant differences between the regression coefficients of the individual steers but no significant difference among the three group regressions. In regard to weight, neither individual nor group regressions were significantly different.

TABLE 1

Correlation and regression coefficients for weight-age and height-log age relationships for individual steers and for groups

	Weight-age		Height-log age	
	Regression	Correlation	Regression	Correlation
Steer 49	26.79	0.993	19.54	0.992
Steer 50	27.63	0.995	23.11	0.995
Av.—group 1	27.21	0.994	21.33	0.989
Steer 51	28.56	0.993	22.04	0.991
Steer 52	25.25	0.988	20.30	0.995
Av.—group 2	26.90	0.989	21.17	0.991
Steer 53	24.65	0.997	20.89	0.991
Steer 47	27.73	0.986	22.88	0.996
Av.—group 3	26.19	0.981	21.88	0.991
Av.—all animals	26.77	0.988	21.46	0.992

In both the height and weight data, the last three measurements were estimated for steer 50 by the missing plot technique (13), and the degrees of freedom for this steer and for error thus were reduced by 3.

Composition of bones and teeth. The calcium, phosphorus and fluorine content of the samples of bone taken from the left radius, and the fluorine content of the lower left first molar and the lower left third molar are presented in table 2. Statistical analysis indicated highly significant differences among the three groups in the calcium content of the bone samples. The group receiving only 1 per cent of the phosphate in the grain mixture had the highest calcium content in the bone, and this difference is chiefly responsible for the statistically significant difference. The differences in phosphorus content of these bone samples among the groups were not significant.

The fluorine content of the bone samples showed a considerable accumulation of fluorine in the bones, as represented by this one sample, when large amounts of fluorine were fed in the ration. The difference between groups 1 and 2 was statistically significant; the difference between groups 1 and 3 was highly significant; and the difference between groups 2 and 3 was not significant.

TABLE 2

Average composition of bone sample from left radius and of the lower left first and third molar teeth

	Bone—left radius			First molar fluorine	Third molar fluorine
	Calcium	Phosphorus	Fluorine		
	(%)	(%)	(%)	(%)	(%)
Steer 49	27.0	12.0	0.15	0.17	0.15
Steer 50	26.7	12.8	0.14	0.20	0.19
Av.—group 1	26.8	12.4	0.14	0.18	0.17
Steer 51	27.5	12.3	0.36	0.39	0.38
Steer 52	27.5	12.6	0.42	0.35	0.34
Av.—group 2	27.5	12.4	0.39	0.37	0.36
Steer 53	26.7	12.1	0.38	0.27	0.25
Steer 47	26.5	12.0	0.61	0.37	0.40
Av.—group 3	26.6	12.0	0.50	0.32	0.32

There was a rather high correlation between the fluorine content of the first and third molars of the same animal. The difference between groups 1 and 2 was significant for both the first and third molar. The difference between groups 1 and 3 was significant for the first molar but not significant for the third molar. The difference between groups 2 and 3 was not significant for either the first or the third molar. The mean values for both first and third molars were slightly lower for group 3 (receiving the most fluorine in the ration) than for group 2 (receiving only half as much).

Examination of bones and teeth. Only one of the bones showed any abnormal characteristics; this was the metacarpal from steer 47 of group 3. The measurements of all of the bones indicated no significant differences, but this one bone (no. 47) was somewhat larger in circumference, both at the smallest

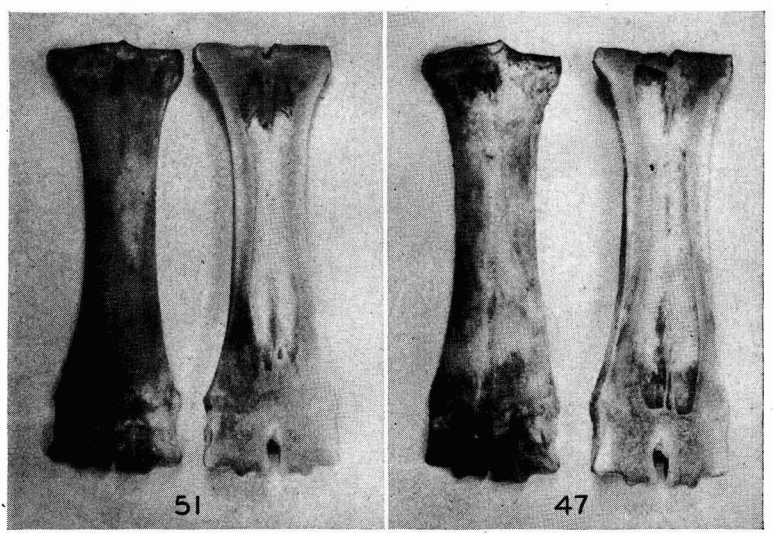

FIG. 1. Enlargement of metacarpal bone due to high fluorine intake of steer 47 compared with the apparently normal bone of steer 51 which was on the borderline level of fluorine intake.

diameter of the diaphysis and the circumference in the region of the epiphyseal-diaphyseal junction. In cleaning this bone, when the periosteum was being removed, a layer of rather porous bone came off with the periosteum in one place. Below this porous layer the bone seemed to be similar in hardness to the bones of the other steers. Careful macroscopic examination showed no differences other than this which could be attributed to the fluorine in the rations. Photographs of the metacarpal bones of steers 51 and 47 are shown in figure 1, to illustrate the difference between an apparently unaffected structure and the enlarged, more porous bone. In the periodic examinations of the steers, no abnormalities of any of the leg bones or ribs, such as exostoses, were observed which could be attributed to the fluorine-containing rations.

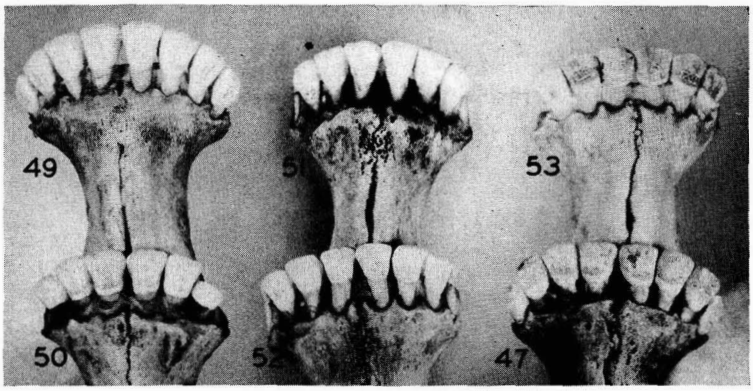

FIG. 2. Comparison of roughness, discoloration and softening of incisor teeth on a low fluorine intake (49 and 50), borderline fluorine intake (51 and 52) and high fluorine intake (53 and 47).

The periodic examinations of the incisor teeth indicated that the deciduous teeth of both steers in group 1 showed no abnormalities in the way of roughness or discoloration. The permanent incisors, however, did show some roughness and streaks, especially near the gum line. This is shown in figure 2. Group 2 showed some increased wear and irregularities of the cutting edges of the de-

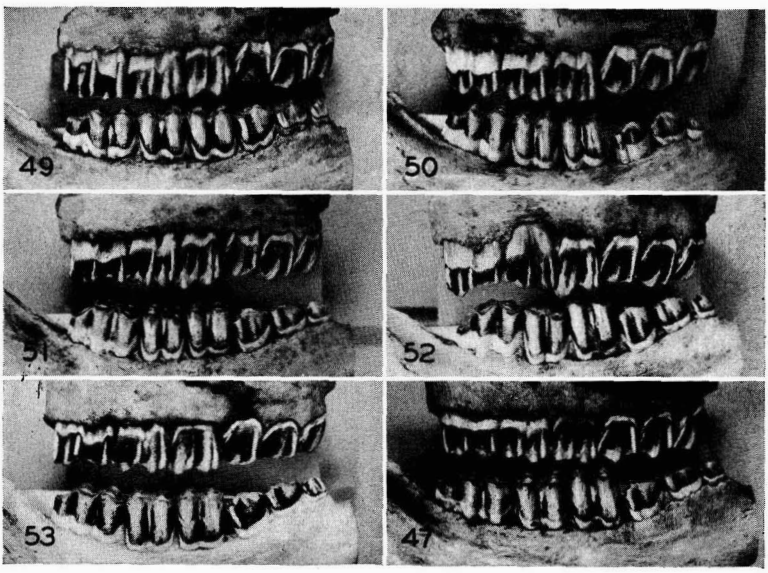

FIG. 3. Comparison of the abrasion of molar and premolar teeth on a low fluorine intake (49 and 50), borderline fluorine intake (51 and 52) and high fluorine intake (53 and 47).

ciduous incisors, and some mottling near the gum. Steer 52 showed somewhat greater mottling than steer 51 and showed wear on the anterior surface of two of the teeth. In group 3 there was definite pitting of the deciduous teeth of both steers and definite roughness and mottling of the permanent teeth. The wear on the anterior surface of the teeth of steer 47 was more pronounced than in steer 52 and was principally on the first pair of teeth.

Photographs of the molar and premolar teeth of the six steers are shown in figure 3. There seemed to be no abnormal wear in either steer of group 1. The abrasion of the teeth of groups 2 and 3 did not differ greatly. The most pronounced wear was found in steer 52 of group 2. Also in this steer the second upper molar on both sides had an abscess at the base, with erosion of the bone surrounding the root. The whole tooth also was discolored, being darker in color than the other teeth.

Examination of internal organs. Steer no. 50 died from ingested wire before the expiration of the experiment, but an autopsy failed to show any noticeable effects of fluorine. Examination of the liver, spleen, kidneys and suprarenal glands of the other five steers failed to show any abnormalities except that the organs were smaller than normally would be found in steers of this size and age. This could be due to the limited amount of exercise allowed. Cysts approximately 0.4 in. in diameter were found in the right atrio-ventricular valves of steers 51 of group 2 and 47 of group 3.

Blood serum. Analysis of the blood serum for calcium and inorganic phosphorus gave values within the normal ranges.

DISCUSSION

Fluorine at the levels studied in this experiment did not affect significantly the rate of growth of steers. At the same age, the heifers in the experiment of Phillips *et al.* (11) showed a definite decrease in weight as compared with the control groups, but these animals had been under the added strain of gestation and lactation. A longer period of development for the steers might have disclosed similar differences.

The statistically significant difference between groups in the calcium content of the bones is difficult to interpret satisfactorily, especially since the group with the highest bone calcium was the one receiving the least calcium supplement in the ration. However, the difference seems to have little practical significance in this experiment. The fluorine content of the bone samples was not strictly proportional to the fluorine intake, but did increase with the increased intake. The fluorine content of the molars did not show a similar proportional relationship, though groups 2 and 3 were higher than group 1.

The incisor teeth of steers 49 and 50 in group 1 did not seem to show any abnormal wear, even though there was a slight roughening and discoloration on the anterior surface. Since these animals had been receiving fluorine almost from birth and through the formative stages of these teeth, which is usually considered the most critical time for such abnormalities to develop, with only

these slight indications of fluorosis, this level of fluorine intake might be considered as a safe level.

Mitchell (10) has emphasized the fact that with the possible exception of very young animals, the phosphorus requirements nearly always are met when a ration contains enough suitable protein supplement to meet the protein requirements of the animal. However, to be on the safe side, many recommendations include some phosphorus supplement in the ration. Such recommendations are seldom for more than 1 per cent of a supplement containing phosphorus at levels comparable to bone meal. In this experiment the low-fluorine ration contained 2 per cent of the fused tricalcium phosphate containing 0.24 per cent fluorine. Apparently, this fused phosphate can be considered as a safe phosphorus supplement, if fed as only 1 per cent of the grain ration.

Since the teeth, both incisors and molars, of the group receiving 0.02 per cent of fluorine in the grain mixture showed definite signs of fluorosis, and the wear of the molars was as severe as for the higher level of fluorine intake, this level should be considered unsafe for dairy animals, especially since milking cows generally would receive larger amounts of grain and consequently a larger total intake of fluorine.

SUMMARY

Six dairy steers received three levels of fluorine in their grain rations (0.0048, 0.02 and 0.04 per cent) from shortly after birth to 3.5 years of age from fused tricalcium phosphate and raw rock phosphate.

The rate of growth of the three groups, as measured by weight and height-at-withers, did not differ significantly.

The fluorine content of the radial bone and the first and third lower molar teeth indicated a definite increase in the deposition of fluorine in these tissues on the fluorine rations, but the increased deposition was not proportional to intake.

Roughness, discoloration and abrasion of the teeth were increased markedly in the two groups receiving the higher levels of fluorine.

It was concluded that fused tricalcium phosphate with 0.24 per cent fluorine is a safe phosphorus supplement if used with discretion.

A grain mixture containing 0.02 per cent or more of fluorine was found to be unsafe for feeding to dairy cattle.

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THE NATURE OF REPRODUCTIVE FAILURES IN COWS OF LOW FERTILITY[1]

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A realization of the importance of the problem of infertility in dairy cattle has been accentuated by the rapid growth of artificial breeding. The necessary emphasis upon breeding records has made dairymen more aware of the cows which are bred repeatedly and fail to conceive. That approximately 50 per cent of all artificial inseminations are infertile signifies the magnitude of this economic problem.

This study was designed to determine the incidence of fertilization and of early embryonic mortality in "repeat-breeding" cows showing no detectable genital abnormalities. While an attempt was made to investigate some factors associated with infertility in these cows, the major emphasis was given to a consideration of the nature of the interruptions of the reproductive processes.

METHODS AND PROCEDURES

The study was made during the period from November 15, 1947, to April 9, 1948, at the Badger Breeders Cooperative research farm located near Shawano, Wisconsin. A total of 104 repeat-breeding cows, 55 Holsteins and 49 Guernseys, was assembled from 14 of the 23 northeastern Wisconsin counties served by Badger Breeders Cooperative.

The cows were selected by the staff veterinarians of the Cooperative on the following basis: (a) a minimum of four infertile services which would exclude approximately 95 per cent of the cows bred (1), (b) a minimum of one calving which would exclude congenital abnormalities preventing conception, (c) a maximum age limit of 10 years which would exclude infertility resulting from senility, (d) a maximum limit of two cows from any one herd which would minimize the effect of any particular herd management and environment, (e) rejection of cows with gross genital abnormalities detectable by rectal palpation which would tend to exclude cows in which fertilization would be mechanically impossible, (f) exclusion of cows displaying purulent discharges which would screen out obvious conditions for which treatment is indicated, (g) normal lengths of estrual cycles which would eliminate apparent endocrine dysfunction, and (h) normal intervals between breedings which would tend to ex-

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clude infectious diseases such as trichomoniasis. In essence, only those repeat-breeding cows were used which appeared "normal" in all respects at barn examination.

After the cows arrived at the research farm they were checked twice daily for estrual behavior; standing for mounting by other cows was the criterion used to determine estrus. Due to the shortness of the days, heat checks were made at approximately 8:00 a.m. and 4:00 p.m., or at intervals of 8 and 16 hours, respectively. With the observation of the onset of estrus at a particular check, each animal was inseminated at the time of the next check for heat. To standardize the insemination technique one inseminator made the major portion of the inseminations. The semen was diluted with synthetic pabulum (2) at the ratio of 1:30, and the insemination volume kept constant at 1.0 ml. diluted semen. The deposition of semen was confined to the second and third rings of the cervix. Other than meeting the requirements of using "day-of-collection" semen, a random sample of the semen available for field use on that day was used.

The cows then were scheduled to be slaughtered alternately on the third or thirty-fourth day after the experimental breeding, except for deviations necessitated by slaughter facilities. By the third day after breeding fertilization or cleavage of the egg is detectable, yet the ova are in the oviducts which facilitates their recovery. Further, fertilized ova recovered at a later stage could not be distinguished from fragmenting unfertilized ova with much surety. By the 34th day the embryo is of sufficient size to permit gross measurement and macroscopic observation for normality. For the cows which were permitted to go for 34 days before slaughter, routine palpations were made on the 21st, 24th, 27th, 30th, and 33rd day after breeding. The object of this series of palpations was to detect any quiet ovulations which might occur about the time of an expected estrual period.

The cows which, though scheduled for slaughter on the 34th day, returned to estrus were rebred at the time of the next heat check and slaughtered on the third day following. From cows slaughtered on the third day after either the first or second experimental breeding, the reproductive tract was removed, the oviducts severed from the mesosalpinx and flushed with physiological saline solution to recover the ova. When ova were recovered, fertilization was determined by the presence of two or more blastomeres of equal size, as microscopically observed at 440 magnifications. In the cows slaughtered on the 34th day after experimental breeding, the uterine horns were dissected to determine the status of the embryo, if present.

RESULTS

One group of cows was intended to contribute information on the percentage of breedings resulting in normal embryos on the 34th day. A total of 53 animals originally was allotted to this group (table 1), but in the end, one was eliminated and, as a result, the embryo studies included a maximum of 52 cows. The one cow eliminated was found at slaughter to have bilateral tubal obstructions which in itself would have prevented fertilization and embryo formation.

Of the 53 cows, only 26 actually were slaughtered on the 34th day, since the remainder returned to estrus between the 11th and 32nd day after the first experimental breeding. Normal embryos were present in only 12 of the 26 cows on the 34th day; the crown-rump measurements on these embryos ranged from 12–17 mm.[3] Abnormal embryos were present in eight other cows, four with embryos normal in size but showing a more or less generalized hemorrhagic condition and the other four with embryos small in size (7–11 mm.) and obviously disintegrating. Six cows slaughtered at 34 days had no embryos; two of these had pyometra. Of the other four, one had a quiet ovulation between the time of breeding and time of slaughter. No such occurrence was detected in the other cows by palpations of the ovaries on the 21st, 24th, 27th, 30th, and 33rd days after experimental breedings.

A group of 27 cows returned in estrus 11 to 32 days after breeding (table 1),

TABLE 1

Cows intended to furnish information on normality of embryo on the 34th day

Pregnancy status of cow	Guernsey		Holstein	
	No.	%	No.	%
Normal embryo	5	20.0	7	25.0
Hemorrhagic embryo	2	8.0	2	7.1
Disintegrating embryo	2	8.0	2	7.1
No embryo	2	8.0	2	7.1
Pyometra	1	4.0	1	3.6
Estrual recurrence (before 34th day)	13	52.0	14[a]	50.0
Total	25	100.0	28	99.9

[a] Includes one cow which was eliminated from the study of embryo normality at the 34th day, because when slaughtered 3 days after the second experimental breeding she was found to have bilateral tubal obstructions.

indicating that the breedings on them resulted in no embryos by 34 days. These animals then were experimentally rebred with the intention of their yielding information on the rate of fertilization when later slaughtered on the third day.

A second group of 51 cows (table 2) was slaughtered on the third day after the first experimental breeding to yield information on the rate of fertilization. Obvious causes for the failure of fertilization formed the basis for eliminating five of these animals: one failed to ovulate, one had bilateral tubal obstructions, and three had unilateral tubal obstructions on the ovulating side. Six other cows were eliminated because of a lack of information on fertilization. In three of these animals no ova were found, although the animals were free of genital abnormalities, and in the other three only empty zona pellucidas, two of which contained embedded spermatozoa, were found. Elimination of these latter six animals, in effect, charges the lack of fertility information on them to imperfec-

[3] Acknowledgement is gratefully made to Dr. S. H. McNutt, of the Department of Veterinary Science, for his assistance in determining the normality of embryos.

tions of the recovery technique rather than to some factor which also affects fertilization rate. There remained, then, 40 animals slaughtered on the third day after the first experimental breeding that actually furnished fertilization information.

Another group of cows yielding fertilization information was mentioned above in connection with the animals intended to furnish information on the percentage of embryos at 34 days. These animals were the 27 cows which returned to estrus before the allotted 34 days and were experimentally bred for the second time (table 2). Actually only 19 of these animals furnished information on fertilization rate. Of the remaining eight animals, no ova were recovered from four cows free of genital abnormalities on the ovulating side.

TABLE 2

Cows intended to furnish information on fertilization rate

Fertilization data	Slaughtered 3 days after: 1st exptl. breeding				2nd exptl. breeding			
	Guernsey		Holstein		Guernsey		Holstein	
	No.	%	No.	%	No.	%	No.	%
Contributing information:								
Fertilized or unfertilized ova								
No genital abnormality	20	83.3	19	70.4	11	84.6	8	57.2
Unilateral tubal abnormality (non-ovulating side)	0	0.0	1	3.7	0	0.0	0	0.0
Contributing no information:								
Empty zona pellucida	1	4.2	2	7.4	0	0.0	0	0.0
No ova recovered:								
No genital abnormality	2	8.3	1	3.7	2	15.4	1	7.1
Unilateral tubal abnormality (non-ovulating side)	0	0.0	0	0.0	0	0.0	1	7.1
Unilateral tubal abnormality (ovulating side)	1	4.2	2	7.4	0	0.0	1	7.1
Bilateral tubal abnormality	0	0.0	1	3.7	0	0.0	1	7.1
Ovulation failure	0	0.0	1	3.7	0	0.0	2	14.3
Totals	24	100.0	27	100.0	13	100.0	14	99.9

Ovum recovery was not possible in the other four cows due to a unilateral tubal obstruction on the ovulating side in one, bilateral tubal obstructions in another, and ovulation failure in two.

Altogether then, the pregnancy and fertilization studies utilize data on a maximum of 52 animals on the 34th day and 59 animals on the third day after experimental breeding. Of the 59 animals, the first experimental breeding yielded data on 40 cows. The other 19 had previously contributed information on the normality of embryos at 34 days and now contributed information on the rate of fertilization on the third day after the second experimental breeding.

In subsequent analyses summary totals which fall short of these two maximum totals, 52 and 59, or a grand total of 111, for the 3-day and 34-day post-breeding groups, respectively, are due to a lack of collateral information for the particular criterion under consideration.

TABLE 3
Genital Abnormalities

Genital Abnormalities	Guernsey		Holstein	
	No.	%	No.	%
Absent	48	98.0	45	81.8
Present				
Ovulation failure			3	5.5
Tubal obstructions				
Unilateral	1	2.0	5	9.1
Bilateral			2	3.6
Totals	49	100.0	55	100.0

Genital abnormalities. An attempt was made to exclude cows having gross abnormalities from the sample of animals selected for this study. Tubal obstructions not readily palpable appeared, however, in eight of the 104 cows slaughtered (table 3). Six of these were unilateral, and two were bilateral, and each of sufficient severity to act as a definite barrier to the transport of the ovum or sperm through the oviduct. These cases fell in the general categories of salpingitis, hydrosalpinx and bursitis. In addition to these presumed permanent tubal abnormalities, there were three cases of ovulation failure which may be considered as temporary barriers to fertilization.

Altogether, out of a total of 104 cows, 10.6 per cent were found with genital abnormalities. Their distribution between breeds was strikingly unequal. Genital abnormalities occurred in 18.2 per cent of the Holsteins, whereas such occurrences were found in only 2.0 per cent of the Guernseys.

Breeds. Slaughter data on 31 Guernseys and 28 Holsteins (table 4) have shown, first, that there are no statistically significant differences between the fertilization rates of ova recovered on the third day after the first and the second experimental breedings (60.0 vs. 63.6 for the Guernsey and 70.0 vs. 75.0 for the Holstein). On this basis, all cows slaughtered on the third day after experimental breeding, whether first or second, have been pooled.

With the division by breeds, the combined rates of fertilization for first and second experimental breedings were 61.3 for the Guernsey and 71.4 for the Holstein. The percentages of normal embryos at 34 days were 20.0 and 25.9

TABLE 4
Breeds

Breed	3 days after:				34 days after:	
	1st exptl. breeding		2nd exptl. breeding		1st exptl. breeding	
	Total no. cows	% with fertilized ova	Total no. cows	% with fertilized ova	Total no. cows	% with normal embryos
Guernsey	20	60.0	11	63.6	25	20.0
Holstein	20	70.0	8	75.0	27	25.9
Totals	40	65.0	19	68.4	52	23.1

for the respective breeds. Statistical significance of the differences between breeds could not be established on the present numbers.

In view of the insignificant differences between the breeds, subsequent analyses will be made by pooling all cows of both breeds furnishing information at the same stage.

Bang's disease. For the 110 cows tested for Bang's disease the following percentages were observed: 71.8 negative, 20.9 positive, and 7.3 suspect. A higher incidence of Bang's disease (positive and suspect reactors) occurred in the Holstein breed (37.0) than in the Guernsey breed (19.6). No explanation for this difference is apparent. All cases of anatomical genital barriers were found in cows which were negative to Bang's disease.

Agglutination test information for Bang's disease was available on 58 cows of the 3-day group and on 52 cows of the 34-day group. No difference in the fertilization rates was found between the 41 cows of the negative group (65.9) and the 17 cows of the positive-suspect group (64.7). Among the cows slaughtered on the 34th day after the experimental breeding the percentage of the 38 cows in the negative group having normal embryos was 26.3, and of the 14 in the positive-suspect group, 14.3. This difference is not statistically significant.

Number of previous services. While the minimum number of infertile services specified for the experimental animals was four, the average number per cow prior to the experimental breeding was 6.4. The Guernseys averaged 6.8 with a range of 4 to 11, while the Holsteins had an average of 6.0 with a range of 4 to 13.

The 59 cows of the 3-day group and the 52 cows of the 34-day group were pooled and arrayed on the basis of the number of services which each had received previously. Division of these 111 cows into low and high groups, as nearly equal in size as possible without splitting the median class, fell between six and seven previous services. No significant difference in the fertilization rates was found between the 34 cows with four to six previous services and the 25 cows with seven to thirteen previous services (67.6 vs. 68.0 on the third day). The percentage of normal embryos on the 34th day in the 34 cows of the low group was 23.5 and in the 18 cows of the high group, 22.2.

Number of previous calvings. The 107 cows for which reproductive histories were available averaged 2.8 previous calvings with an insignificant difference between breeds, being 2.9 for the Guernsey and 2.7 for the Holstein. These cows were arrayed according to their respective number of calvings and divided into a low group (1 and 2 calvings) and a high group (3 to 6 calvings). The 28 cows with a low number of calvings and slaughtered on the third day showed a higher fertilization percentage than did the 28 cows with a high number of calvings (75.0 vs. 57.1), but this difference is not statistically significant ($P = 0.2–0.1$). Similarly, in the group of cows slaughtered on the 34th day, a higher percentage having normal embryos was found in the 25 cows with a low number of calvings (32.0) than in the 26 cows with a high number of calvings (15.4). This difference of 16.6, again, is not statistically significant ($P = 0.3–0.2$).

Herd size. The average size of herd from which the experimental animals

originated was 20.6 cows. The Guernsey herds ranged from 3 to 63 cows with an average of 20.5, as compared with the Holstein herds which ranged from 7 to 70 cows with an average of 20.8. Again all the cows were pooled and arrayed according to the size of herd from which they came. There were 56 cows from small herds (3–19 cows) and 55 cows from large herds (20–70 cows). The fertilization percentage in the 33 cows from small herds was 60.6 and in the 26 cows from the large herds, 73.1. This difference of 12.5 per cent is not statistically significant (P = 0.5–0.3). In the 23 and the 29 cows slaughtered on the 34th day from small and large herds, respectively, the percentages with normal embryos were 26.1 and 20.7, with a difference of 5.4 which also is insignificant.

Herd breeding index. To obtain a more accurate differentiation between "problem cows" from good breeding herds and representative cows from "problem herds," herd indexes were calculated for the calendar year 1947. These indexes were simply the percentages of cows receiving "first services" for which no repeat breedings were required during a minimum period of 3 months. The average herd index was 43.3 per cent. Based on their respective herd breeding indexes, all cows were arrayed and divided into a low- and a high-index group. The low-index group consisted of 56 cows from herds with breeding efficiencies ranging from 0.0 to 45.5, while the 55 cows comprising the high-index group came from herds with breeding efficiencies ranging from 46.2 to 75.0. Twenty-nine cows from low index herds had a fertilization rate of 44.8 and thirty cows from high index herds, 86.7. This difference of 41.9 is highly significant statistically (P = 0.01). On the other hand, there is no significant difference between the percentages of cows having normal embryos at 34 days from the low index herds (22.2 on 27 cows) and from the high index herds (24.0 on 25 cows).

DISCUSSION

Cows with genital abnormalities detectable by palpation intentionally were excluded from this study so that chief emphasis could be given to a determination of the relative importance of failure of fertilization and early embryonic death. The finding of 7.7 per cent of the animals with tubal abnormalities was not expected. Inasmuch as these abnormalities probably are permanent and not amenable to treatment, the problem of their diagnosis is particularly important so that such cows can be removed from the herd. Rowson (3) has outlined procedures for detecting these conditions, which, if applied skillfully, should eliminate many such animals. The frequency observed in this study undoubtedly represents only the less readily detectable abnormalities. It will be assumed, however, that this frequency can serve as an index of the relative frequencies of all grades of the abnormalities in groups of animals under comparison.

The higher incidence of genital abnormalities in the Holstein breed (18.2 per cent) than in the Guernsey breed (2.0 per cent) is surprising in view of the generally-recognized lower breeding efficiency of the Guernsey than of the Holstein. The fact that no tubal abnormalities were found in cows reacting to

the agglutination test for Bang's disease also is at variance with the idea that brucellosis increases the incidence of salpingitis.

The comparison of different groups can be seen better if estimates of embryonic degeneration and mortality are available alongside the data on fertilization rate. These estimates (table 5) are derived from the differences observed in the original data between the fertilization rate and the percentage of cows with normal embryos at 34 days. This difference, for example, with cows negative to the test for Bang's disease (see above) was 65.9 minus 26.3 or 39.6. From this figure then an estimate of the percentage of embryonic death and abnormalities at the 34th day, 60.1 $\left(\frac{39.6}{65.9}\times 100\right)$, is calculated (table 5).

In practically all artificial breeding associations the Guernsey breed consistently has maintained a lower breeding efficiency than the Holstein breed.

TABLE 5
Group Comparisons

Comparison	Fertilization rate (%)	% embryonic death and abnormality at 34 days
Breed (Guernsey vs. Holstein)	61.3 vs. 71.4	67.4 vs. 63.7
Bang's Disease (Negative vs. Positive-Suspect)	65.9 vs. 64.7	60.1 vs. 77.9
Number previous services (4–6 vs. 7–13)	67.6 vs. 68.0	65.2 vs. 67.4
Number previous calvings (1–2 vs. 3–6)	75.0 vs. 57.1	57.3 vs. 73.0
Herd size (3–19 vs. 20–70)	60.6 vs. 73.1	56.9 vs. 71.7
Herd breeding index (0.0–45.5% vs. 46.2–75.0%)	44.8 vs. 86.7	50.4 vs. 72.3

The differences observed in this study between the breeds fail to explain this field condition. The higher rate of fertilization and the lower rate of embryonic mortality in the Holstein (table 5) are offset by the higher percentage of genital abnormalities noted above.

The view commonly is held that infection of a herd by Bang's disease causes a reduction in the conception rate. In this study, little difference was found between the fertilization rates of the negative group (65.9) and the positive-suspect group (64.7). However, a suggestion of a higher rate of embryonic mortality in the positive-suspect group (77.9) than in the cows of the negative group (60.1) was observed.

Barrett *et al.* (1) found a progressively declining rate of pregnancy (as diagnosed by manual palpation at 34 to 50 days after breeding) with each additional service. Such a trend was not noted in this group of experimental cows. In fact, no appreciable differences (table 5) between the fertilization rates or between the rates of embryonic mortality were found between the cows with a low number of previous services (4–6) and those with a high number of services (7–13). The exclusion of cows with genital abnormalities from the present

population but not from that studied by Barrett and associates may account for the difference in the performance of the two populations.

The most obvious effects upon both fertilization rate and embryonic mortality appear to be produced by the three factors: age of cow (number of previous calvings), herd size and herd breeding index. The younger animals had a higher fertilization rate and at the same time a lower embryonic death rate than the older animals (table 5). Cows from the larger herds and from herds with higher breeding indexes had the higher fertilization rates. Concurrently, however, the same cows had the higher embryonic death rates, which by the 34th day left them with no more normal embryos than cows from small herds and from herds of low breeding indexes.

The small number of animals used in this study makes it difficult to study the interactions of the various factors upon fertilization rate and embryonic death rate. The most interesting finding resulting from attempts at further study has been the correlation between age of animal and size of herd from

TABLE 6

The nature of reproductive failures in cows of low fertility

			%
Failure of fertilization			39.7
Physical barriers absent		31.0	
Physical barriers present		8.7	
Bilateral	1.9		
Unilateral (ovulating side)	3.9		
Ovulation failure	2.9		
Embryonic abnormalities or mortality before 34 days			39.2
Embryos still normal at 34 days			21.1
Total			100.0

which it came. The larger herds furnished 64.2 per cent of the younger animals and the smaller herds furnished 58.9 per cent of the older animals. Other factors showed less association among themselves. There is then some confounding of youngness of animal and largeness of herd. Additional data will be necessary for further pursuance of this analysis.

Cows of low fertility may be divided into three main categories on the basis of their reproductive performance during the first 34 days after breeding: (a) failure of fertilization, (b) embryonic abnormalities and mortality before 34 days, and (c) embryos still normal at 34 days (table 6).

Throughout the major portion of this study the cows showing definite physical barriers to fertilization have been excluded. It can be seen from table 3 that out of 104 cows there were three cases of ovulation failure, two of bilateral tubal obstructions and six of unilateral tubal obstructions, but of the last only four (table 2) were on the ovulating side. A failure of fertilization in 8.7 per cent of the cows would be expected because of physical barriers: 1.9 per cent because of bilateral tubal obstructions, 3.9 per cent because of unilateral tubal obstructions on the ovulating side and 2.9 per cent because of ovulation failure.

Subtraction of the percentage of physical genital barriers (8.7) from all animals would leave 91.3 per cent of the animals mechanically capable of fertilization. Since the pooled fertilization percentage for genitally-normal animals of both breeds (3 days after the first and second experimental breedings) is 66.1 (table 4), its complementary percentage for non-fertilization in genitally-normal animals is 33.9. In terms of all animals as a whole, this becomes 33.9×91.3, or 31.0 per cent which fail to conceive although genital barriers are absent (table 6), or a combined percentage of 39.7 ($31.0 + 8.7$) with and without physical obstructions in which there is fertilization failure.

The percentage of cows having normal embryos at 34 days was 23.1 (table 4). Again in terms of the whole population of cows, 91.3×23.1 or 21.1 is the calculated percentage with normal embryos at 34 days.

From the percentage of fertilization failure (39.7) and the percentage of cows with normal embryos at 34 days (21.1) the rate of embryonic mortality may be derived by subtracting the sum of these two percentages from 100. The remainder, 39.2, is an indirect estimate of embryonic abnormalities and mortality by the 34th day after breeding.

SUMMARY

The study covered 104 cows, 49 Guernsey and 55 Holstein, each of which had been bred from 4 to 13 times without conceiving. The percentage of genitally-normal cows having fertilized ova when slaughtered at 3 days was 66.1, but at 34 days the percentage having normal embryos had dropped to 23.1, for an embryonic death rate of 65.1. Estimates were made of the effect of the following factors on fertilization and embryonic death: (a) breed, (b) Bang's disease, (c) number of previous services, (d) number of previous calvings, (e) herd size, and (f) herd breeding index. Appreciable effects upon the fertilization rate were noted for the first and the last three factors, and upon embryonic death rate for the second and the last three. Visible genital abnormalities were found in 10.6 per cent of the cows at the time of slaughter; these abnormalities were not detected by clinical examination. There was a higher occurrence in the Holsteins (18.2 per cent) than in the Guernsey (2.0 per cent). The total percentage of cows with genital abnormalities, 10.6, included 8.7 per cent in which the abnormality constituted a physical barrier to fertilization. Considering the group of cows as a whole, including those with genital abnormalities, division may be made into three main categories on the basis of reproductive performance during the first 34 days after breeding: (a) failure of fertilization, 39.7 per cent; (b) embryonic abnormalities and mortality before 34 days, 39.2 per cent; and (c) embryos still normal at 34 days, 21.1 per cent.

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GROWTH AND PRODUCTION OF INBRED AND OUTBRED HOLSTEIN-FRIESIAN CATTLE[1]

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One of the greatest limitations to genetic improvement of our livestock for economically important characters is the relative inaccuracy in evaluating the individual animal's transmitting ability. The most complete information on the genetics of the sire or dam, if they are not inbred, supplies limited evidence of the breeding worth of an individual offspring. The individual's own appearance or performance may supply more information on its transmitting ability, but this also usually is far from an accurate guide.

On theoretical grounds, selection alone generally should improve the average desirability but have little effect on the uniformity of the offspring. Inbreeding alone has been found to cause individuals to breed more nearly true for good and poor inheritance alike. This means that there is less genetic variation in estimates of transmitting ability. Hence, selection combined with inbreeding should be a means of producing animals which transmit desirable traits more uniformly than would selected or unselected outbred individuals.

The rate of inbreeding which can be practiced without danger of deterioration depends on the degree of dominance (including overdominance), the real merit of the foundation stock and the heritabilities and intensities of selection for the desired traits. The possibilities and limitations of selection and inbreeding as a method for the improvement of dairy cattle could be determined by a carefully planned selection and inbreeding program with the best foundation stocks available. Until this is done systematically, some evidence can be secured from herds in which inbreeding has been practiced and in which there are enough non-inbred control data to warrant conclusions.

Data of this kind are presented in this paper for Holstein-Friesian cattle in three unrelated herds. Comparisons are made between non-inbred cattle and inbreds of different degrees of inbreeding on body dimensions at 6 months of age, 18 months of age and at maturity. Similar data also are presented on milk and butterfat production and butterfat test.

REVIEW OF LITERATURE

An inbreeding experiment with Jersey cattle, started by Regan at New Jersey and then moved to California, had as its objective the development of bulls prepotent for high production. It was reported in 1944 (1) that out of 50 bulls that had progeny tests, only four failed to raise the production of their daughters

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over dams. The average increase in butterfat production of daughters over dams was 88 lb. During the course of the inbreeding experiment, several heritable defects have been reported (7, 8, 10, 11, 12, 15, 16).

Baker *et al.* (2) studied the growth curves of inbred and outbred daughters of one Holstein-Friesian bull and found significant decreases in height at withers, weight and heart girth with increases in the coefficient of inbreeding.

Bartlett *et al.* (3, 4, 5, 9) attempted to establish a herd of Holstein-Friesian cattle possessing genetic factors for high milk production and high fat test. Four sires and 45 high-producing cows were selected as foundation animals, but only one of the four sires withstood the inbreeding tests. The descendants of this one sire were inbred up to 20 per cent (Wright's (21) coefficient). They did not differ significantly in body weight or body dimensions at any stage from birth to maturity. Females inbred more than 20 per cent were smaller at maturity than the outbreds used as controls. The average milk and butterfat production of the outbred daughters of four sires was significantly larger than that of the inbreds, but the average fat tests for the two groups were about the same.

Starting with cows of average production, Woodward and Graves (20) used inbreeding to determine whether a good dairy herd could be developed from an ordinary one by the use of only one foundation sire. Grade cows were bred either to a purebred Guernsey or purebred Holstein-Friesian bull, and the daughters bred back to their sires for successive generations. They found that intense inbreeding (25 to 50) resulted in lighter birth weights and a reduction in mature weights. A few inbred Guernseys were deformed at birth and the average mortality of the calves after birth was greater among the inbreds. The effects on production of inbreeding and breed differences were confused in these data.

Plum (13) found a negative intra-sire correlation between inbreeding and butterfat production in one Jersey herd in which the inbreeding ranged from 0 to 22 per cent.

Tyler *et al.* (19) found an average intra-sire decline of 0.28 lb. in birth weight for each increase of 1 per cent inbreeding in data from three unrelated herds.

EXPERIMENTAL PROCEDURE

The data were collected from 1937–1945 on the Holstein-Friesian cattle of the three herds described in a previous paper (19).

Body dimensions were taken at approximately 6 and 18 months of age, and 1 month following each calving. Only females were measured at ages beyond 6 months. The dimensions taken were height at withers, circumference of shin bone, heart girth and width at hips. The measurements that were taken after each calving were adjusted to a 60-months-of-age basis.

The monthly milk weights and fat tests were taken directly from the D.H.I.A. herd books, and the summation of the first ten testing-day values multiplied by 30.5 was used to estimate 305 days' production (18). All production records were standardized to a mature equivalent, twice-a-day milking, 305-day basis.

The suggested breeding plan in each herd was to mate the herd sires to their

TABLE 1

Means of the body dimensions of inbred and outbred males at 6 months of age

	No. of animals	Dimension			
		Height at withers	Circumference shin bone	Heart girth	Width at hips
		(*cm*)	(*cm*)	(*cm*)	(*cm*)
Inbreds					
6 to 11	10	104	14.2	124	29.9
12 to 17	15	104	14.0	123	29.7
18 to 23	7	105	14.2	122	30.1
24 to 29	10	101	13.8	119	29.4
30 to 37	1	105	14.5	125	32.5
All inbreds	43	103	14.1	122	29.8
Outbreds	17	104	13.9	123	30.1

daughters or close collateral relatives as a test of their actual breeding worth, and during the same period mate them to unrelated cows to produce offspring or control animals by the same sire. The inbreeding percentages were calculated by Wright's method, and during the period of investigation these inbreeding percentages varied from 0 to 37. For comparative purposes animals whose inbreeding percentages were less than six were classified as outbreds. Individuals whose inbreeding percentages were six or larger were designated as inbreds.

RESULTS

Dimensions at 6 months of age. There were 60 males and 193 female calves that were measured at approximately 6 months of age. The number of calves and the means of the four body dimensions for the outbred and inbred groups are given for males in table 1 and for females in table 2. The inbred animals were divided into five groups on the basis of the size of their inbreeding percentages. The figures in tables 1 and 2 suggest that, with the exception of the circumference of shin bone measurement for male calves, the average inbred was slightly smaller than the average outbred individual.

In order to remove any bias that sire, dam and herd may have had on these

TABLE 2

Means of body dimensions of inbred and outbred females at 6 months of age

	No. of animals	Dimension			
		Height at withers	Circumference shin bone	Heart girth	Width at hips
		(*cm*)	(*cm*)	(*cm*)	(*cm*)
Inbreds					
6 to 11	39	100	13.1	119	29.4
12 to 17	43	102	13.2	120	30.1
18 to 23	18	101	13.0	119	29.5
24 to 29	12	100	13.0	119	30.0
30 to 37	5	101	13.3	118	29.5
All inbreds	117	101	13.1	119	29.7
Outbreds	76	102	13.4	122	30.5

TABLE 3

Intra-sire correlations and partial regression coefficients (holding mature body dimension of dam constant) between body dimensions of male and female calves at 6 months of age and inbreeding

Dimension	Males			Females		
	Degrees of freedom	Correlation between dimension and inbreeding	Partial regression of dimension on inbreeding	Degrees of freedom	Correlation between dimension and inbreeding	Partial regression of dimension on inbreeding
Height at withers	55	−0.138	−0.055	180	0.084	0.045
Circum. of shin bone	55	−0.078	−0.002	180	−0.016	−0.001
Heart girth	55	−0.205	−0.148	180	−0 003	−0 002
Width at hips	55	−0.093	−0.017	180	−0.019	0.005

average values, the influence of inbreeding on each body dimension was measured by the within-sire partial regression of dimension on inbreeding, holding the same mature dimension of the dam constant. The correlation and partial regression coefficients for each dimension are given separately for each sex in table 3. The partial regression coefficients for the males were negative, but small and not statistically significant. In the case of the heifer calves, the small negative and positive within-sire partial regression coefficients were not significant. Hence, inbreeding was not demonstrated to have influenced the size of male and heifer calves at 6 months of age by these data.

Dimensions at 18 months of age. A total of 152 females were measured at 18 months of age. Sixty-four of these heifers were outbred. The inbreeding percentages on the other 88 animals ranged from 6 to 34 with an average of 14. The number of heifers and the means of the four body dimensions at 18 months of age are given for the outbred and inbred heifers in table 4. The average dimensions of all inbreds were the same as those for the outbreds. The within-sire partial regression coefficients of each dimension on inbreeding, holding constant the same dimension of the dam at maturity, were calculated and are given

TABLE 4

Means of the body dimensions of inbred and outbred females at 18 months of age

	No. of animals	Dimension			
		Height at withers	Circumference shin bone	Heart girth	Width at hips
		(*cm*)	(*cm*)	(*cm*)	(*cm*)
Inbreds					
6 to 11	27	125	17.0	169	44.6
12 to 17	36	125	16.9	169	45.1
18 to 23	17	125	16.7	169	44.9
24 to 29	5	122	17.1	163	44.0
30 to 34	3	126	17.0	165	45.3
All inbreds	88	125	16.9	168	44.9
Outbreds	64	125	16.9	168	44.9

TABLE 5

Intra-sire correlations and partial regression coefficients (holding mature body dimension of dam constant) between body dimensions of heifers at 18 months of age and inbreeding

Dimension	Degrees of freedom	Correlation between dimension and inbreeding	Partial regression of dimension on inbreeding
Height at withers	141	0.048	0.007
Circumference of shin bone	141	0.021	0.002
Heart girth	141	−0.167[a]	−0.106[a]
Width at hips	141	−0.012	−0.002

[a] $P < .05$.

in table 5. The partial regression of heart girth on inbreeding was significant and negative, but the other regressions were not significant. The reduction in heart girth amounted to about 1 cm. or about 20 lb. in body weight for an increase of 10 per cent in inbreeding.

Dimensions at maturity. The average of the four dimensions for the 56 inbred and 55 outbred cows that were measured after one or more calvings are given in table 6. The inbreds were smaller on the average than the outbreds in circumference of shin bone, heart girth and width at hip measurements. The correlations and partial regressions of dimensions on inbreeding, holding dam's mature dimension constant, are shown in table 7. The within-sire regression coefficients are negative, but not statistically significant.

Production records. Milk and butterfat production records were available on 47 outbred and 42 inbred daughters of 5 sires. The average milk and butterfat production and butterfat test for outbred and inbred daughters by each sire and the partial regression of production and test on inbreeding, holding corresponding performance of the dam constant, are given in tables 8, 9 and 10.

The outbred daughters produced more milk than their inbred half-sisters in four out of five sire groups. The partial regressions were significant for one of

TABLE 6

Means of the body dimensions of inbred and outbred females at maturity

	No. of animals	Dimension			
		Height at withers	Circumference shin bone	Heart girth	Width at hips
		(*cm*)	(*cm*)	(*cm*)	(*cm*)
Inbreds					
6 to 11	16	136	17.5	190	57.2
12 to 17	19	137	17.1	189	56.5
18 to 23	13	139	17.3	186	56.1
24 to 29	7	134	17.8	191	56.8
30 to 34	1	138	18.7	201	61.6
All inbreds	56	137	17.4	189	56.8
Outbreds	55	136	17.9	193	57.8

TABLE 7

Intra-sire correlations and partial regression coefficients (holding mature body dimension of dam constant) between body dimensions at maturity and inbreeding

Dimension	Degrees of freedom	Correlation between dimension and inbreeding	Partial regression of dimension on inbreeding
Height at withers	104	0.013	−0.003
Circumference of shin bone	104	−0.067	−0.011
Heart girth	104	−0.085	−0.082
Width at hips	103	−0.175	−0.026

TABLE 8

Number and mean milk production of outbred and inbred daughters and their dams by sires, and the partial regressions (holding milk production of the dams constant) of milk production on inbreeding (mature equivalent, twice-a-day, 305-day records)

Sire	Outbred			Inbred				Partial regression BF on inbreeding
	No. daughters	Daughter av.	Dam av.	No. daughters	Av. % inbreeding	Daughter av.	Dam av.	
		(*lb.*)	(*lb.*)			(*lb.*)	(*lb.*)	
1	4	15,394	13,818	13	16	12,685	13,486	−149.2[a]
2	5	12,368	12,637	15	9	12,132	13,116	− 93.0
3	4	12,739	11,606	4	11	11,128	11,444	−154.6
4	31	11,447	12,066	6	24	10,306	10,188	− 51.0
5	3	10,988	10,882	4	12	11,068	11,969	12.9
Total	47	.....	.....	42	14	.....	.....	− 73.8[b]

[a] $P > 0.01 < 0.05$.
[b] $P < 0.01$.

TABLE 9

Number and mean butterfat production of outbred and inbred daughters and their dams by sires, and the partial regression (holding dams' butterfat production constant) of butterfat production (BF) on inbreeding (mature equivalent, twice-a-day, 305-day records)

Sire	Outbred			Inbred				Partial regression BF on inbreeding
	No. daughters	Daughter av.	Dam av.	No. daughters	Av. % inbreeding	Daughter av.	Dam av.	
		(*lb.*)	(*lb.*)			(*lb.*)	(*lb.*)	
1	4	518	485	13	16	446	471	−6.81[a]
2	5	496	484	15	9	512	459	−0.66
3	4	432	383	4	11	372	378	−6.22
4	31	381	405	6	24	338	363	−1.82
5	3	386	371	4	12	382	377	−0.30
Total	47			42	14			−2.32[b]

[a] $P > 0.01 < 0.05$.
[b] $P < 0.01$.

the sires, while the average decline (within-sire) for all sires was 74 lb. of milk for each 1 per cent increase in inbreeding. The average regression coefficient was highly significant.

The average butterfat production of the outbreds was greater than that of the inbreds for four of the five sires, but the partial regressions were significant for only one sire (table 9). The average regression within-sire was significant and amounted to 2.3 lb. decrease for every 1 per cent increase in inbreeding. The average butterfat production for the inbred daughters of sire 2 was 16 lb. more than the outbreds. This superiority was caused by the high fat production of the slightly inbred (6 per cent) daughters. However, the more highly inbred cows were lower producers which led to the negative regression for this group of 20 daughters.

TABLE 10

Number and average fat percentage of outbred and inbred daughters and their dams by sires and the partial regressions (holding dams' fat percentage constant) of fat percentage on inbreeding

Sire	Outbred			Inbred				Partial regression fat test on inbreeding
	No. daughters	Daughter av.	Dam av.	No. daughters	Av. % inbreeding	Daughter av.	Dam av.	
		(%)	(%)			(%)	(%)	
1	4	3.36	3.51	13	16	3.52	3.49	0.006
2	5	4.01	3.83	15	9	4.22	3.50	0.014
3	4	3.39	3.30	4	11	3.34	3.30	−0.008
4	31	3.33	3.36	6	24	3.28	3.56	−0.010[a]
5	3	3.51	3.41	4	12	3.45	3.15	0.012
Total	47			42	14			0.0053

[a] $P > 0.01 < 0.05$.

The mean fat test of the outbreds was higher in 3 out of the 5 daughter groups (table 10). The increase, however, was significant for only one sire. The average regression of fat test on inbreeding was positive but not statistically significant.

DISCUSSION

The body size of the inbred Holstein-Friesian cattle in the three herds studied was slightly less than the size of outbred animals in the same herds at ages of 6 months, 18 months and maturity when the records were adjusted to a standard mature size of their dams.

The influence of inbreeding on birth weights of animals in these three herds had been found previously to be significant and amounted to a decrease of 2.3 lb. for a 10 per cent increase in the coefficient of inbreeding (19). The lack of evidence of an inbreeding effect at 6 months of age may have been due to the small numbers, the low degree of inbreeding in most comparisons, the differential culling between inbreds and outbreds or, of course, an absence of an inbreeding depression in some of these progeny groups. Selection does not appear to have been a factor, however. For example, there was no evidence that the difference between the

birth weights of inbred culls and inbred non-culls was any larger than the difference between the birth weights of outbred culls and outbred non-culls. However, the percentages of culling for inbred and outbred groups of males were 61 and 40, respectively. For the female groups the percentages were 31 and 24. This may mean that differential culling between inbreds and outbreds, as far as dimensions at older ages are concerned, has occurred even though it is not apparent from the birth weights of culls and non-culls.

The inbred and outbred cattle, in general, were similar in body size at the various ages to the "normal" size of Holstein-Friesian animals as given by Ragsdale's (14) tables for height at withers, circumference of chest (heart girth in this study) and width at hips. The exceptions to the normal were: (a) Both inbred and outbred male calves and inbred female calves at 6 months of age were about 4 per cent smaller in heart girth. (b) The inbred and outbred heifers at 18 months of age were approximately 2 per cent larger in heart girth. (c) At maturity the inbred cows average 2 per cent narrower at the hips and 3 per cent smaller in heart girth measurement than the normal animal.

The results gave no evidence of an effect of inbreeding on butterfat test but did indicate that the milk and butterfat production of the inbred cows was significantly lower than the production of the outbred cows by the same sires. On the basis of these results, the average rate of decline of butterfat production in these cows was sufficiently low so that with moderate inbreeding (6 per cent per generation) the decrease in production should be 14 lb. of butterfat per generation. The standard deviation of butterfat in these cows is about 80 lb., and if heritability is 0.2, the parents would need to average $14 \div (80 \times 0.2) = 0.87$ of a standard deviation above the group average to counteract this inbreeding depression. Production can be measured only in the female, and culling among cows based on their own records has limited possibilities (17). This means that bulls and heifers saved for breeding would need to come from dams averaging $14 \div (80 \times 0.1)$ or $2 \times 0.87 = 1.74$ standard deviations above the average or from the best fifth to tenth of the cows. It would be impossible to do this even if selection of bulls and heifers were based entirely on production records, which it cannot be. A more realistic intensity of inbreeding that could be offset by selection would be about 3 per cent per generation. However, there was considerable variation between sires in the effect of inbreeding on production, presumably because of the level of transmitting ability of the sire relative to the average of the unrelated dams to which he was mated. Thus, by linebreeding to the superior sires, linebred families of increased uniformity of transmitting ability probably could be produced without much loss of production.

SUMMARY

Growth and production data of inbred and outbred progeny of Holstein-Friesian sires in three Wisconsin State Department of Public Welfare herds were collected and analyzed to determine the effect of inbreeding on body dimensions (height at withers, circumference of shin bone, heart girth and width of hips) at 6 and 18 months of age and at maturity and on milk and butterfat production

and butterfat test. The average intra-sire partial regression (holding mature size of dam constant) of dimension on inbreeding was used to measure this effect. The partial regression of dimensions on inbreeding was essentially zero, except for heart girth at 18 months and maturity, which was significant only at 18 months. Intra-sire partial regressions (holding dam's record constant) of milk and butterfat production of 42 inbred and 47 outbred cows on inbreeding were significant and amounted to an average decrease of 74 lb. of milk and 2.3 lb. of butterfat for each 1 per cent increase in inbreeding. No evidence of an effect of inbreeding on butterfat percentage was indicated.

Considerable variation was found in the partial regression coefficients of sires, indicating that offspring of some sires could be inbred without any apparent decrease in body size or production, possibly in part because the beneficial effect of increasing the relationship to a sire transmitting a superior level of production tends to offset detrimental effects of inbreeding to him.

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A STUDY OF FEEDING LOW LEVELS OF THYROPROTEIN TO DAIRY COWS FOR A PERIOD OF FIFTY-TWO WEEKS[1, 2]

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Considerable research has been conducted in efforts to determine the value of synthetic thyroproteins in the rations of dairy cows (1, 2, 3, 4, 6, 7, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29). In the majority of these reports, it appears that with the levels of thyroprotein fed there were losses in body weight, increases in heart and respiration rates, increases in body temperature and irregular responses in milk and milk fat production.

As a result of the previous review of available literature, it appeared to be desirable to conduct experiments for a minimum of 1 year using somewhat lower levels of thyroprotein feeding than those reported previously. The effects upon milk and milk fat production, as well as upon body weights, body temperatures, heart rates, respiration rates and breeding efficiency, were observed and are presented in this report.

EXPERIMENTAL PROCEDURE

Two trials were conducted to determine the effects of feeding levels of 0.625, 1.25 and 5.0 g. of iodinated casein daily to cows for a period of 52 weeks. Two separate experiments were conducted under somewhat different feeding conditions and the results are presented in this report.

Trial I. Four groups of three Holstein cows each were selected so as to be comparable in age, stage of lactation, stage of gestation, body weight and milk production. The four groups were assigned at random to the three levels of thyroprotein as well as to a control group. All cows in this trial were maintained at a T.D.N. intake of 125 per cent of Morrison's (10) recommended standards for good cows under usual conditions. The rates of feeding were recalculated every 2 weeks on the basis of body weight and milk and milk fat production during the previous 3 days. The cows were milked twice daily and their production recorded for each milking. The percentage of milk fat was determined once each month by the Babcock method. Body weights were checked every 2 weeks and rectal temperatures every 3 months. A stethoscope was used to check the heart rates every 3 months. Management conditions were controlled as carefully as possible so as to be similar for all cows on the trial.

Trial II. Four groups of eight cows, each including the Ayrshire, Brown

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[1] Taken from data presented in a thesis to the graduate faculty of The Pennsylvania State College by R. G. Swanson in partial fulfillment of the requirements for the Degree of Master of Science. Authorized for publication on August 23, 1948 as paper No. 1464 in the journal series of the Pennsylvania Agricultural Experiment Station.

[2] The authors wish to express their appreciation to the Whitmoyer Laboratories, Inc., Myerstown, Pa. for a research grant and to the Cerophyl Laboratories, Inc. which supplied the iodinated casein for this study.

Swiss and Guernsey breeds, were selected and assigned at random to the same levels of thyroprotein with a control group as in trial I. All cows in this trial were maintained at a T.D.N. intake of 110 per cent of Morrison's (10) recommended standards for good cows under usual conditions. As in trial I, the rates of feeding were recalculated every 2 weeks on the basis of body weight and milk and milk fat production during the previous 3 days. The cows were milked three times per day, and milk weights were recorded at each milking. Fat tests were determined at 10-day intervals during the trials and for 8 days before and after the withdrawal of thyroprotein from the ration. Body weights were checked every 2 weeks and rectal temperatures were taken every month. Heart and respiration rates also were checked once a month with a stethoscope. Management conditions were controlled carefully so as not to be a variable in the experiment. All milk records in both trials were converted to a 4 per cent fat-corrected milk, mature equivalent basis (5, 8). All data were analyzed according to the methods of Snedecor (23) wherever applicable.

EXPERIMENTAL RESULTS

Trial I. When milk production of the groups receiving thyroprotein was compared to the control group, no statistically significant differences were observed. There was considerable variation between cows within groups. In table 1 are presented the average total milk production and the average number of days milked during the feeding trial.

TABLE 1

Mean total milk production[a] trial I

Thyroprotein fed	Milk	Milk fat	Days in milk
(*g.*)	(*lb.*)	(%)	
0 (control)	14,708	3.76	328
0.625	14,311	3.71	305
1.25	13,844	4.04	309
5.0	14,456	4.09	300

[a] Expressed as 4 per cent F.C.M., M.E.

The analysis of variance of the data relative to the milk production of the cows in trial I is presented in table 2. While there apparently were differences

TABLE 2

Analysis of variance of milk production of cows in trial I

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	143	68,522,262	
Cows	2	727,489	363,745
Months	11	18,616,342	1,692,395[a]
Treatments	3	771,770	257,257
C × M	22	8,291,638	376,893
C × T	6	10,027,368	1,671,228[a]
T × M	33	6,209,044	188,152
Remainder	66	23,878,611	361,797

[a] = Significant at 1 per cent level.

TABLE 3

Mean body weights in trial I

Thyroprotein fed	Initial	Final	Mean gain
(*g./day*)	(*lb.*)	(*lb.*)	(*lb.*)
0 (control)	1,418	1,608	190
0.625	1,334	1,509	175
1.25	1,247	1,388	141
5.0	1,395	1,522	127

in the fat content of the milk produced by the various groups, when the production was converted to 4 per cent fat-corrected milk these differences were not significant.

All cows in this trial gained in body weight during the experiment. The average group weights and gains are presented in table 3. The differences in the average group gains are not statistically significant, but a trend appears, since the group receiving the most thyroprotein gained the least and the control group gained the most. The analysis of variance of the data relative to the effects upon body weight in trial I are presented in table 4.

TABLE 4

Analysis of variance of body weights in trial I

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	323	5,802,954	
Periods	26	770,528	29,636[a]
Cows	2	30,982	15,491
Treatments	3	1,248,055	416,018
Treatments × Cows	6	2,026,303	337,717[a]
Treatments × Periods	78	188,560	2,417
Cows × Periods	52	396,785	7,630
Sampling error	156	1,141,741	7,319

[a] = Significant at 1 per cent level.

While there was a highly significant difference in the body weights of the cows during the different periods, the differences between cows and between treatments were not statistically significant. The interaction of treatments and cows was highly significant.

Body temperatures, heart rates and respiration rates of the cows in this trial, as presented in table 5, showed no significant increases or changes when compared

TABLE 5

The effect of thyroprotein feeding upon body temperature, heart rate, and respiration rate in trial I

Thyroprotein fed	Body Temperature	Heart rate	Respiration rate
(*g./day*)	(° *F.*)	(*min.*)	(*min.*)
0 (control)	101.6	65.8	23.3
0.625	101.7	65.7	24.1
1.25	101.8	65.3	24.9
5.0	101.6	65.1	23.8

TABLE 6

Mean total milk production[a] in trial II

Thyroprotein fed	Milk	Milk fat	Days dry
(*g./day*)	(*lb.*)	(%)	
0 (control)	9,876	4.3	77
0.625	12,564	4.4	46
1.25	11,461	4.3	61
5.0	10,586	4.4	59

[a] Expressed as 4 per cent F.C.M., M.E.

with the control cows. There was no indication that the amounts of thyroprotein fed had any adverse effect on breeding efficiency.

Trial II. Although there was some differences in the average total milk production (table 6) between the groups receiving thyroprotein and the control group, an analysis of variance (table 7) showed these differences to be non-

TABLE 7

Analysis of variance of milk production of cows in trial II

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	383	91,616,562	
Cows	7	1,522,412	217,487
Treatments	3	2,444,977	814,992
Months	11	16,886,612	1,535,146[a]
C × T	21	6,922,541	329,644[b]
C × M	77	18,960,403	246,239[b]
T × M	33	2,719,377	82,405
Sampling error	231	42,160,240	182,511

[a] = Significant at 1 per cent level.
[b] = Significant at 5 per cent level.

significant statistically. Some of the variation between groups can be accounted for by the differences in the length of dry periods (table 6).

On the basis of the mean per cent of milk fat, there was no effect of feeding these levels of thyroprotein upon the fat content of the milk produced. There were no significant differences between cows relative to milk production in trial II.

In table 8 are presented data relative to the milk production of five cows of the

TABLE 8

Average milk production of comparable groups of 5 cows from each group during first 4 months of thyroprotein feeding in trial II[a]

Thyroprotein fed	Av. production 10 d. prior to start	Av. total production (4 mo.)	Av. production 10 d. prior to end
(*g./day*)	(*lb.*)	(*lb.*)	(*lb.*)
0 (control)	44.6	4,682	34.1
0.625	45.5	5,540	44.6
1.25	43.8	5,084	38.6
5 0	43.4	5,241	43.2

[a] = Expressed as 4 per cent F.C.M.

TABLE 9

Analysis of variance of data of 4 months milk production at beginning of trial

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	239	2,320,113	
Days	11	27,298	2,482[a]
Cows	4	1,216,734	304,183[a]
Treatments	3	128,429	42,809
T × C	12	691,029	57,586[b]
T × D	33	35,329	1,071
C × D	44	49,469	1,124
Sampling error	132	171,825	1,302

[a] = Significant at 5 per cent level.
[b] = Significant at 1 per cent level.

various groups during the first 4 months of trial II. These cows were selected solely on the basis of obtaining groups which were comparable in milk production, stage of lactation, stage of gestation and age. On the basis of the mean data it would appear that some stimulation of milk production occurred as a result of thyroprotein feeding. However, an analysis of variance (table 9) of the data proved the differences to be non-significant statistically.

Near the conclusion of the 52-week feeding period, cows in the same stage of

TABLE 10

Comparison of production during the 35 days preceding and following withdrawal of thyroprotein from the ration

Thyroprotein fed	Mean decline in production per cow[a]
(*g./day*)	(*lb.*)
0 (control)	31
0.625	66
1.25	42
5.0	152

[a] Expressed as lb. 4 per cent F.C.M., M.E. during the 35-day period.

lactation were selected from each group and compared for 35 days before and after the withdrawal of thyroprotein from the ration. The results of this study (table 10) indicate that there may have been some stimulation to milk production as a result of thyroprotein feeding. When thyroprotein was withdrawn from the ration there was no decline in the per cent of milk fat. This seems to indicate that, at these levels of intake, thyroprotein did not increase the percentage of milk fat.

TABLE 11

Mean body weights in trial II

Thyroprotein fed	Initial	Final	Mean gain
(*g./day*)	(*lb.*)	(*lb.*)	(*lb.*)
0 (control)	1,140	1,285	145
0.625	1,130	1,230	100
1.25	1,180	1,240	60
5.0	1,155	1,235	80

TABLE 12

Analysis of variance of body weights in trial II

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	831	30,940,085	
Weeks	25	1,159,127	46,365[a]
Cows	7	17,828,396	2,546,914
Treatments	3	79,099	26,366
T × C	21	9,845,110	468,815[a]
T × W	75	109,643	1,461
C × W	175	715,418	4,088[a]
Sampling error	525	1,203,292	2,292

[a] = Significant at 1 per cent level.

All cows in trial II gained in weight during the experiment (table 11). Analysis of variance (table 12) of body weights showed that there were no significant differences between treatments. The interaction of treatments and cows was highly significant, indicating that individual cows responded differently in weight gains to thyroprotein feeding

In trial II the body temperatures, heart rates and respiration rates were checked once each month. Body temperatures varied considerably from month to month but not between treatments. Heart and respiration rates of the cows receiving thyroprotein were not significantly different from those in the control

TABLE 13

Mean body temperature, heart and respiration rate (trial II)

Thyroprotein fed	Body temperature	Heart rate	Respiration rate
(*g./day*)	(° *F.*)	(*min.*)	(*min.*)
0 (control)	101.6	74	32
0.625	101.6	76	33
1.25	101.5	76	31
5.0	101.6	76	32

group; however, individual cows showed variations in response as indicated by a highly significant interaction between treatments and cows. The groups as a whole, however, did not exhibit these differences (table 13).

In trial II, as in trial I, no indication of lowered breeding efficiency could be observed due to thyroprotein feeding.

SUMMARY

Thyroprotein in the form of iodinated casein fed at levels of 0.625, 1.25 and 5.0 g. daily to dairy cows with a T.D.N. intake of 125 per cent of Morrison's standards for good cows under usual conditions (10) produced no significant stimulation of milk production, milk fat production, heart rates, respiration rates or body temperatures. Gains in body weight and breeding efficiencies were not affected significantly.

These same levels of thyroprotein feeding accompanied by a T.D.N. intake of 110 per cent of Morrison's standards for good cows under usual conditions (10)

produced increases in milk production which approached statistical significance. Milk production appeared to decline when thyroprotein was withdrawn from the ration. Mean milk fat percentage, body weights, heart rates, respiration rates, body temperatures and breeding efficiencies were not affected significantly by these levels of thyroprotein administration.

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VOLUME XXXII MARCH, 1949 NUMBER 3

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ABSTRACTS OF LITERATURE

BOOK REVIEW

141. Annual review of microbiology, Vol. II. Edited by C. E. CLIFTON. Annual Reviews, Inc., Stanford, Calif. 532 pp. $6.00. 1948.

Microbiologists and those who are concerned with the relationships of microbiology to other fields will welcome the second volume of this series of publications. The eighteen reviews cover the wide range of topics which one has come to expect in the Annual Reviews series. The discussions are presented well, the citations to the literature are numerous and the volume is well-prepared and well-indexed.

The reviews cover the following topics: Yeasts, Genetics of the Fungi, Bacterial Metabolism, the Metabolism of Malarial Parasites, Growth Factors for Microorganisms, Antibiotics, The Mode of Action of Chemotherapeutic Agents, Inheritance of Immunity in Animals, Complement, The Nature of Antibodies, Pathogenic Streptococci, the Spirochetes, The Neurotropic Viruses, Bacteria as Plant Pathogens, Chemical Disinfectants, Microbiology of Drinking Water and Sewage, Microbiology of Soil and Biological Nitrogen Fixation.

F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

142. Vibrio foetus infection in cattle. A. S. CANHAM, Allerton Laboratory, Pietermarityburg, South Africa. J. S. African Vet. Med. Assoc., **19,** 3: 103. Sept., 1948.

Several case histories of *Vibrio foetus* infection in cattle are reported. The disease is not contagious, therefore does not appear to be as serious as contagious abortion. Cows aborted from 4 to 7 mo. after pregnancy and in all cases retained placenta were observed. All cows were isolated until cleaned and treated with daily irrigation of saline. When the animals appeared normal they were bred. Normal pregnancies and births of living calves followed in all cases. The disease is widespread in South Africa. However, in no herds has there been a complete loss of a calf crop and few cows fail to recover and breed normally after their first abortion.

K. M. Dunn

143. The diagnosis of east coast fever in South Africa. A. M. DIESEL AND G. C. VAN DRIMMELEN. Institute of Onderstepoort, Pretoria, South Africa. J. S. African Vet. Med. Assoc., **19,** 3: 81. Sept., 1948.

A review of the disease, covering its history and methods of diagnosis, is presented. The authors point out the similarity of this disease to those caused by protozoal diseases such as *T. parva* and anaplasmosis. The present methods of diagnosis are based on both epizootological and protozoological studies. Detection of the parasites is a difficult job and requires well-trained personnel both in collecting samples and for microscopic examinations. K. M. Dunn

144. Treponema theileri of a donkey and its transmission to a calf. V. R. KASCHULA, Pietermarityburg, South Africa. J. S. African Vet. Med. Assoc., **19,** 3: 100. Sept., 1948.

A successful attempt to transmit *Treponema theileri* from the donkey to a calf by blood transfusion is reported. It required 18 d. for the calf to show fever and for *T. theileri* to appear in the blood as detected from smears. No rough or scaly skin was observed in the calf.

K. M. Dunn

BUTTER

O. F. HUNZIKER, SECTION EDITOR

145. Das physikalische Bild der Butter. (The physical aspect of butter). English summary. N. KING AND W. FRITZ. Die Milchwissenschaft **3,** 3: 75–82. 1948.

The continuous phase in butter consists of free, non-spherical fat. The free fat is made up of (a) mechanically broken fat globules and (b) butter oil formed in low-cooled cream. In cooling cream, the crystallizing fat migrates to the outer part of the fat globule, forming a solid circle which on prolonged cooling exerts pressure upon the inner liquid portion resulting in rupture of the globule and the liberation of the butter oil. The butter oil then forms crescent-shaped layers around the solid fat portion.

The % free fat is highest in machine butter (62%) and consists chiefly of broken fat globules. In churn butter the free fat amounts to 48% and

is made up of broken fat globules and butter oil. In Alfa butter the free fat (12%) consists mostly of butter oil. The % free fat in butter is reduced by (a) low churning temperature and (b) use of large churns. The consistency of butter is determined by (a) composition of free fat and (b) size and number of fat crystals. High butter oil and few small crystals result in sticky butter, whereas low butter oil and many large crystals result in crumbly butter. Deep cooling of winter cream prevents crumbliness, whereas in summer cream it may result in sticky butter. "Oily" butter results from churning low-cooled high-fat cream. I. Peters

146. Das physikalische Bild der Butter. (The physical aspect of butter). N. KING AND W. FRITZ. Die Milchwissenschaft, **3,** 4:102–107. April, 1948.

Whereas the free fat phase plays an important role in the consistency of the butter, the aqueous phase determines primarily the initial and keeping quality of the butter, with some possible influence upon its consistency. The buttermilk droplets with a diam. below 15 μ constitute the major part of the moisture in butter, with the droplets of added water, 100 to 1,200 μ in diam., making up the rest. The washing of butter granules neither dilutes the buttermilk droplets nor decreases their size. The average size of the water droplets decreases in the following order of manufacturing procedures: butterworker → machine butter → Alfa butter. The size of the water droplets decreases in machine butter with (a) increasing output per hr. (79 to 470 kg. per hr.) and (b) lowering of churning temperature (11.5 to 5.8° C.) The finer distribution of water droplets improves the texture and assures greater keeping quality of the finished butter. I. Peters

147. Butter cutter. E. A. ANDERSON. U. S. Patent 2,454,421. 4 claims. Nov. 23, 1948. Official Gaz. U. S. Pat. Office, **616,** 4: 1016. 1948.

Butter is cut into prints by wire frames which are forced down and across through the block as it is supported on a platform. R. Whitaker

Also see abs. no. 155, 160, 162.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

148. Comparisons of the resazurin test with other tests for the quality of milk. J. C. BOYD AND H. C. HANSEN, Univ. of Idaho, Moscow. Milk Dealer, **38,** 1: 200–208. Oct., 1948.

The results of a comparison, on the same milk samples, of freshly-made solutions of methylene blue and resazurin with those 2, 3, 4 and 5 d. old do not indicate any changes in the solutions that would affect the grading of milk. A comparison of the liquid resazurin with the dry resazurin showed a correlation of almost 100%. Of 144 samples graded A by the resazurin test, 79% had a standard plate count (SPC) of $<$ 100,000/ml. and of 236 samples graded A by the methylene blue, 71% had a SPC of $<$ 100,000/ml.; 2.7% of those graded A by the resazurin test and 3.7% by the methylene blue had SPC's $>$ 500,000/ml. The resazurin test placed more samples having a SPC of $<$ 100,000/ml. in B and C grades than the methylene blue, and of the D grades the number of samples was insufficient, but the resazurin test had a tendency to place in D grade a few samples with SPC's of $<$ 100,000/ml.

A comparison of the resazurin test and the direct microscopic count (DMC) on 408 samples showed that 65.2% of the 205 samples graded A by the 1-hr. resazurin test had a DMC of $<$ 1 million/ml. Also 6.3% had a DMC $>$ 5 million/ml. Of the samples graded D, 8% had a DMC of $<$ 1 million/ml. The results of a comparison of the 1-hr. resazurin test with the 5.5-hr. methylene blue test show that the 1-hr. resazurin test will place fewer samples in A grade and more samples in D grade than will the methylene blue test. A comparison of the 1-hr. resazurin test and the alcohol test showed no correlation. A comparison of the 1-hr. resazurin test to titratable acidity showed that the samples placed in D grade by the resazurin test had titratable acidities varying from 0.16 to 0.26%, but no sample placed in A grade had a titratable acidity $>$ 0.2%. A comparison of the 1-hr. resazurin test with the sediment test showed 14.3% of the samples graded no. 4 by sediment and 58.5% graded D by the resazurin test.

C. J. Babcock

149. Die Verwendung der Milchzucker-Fuchsin-Papier-Platte fur den Nachweis von *Bact. Coli* und die Bedeutung des Coliquotienten in der Milch. (The use of lactose fuchsin-paper disk evidence of *Bact. coli* and the significance of the coli quotient in milk.) English summary. G. MÜLLER. Die Milchwissenschaft, **3,** 3: 82–83. 1948.

The use of membrane filters in removing bacteria from water supplies and the successful growth of these organisms by placing the membrane upon an endo agar medium induced the author to adapt this method for the examination of milk. The agar medium was replaced by a paper disk saturated with a suitable nutrient, such as lactose-fuchsin; this resulted in a considerable

saving of medium. Typical coliform colonies, 2–4 mm. in diam., were obtained after 20 hr. incubation at 37° C. The method also could be used to obtain total counts in milk. The ratio of coliform count to total count (coliform ÷ total count × 100) was expressed as the coliform quotient and should not exceed 0.02% in grade A milk. Samples of pasteurized milk examined showed quotients up to 10%, indicating recontamination of milk or prolonged storage at improper temperatures. I. Peters

150. Control of thermoduric bacteria in milk. Ann. Rpt. N. Y. State Assoc. Milk Sanit., **21:** 53–70. 1948.

From the standpoint of the health department. P. Corash, N. Y. City Dept. Health. Pp. 53–56. The thermoduric bacteria problems developed with the adoption of tryptone-glucose-milk agar for plating, as these bacteria grow on this medium. Although thermoduric bacteria are found in the cow's udder, it is doubtful if this source is very important. Thermoduric bacteria are not pathogenic but good sanitation requires that their numbers be low. Our research has shown no growth of thermodurics in holding tanks under proper refrigeration, but homogenization gave increased counts from 3.5 to 34% in one series of tests and an average increase of 64% in another series of experiments. Raw milk must be pasteurized down to 20,000 per ml. to be acceptable.

From the standpoint of the plant laboratory. P. E. Carney, Sheffield Farms, Inc., New York, N. Y. Pp. 57–58. Stress was given to the correct sampling procedure for milk for bacterial counts. With the increased use of high temperature pasteurization in plants, the same method might be used in the laboratory. The oval tube method of determining thermoduric bacteria was recommended to save time, agar, incubator space, and glassware.

From the standpoint of the dairy serviceman. D. T. Baker, Dairymen's League Coop. Assoc., Poughkeepsie, N. Y. Pp. 59–61. In looking for the source of high thermoduric counts, the milk cans should be given first attention. Possible causes on the farm are (a) dust contamination, (b) dirty cows, (c) filth and moisture in air lines, (d) casein in milking utensils, (e) cracked or porous rubbers, (f) cracks, rust, or dents in metal parts and (g) milk cooler not properly functioning. Suggestions were offered for correction of faulty conditions on the farm.

From the standpoint of the milking machine manufacturer. L. E. Bober, Babson Bros. Co., Chicago, Ill. Pp. 61–66. Unfortunately for the solution of the thermoduric problem, there has never been close agreement within the industry and the colleges as to what constitutes good milk machine sanitation. In our survey of 1944–1945 involving 46 farms, 8 of the 10 farms with the highest quality rating scrubbed every part of their milkers at least once a day; of 120 milk samples taken only 2 had thermoduric counts over 15,000 per ml. The other group of 36 farms flush-washed their milkers. Out of 120 samples of their milk, 69 gave thermoduric counts over 30,000 per ml. It should be apparent that machines should be scrubbed clean.

From the standpoint of the milking machine manufacturer. G. H. Hopson, DeLaval Separator Co., New York, N. Y., Pp. 67–70. The personnel caring for the milking machines is extremely important in obtaining clean machines. Some dairymen always desire and try to keep equipment and product clean, some will cooperate when told and shown, but others are chronic offenders. It would appear that an incentive to produce clean milk based upon premium for quality would be very helpful. A. C. Dahlberg

151. Thermoduric microflora of dairy utensils. S. B. Thomas, E. Jones-Evans, L. B. Jones and B. F. Thomas, Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., **1946,** 1: 51–53. 1946.

Farm milking utensils were examined by a rinse technic after sterilization by various methods. In order of bactericidal effectiveness, steam was best, followed by hypochlorite treatment, while washing with warm water was the poorest. Laboratory pasteurization of the rinses showed that the micro bacteria, micrococci, and spore-forming rods were the predominant types found after steam and hypochlorite treatments. Bacteria in pasteurized rinses from utensils washed with warm water were 98% microbacteria. Scrapings of milk-stone from certain utensils showed the presence of micrococci, microbacteria, spore-forming rods and streptococci in much smaller numbers. M. L. Speck

152. Further studies of thermoduric bacteria in milk. D. A. McKenzie, M. Morrison and J. Lambert. Dept. of Agr., Univ. of Leeds. Proc. Soc. Applied Bact., **1946,** 1: 37–39. 1946.

This study was to determine the types of thermoduric bacteria present in farm milk cans, which were found to be a very important source of such bacteria in raw milk. Counts were made on laboratory-pasteurized can rinses and plates were incubated at 30° C. The types found to be present in a total of 654 statistically picked colonies were: microbacteria, 73.1%; micrococci, 10.2%; streptococci, 2.7%; miscellaneous (gram-negative and filamentous rods), 13.9%. M. L. Speck

153. Heat resistant bacteria in farm water supplies. S. B. THOMAS AND M. ROBERTS, Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., **1946,** 1: 44–46. 1946.

A series of 116 farm water supplies was examined for thermoduric and thermophilic bacteria. Thermoduric counts exceeding 100 per ml. were found in 21% of the samples and none was free of these bacteria. Classification of 342 thermoduric colonies selected at random showed the presence of the following: bacilli, 74%; micrococci, 9.1%; actinomycetes, 8.2%; gram-negative rods, 3.8%; yeasts, 2.6%, microbacteria, 2.0% and streptococci, 0.3%. Thermophiles were present in 21% of the samples, but only 2.5% contained more than 10 per ml. M. L. Speck

154. Psychrophilic bacteria in raw and commercially pasteurized milk. S. B. THOMAS AND C. V. CHANDRA SEKAR, Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., 1946, **1:** 47–50. 1946.

Incubation at 3–5° C. required 21 d. to give maximum counts of psychrophiles on yeastrel agar. Raw milk and pasteurized milk both contained psychrophiles, although raw milk laboratory pasteurized at 63° C. for 30 min. showed no survival of these bacteria. Also, none of the pure cultures isolated from raw milk survived this pasteurization. Of 203 psychrophiles isolated from raw and pasteurized milk, 98% were gram-negative rods and the remainder were yellow micrococci. M. L. Speck

155. Studies on starters. V. Effect of pathological or physiologically abnormal milk on acid production by lactic acid streptococci. E. B. RICE. Dairy Division, Dept. of Agr. and Stock, Brisbane, Queensland. Dairy Ind, **8,** 10: 983. Oct., 1948.

The effect of mastitic milk on acid production by lactic acid streptococci differed to quite an appreciable extent in different experiments. More frequently acid formation appeared to be depressed, but in some samples acid developed to the same extent in the normal and in the mastitic milk. Even in the milk after pasteurization slower growth occurred in some samples, showing that the milk itself, and not the growth of the organisms which are heat labile, was responsible.

Development of the lactic streptococci in late lactation milk was appreciably slower than in milk from cows in full lactation. This is attributed in some measure to the high leucocyte content of late lactation milk.

Colostrum itself and milk to which it is added at the rate of 10% or more promotes the acid forming capacity of starter streptococci when incubated at a constant temperature. This probably is associated with the high buffer value of colostrum. G. H. Watrous

156. The lactic acido-proteolytic bacteria and the genotypicity of the bacterial enzymes. CONSTANTINO GORINI. Enzymologia, **12,** 2: 82–87. April, 1947.

The characteristics of acido-proteolytic bacteria and especially the variations in their enzyme systems are reviewed. The possibility that enzyme variation patterns may aid in the classification of these organisms is advanced. F. E. Nelson

157. The activity of certain cationic germicides. G. J. HUCKER, R. F. BROOKS, DOROTHEAE METCALF AND WM. VAN ESELTINE. N. Y. Agr. Expt. Sta., Geneva. Food Tech., **1,** 3: 321–344. July, 1947.

The germicidal action of 13 compounds was investigated against *Escherichia coli,* capsulated and non-capsulated strains of *Aerobacter aerogenes, Micrococcus aureus, Streptococcus cremoris, Bacillus subtilis* and three spore-forming flat sour organisms. One ml. of the germicide solution of 10 × the final concentration desired was added to 9 ml. of broth containing approximately 500 million organisms per ml., then the time required for complete killing was determined. A wide variation existed in the relative germicidal efficiency of the cationic germicides studied. The individual organisms showed considerable variation in resistance to the cationics. In general, *E. coli* was more resistant than *A. aerogenes,* while the non-capsulated strain of *A. aerogenes* usually was much less resistant than the capsulated strain of this organism. The cationic germicides were effective in killing bacterial spores, but a much greater concentration was required than for killing vegetative cells. The detergents studied showed no corrosive action on the metals commonly used in foods and dairy processing plants. E. R. Garrison

158. A study of the germicidal value of glycols, glycol benzoates and related compounds. H. C. HEIM AND C. F. POE, Univ. of Colorado, Boulder. Food Tech., **2,** 1: 23. Jan., 1948.

Ethylene glycol, diethylene glycol, propylene glycol and trimethylene glycol gave phenol coefficients that varied from 0.0 to 0.021, while the six benzoic acids studied showed phenol coefficients that ranged from 0.0 to 7.8 when tested against *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) and *Eberthella tyhpi* (*Salmonella typhosa*). The bactericidal action of 46 derivatives of benzoic acids towards *Escherichia coli* and *Aerobacter aerogenes* was studied.

Enough of each chemical was added to lactose broth to produce saturation, then a fermentation tube of the saturated broth was inoculated with the test organism. The tubes were incubated at 37° C. and examined for growth and gas production after 12, 24, 48 and 72 hr. Most of the compounds studied failed to show any marked bactericidal properties, but the benzoates and salicylates of methyl and ethyl alcohols were very good inhibitors. E. R. Garrison

159. A technique for studying resistance of bacterial spores to temperatures in the higher range. C. R. STUMBO, Food Machinery Corp., San Jose, Calif. Food Tech., **2,** 3: 228–240. July, 1948.

A heat exchanger (thermoresistometer) was designed and used to determine the thermal resistance of bacterial spores. The errors due to temperature lag largely were eliminated by rapidly heating the inoculated medium to process temperature and subsequently cooling rapidly to a non-lethal temperature.

Approximately 0.01 ml. of food and 0.01 ml. of spore suspension were mixed together in a small tin cup, the sample cup was placed in an aluminum boat and both cup and boat were exposed in a steam-heated chamber for the time desired. At the end of the prescribed time, the chamber was automatically exhausted by means of an electric clock with two micro switches and the boat, cup and sample were drawn into a tube of culture medium. The culture containing the heated spores then was covered with a layer of sterile vaseline-paraffin mixture and incubated at a suitable temperature to determine sterility. The temperature of the heating chamber could be controlled to within 0.3° F. of the temperature desired and the heating time could be reproduced with an accuracy of ± 0.001 minute.

The method was developed for accurately determining thermal resistance of bacterial spores to temperatures of 240 to 270° F. but it also is applicable for use at any temperature between 215 and 270° F. A trained technician can process as many as 216 samples in an 8-hr. day by this procedure. E. R. Garrison

Also see abs. no. 141.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

160. Apparatus for breaking and making emulsions. C. E. NORTH AND A. P. NORTH. U. S. Patent 2,455,945. 9 claims. Dec. 14, 1948. Official Gaz. U. S. Pat. Office, **617,** 2: 433. 1948.

Cream can be converted into butter, and milk and cream can be reconstituted from milk fat and dry non-fat milk solids by passing through this equipment. Both processes are accomplished continuously in castings in which specially designed agitators rotate. Emulsions are broken by the use of dished disc-shaped blades, while emulsions are formed by the use of perforated paddle-shaped agitators. R. Whitaker

161. Cream separator and homogenizer. J. B. MCFADDEN. (assigned to United Dairy Equipment Co.). U. S. Patent 2,453,924. 5 claims. Nov. 16, 1948. Official Gaz. U. S. Pat. Office, **616,** 3: 740. 1948.

In a combination separator-homogenizer, whole milk is separated by centrifugal force into skim milk which is immediately discharged and cream which is homogenized by forcing it against vertical surfaces on overlapping rotating blades. R. Whitaker

162. Centrifugal separator. H. O. VOGEL (assigned to International Harvester Co.). U. S. Patent 2,456,347. 9 claims. Dec. 14, 1948. Official Gaz. U. S. Pat. Office, **617,** 2: 535. 1948.

A self-washing cream separator bowl is described. When it is desired to clean the bowl, the washing liquid is admitted to the bowl and directed through the disks by a distributor tube in such a manner that all surfaces are flushed by the cleaning fluid. The side walls of the bowl slope toward a series of outlets or ports through which the cleaning liquid escapes, carrying the milk and slime residues. The ports are open at reduced speeds and closed by means of valves which are operated by the centrifugal force at normal bowl speeds. R. Whitaker

163. Methods used by the dairy industry to improve sanitary control through equipment design. Dairy products and liquid foods. E. H. PARFITT, Sanitary Standards Subcommittee of the Dairy Industry Committee, Chicago, Ill. Food Tech., **2,** 1: 39–44. Jan., 1948.

The development of the dairy industry's interest in equipment design is reviewed. The sanitary standards approved for equipment used in all branches of the dairy industry are now arrived at by the cooperative action of three committees appointed by the Dairy Industry Committee, the International Assoc. of Milk Sanitarians and the U. S. Public Health Service. These three agencies by joint action are endeavoring to standardize the design of dairy equipment, thereby making parts uniform and interchangeable and increasing the ease with

which the equipment can be cleaned, drained and inspected. The work accomplished to date includes the development of sanitary standards for 37 fittings for milk pipes and sanitary standards for milk storage tanks, milk pumps, weigh cans and drop tanks for raw milk and homogenizers. The work in progress includes the development of standards for milk transportation, tanks, milk pails and strainers, milking machines, electric motors, can washers and heat exchangers. E. R. Garrison

164. 100% Freon plant. ANONYMOUS. Ice Cream Rev., **32,** 6: 32, 67. Jan., 1949.

The Euclid-Race Ice Cream Co. in Euclid, Ohio, is the first ice cream plant in the United States using Freon-12 exclusively as the refrigerant. The plant is equipped with two 50 h.p. and two 30 h.p. compressors. These supply refrigeration for the operation of two 150-gal./hr. continuous freezers, a 24-mold brine tank rated at 176 doz. novelties per hr., two 500-gal. mix storage vats, a sweet water cooler, a hardening room maintained at – 20° F. with a capacity of 55,000 gal. of ice cream and a refrigerated storage room for the storage of flavors and raw materials.

Advantages claimed for the use of Freon-12 over ammonia include: (a) greater safety, (b) saving in the amount of space required for the compressors, (c) power economy, (d) maintenance economy through the use of compact equipment and non-corrosive copper lines and (e) water economy. W. J. Caulfield

165. Heating rates of food in glass and other containers. D. G. MERRILL. Hartford-Empire Co., Hartford, Conn. Ind. Eng. Chem., **40,** 12: 2263–2269. Dec., 1948.

An empirical formula of general application is given for the approximate heating rate of cylinders. Data from confirming experiments are given. Constants are derived for application to the heating of food in glass containers so that the established methods for computing sterilization process time in tin may be extended to glass. B. H. Webb

166. Use and care of water softeners. ESKEL NORDELL, The Permutit Co., New York, N. Y. Ann. Rpt. N. Y. State Assoc. Milk Sanit. 21: 111–121. 1948.

The hardness of dairy supply water is very objectionable because it tends to form scale and sludge when heated or concentrated, or upon addition of alkaline washing compounds. Scale is unsightly and difficult to sterilize. Insoluble granulate zeolites soften water by the "base exchange" method. Calcium magnesium, and ferrous iron are removed and replaced by sodium ions. After the zeolite has removed the maximum capacity of hardness, it is necessary to automatically or manually regenerate the system by backwashing, salting and rinsing.

A. C. Dahlberg

Also see abs. no. 173, 181, 182.

DAIRY PLANT MANAGEMENT AND ECONOMICS

167. The future of milk consumption. L. SPENCER, Cornell Univ. Milk Dealer, **38,** 2: 50–62, 86–87. Nov., 1948.

The outlook for milk consumption generally is favorable. The two factors that will have most influence upon future trends of milk consumption are changes in population and fluctuations in consumer incomes. The long-time trend appears to be in the direction of fewer children and more old people, so there is need for more emphasis on the value of milk in the diets of adults, especially those past middle age. During the past few years consumer incomes and purchasing power have been greater than ever before. This is the main reason for the unprecedented rate of milk consumption per capita. The foreign-aid program and large military expenditures are expected to take up any slack in business activity that might otherwise appear during the next year or two. Business forecasts beyond that point are extremely hazardous, but there is good reason to believe that any major depression such as was experienced in the early 1930's would be prevented by government actions of various kinds. Loss of purchasing power by substantial numbers of people whose incomes fail to rise with the cost of living will have a depressing effect upon milk sales if inflation continues.

Other important but minor factors in the outlook for milk consumption are retail prices of milk, the maintenance of satisfactory service and quality, consumer acceptance of milk as a food and the consumer's attitude toward the milk industry. Much more effort should be made to inform consumers about prices, costs and profits in the industry as well as about the food value of milk. C. J. Babcock

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

168. Carotene retention in alfalfa meal. Effect of blanching, packaging, and storage tempera-

ture. W. L. Nelson, J. K. Loosli, G. Lofgreen and N. Yager. Ind. Eng. Chem., **40,** 11: 2196–2198. Nov., 1948.

The blanching of alfalfa before dehydration resulted in the retention of appreciably more carotene in the dried meal. Samples so treated contained an average of 164 μ of carotene per g. of dry alfalfa meal as compared with 43 μ for similar samples of unblanched alfalfa. Blanching pretreatments had no influence on the retention of carotene during storage. The use of moisture vapor-proof laminated foil bags was less effective in preserving carotene either at room temperature or at 40° F. than were cloth or paper bags. The addition of calcium oxide to maintain low moisture in the samples appeared detrimental to carotene retention. Alfalfa meals stored at 40° F. in cloth or paper bags showed a marked rise in moisture content during storage periods up to 6 mo.

B. H. Webb

Also see abs. no. 170.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

169. Experience with pen stables. F. W. Graves, N. Y. Dept. of Health, Albany. Ann. Rpt. N. Y. State Assoc. Milk Sanit., **21:** 29–34. 1948.

Most dairymen with pen stables are pleased with them. The cows remain cleaner and have less udder trouble, fewer leg and shoulder injuries, and fewer rheumatic ailments. Cows in heat are observed more easily. There is a saving in labor for cleaning barns and in feeding.

Unfavorable experiences include too little bedding, resulting in unclean animals. There ought to be at least 10 ft. of head room and at least 70 ft.^{2} of floor space for a Jersey and small Guernsey cow, 80–90 ft.^{2} for a medium-sized cow and 100 ft.^{2} for a large Holstein, Brown Swiss or Shorthorn cow. It sometimes is difficult to remove enough posts in remodeled barns to permit the use of tractors and power forks. Cows with horns cannot be stabled safely in pen barns. The pen barns do not display individual cows to advantage.

Today there are 20 pen stable barns in operation in N. Y. State and more have been approved. The quality of the milk has not been affected by pen stabling, but the number of tests is too small for a final conclusion.

A. C. Dahlberg

170. Calf feeder. C. I. Young. U. S. Patent 2,455,848. 3 claims. Dec. 7, 1948. Official Gaz. U. S. Pat. Office, **617,** 1: 246. 1948.

A bottle with a nipple is supported in a cage which is hinged on the wall to facilitate removal.

R. Whitaker

171. Overhead support for milkers. H. B. Babson (assigned to Babson Bros.). U. S. Patent 2,454,300. 11 claims. Nov. 23, 1948. Official Gaz. U. S. Pat. Office, **616,** 4: 986. 1948.

A stall with an overhead framework designed to support a mechanical milker in position beneath the cow is described. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

172. Liquid sugar in food products. W. R. Junk, O. M. Nelson and M. H. Sherrill. Calif. and Hawaiian Sugar Refining Corp., Ltd., San Francisco, Calif. Food Tech., **1,** 4: 506–518. Oct., 1947.

Some of the more useful published data in the literature together with some original unpublished material of the authors on invert sugar are presented in this paper . Tables and graphs showing weights per gal., refractive indices, viscosities, boiling points, freezing points and other information about sugar solutions are given. To obtain a minimum of color in the finished syrup, the sugar solution should be inverted at the lowest practical temperature and neutralization of the acid in the inverted syrup should be delayed as long as possible before cooling is completed.

E. R. Garrison

173. Mix cooling—a problem in small production units. E. Humphriss, A.R.I.C. Dairy Ind., **8,** 10: 1021–1022. Oct., 1948.

The Ice Cream Regulations (England), 1947, regarding heat treatment, etc., require that all ice cream mix be cooled to 45° F. or lower within 1.5 hr. after pasteurization and held there or below until frozen. Suggestions for mechanical cooling are given, especially for the small manufacturer. Documentary evidence that cooling apparatus is in order constitutes a successful defense against accusations that the Heat Treatment Regulations are not being complied with until May, 1949. This reflects the difficulty in England of securing dairy equipment.

G. H. Watrous

174. Selling ice cream through vending machines. Claron Burnett, Ice Cream Review. Ice Cream Rev., **32,** 6: 34, 66. Jan., 1949.

Factories may prove to be one of the most

important outlets for ice cream cups and bars sold through vending machines. In one group of Milwaukee, Wis., plants, ice cream vending machines have increased sales and have resulted in less interruption in work schedules than when ice cream was sold by vendors passing through the plants. At these plants 25 vending machines for 10¢ chocolate-coated bars are in current operation with 50 more machines to be installed this spring. The installation of one machine for each 200 persons is considered profitable. Daily sales per unit have ranged from $12 to $20. When the number of units is increased to 75 it is estimated that the total sales will average approximately $1,500 per day.

Electric current consumption for the ice cream vending machine was lower than for soft drink vending machines, because the ice cream has been hardened before it is placed in the machine. Electric current for the operation of the machines is furnished by the industrial plants. One cent from each ice cream bar sold goes into an employee fund and the remaining receipts are retained by the company which operates and services the vending machines.

W. J. Caulfield

175. Packaged sundae. F. T. Mosher. U. S. Patent 2,435,094. 1 claim. Jan. 27, 1948. Official Gaz. U. S. Pat. Office, **606,** 4: 627. 1948.

Ice cream from the freezer is placed in a small carton, covered with a layer of fruit or other flavored heavy syrup and closed. The packaged sundae then is hardened while inverted.

R. Whitaker

176. Ice cream scoop. J. J. Deutsch and J. Vigilante. U. S. Patent 2,454,735. 7 claims. Nov. 23, 1948. Official Gaz. U. S. Pat. Office, **616,** 4: 1094.

A wiper, pivotally mounted in a hemispherically-shaped bowl, is made to rotate by rotating a sleeve on the handle of the dipper.

R. Whitaker

Also see abs. no. 164.

MILK

P. H. TRACY, SECTION EDITOR

177. Recent amendments to the U. S. Public Health Service milk code. A. W. Fuchs, U. S. Publ. Health Service, Washington, D. C. Ann. Rpt. N. Y. State Assoc. Milk Sanit., **21:** 135–150. 1948.

During the war the 1939 edition of Public Health Bull. 220 remained in effect and a special committee was appointed in 1947 for the purpose of revision. Provisions of the sanitary code were made enforceable by degrading and by court suspension. Considerable material that was not actually public health was removed from the text and placed in the appendix. Several changes were made in computing sanitary ratings. The code provides for emergency sale of ungraded milk, labelling of Vitamin D milk, and bacterial and temperature standards. Several changes have been made in bacterial standards, including a maximum coliform count of 10 per ml. on pasteurized milk.

All revisions were published in a tentative report in 1947 and the final code will be published in 1948. A. C. Dahlberg

178. Let's sell more fat-free milk. W. L. Foust, Warren Sanitary Milk Co., Warren, Ohio. Milk Dealer, **38,** 1: 44, 80. Oct., 1948.

A description is given of Vitolac, a low-fat milk prepared by concentrating skim milk under high vacuum and low temperature until the total solids are roughly 12.5%. After standardizing to 1.5% B.F., 2,000 units of vitamin A and 400 units of vitamin D are added. The product tastes richer and slightly sweeter than regular milk. A table is presented which shows the comparative nutritional values of milk and Vitolac. C. J. Babcock

179. The influence of conditions of cold storage prior to distribution on the keeping quality of pasteurized milk. G. M. Phillips, Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., **1946,** 1: 40–42. 1946.

Duplicate samples of milk were taken from the filler, subjected to the 0.5-hr. methylene blue and keeping-quality tests. Then one sample was stored in a laboratory refrigerator at 36–43° F. and the other in a cold storage room in which the temperature rose to 50–60° F. on the afternoon of bottling but was 39–43° F. on the following morning. After 24 hr. storage the same 2 tests again were performed. Those stored in the laboratory refrigerator all were satisfactory by the methylene blue test and only 2.3% had a keeping quality less than 48 hr. Of those stored in the cold storage room, 30% reduced methylene blue in less than 0.5 hr. and 57% had a keeping quality of less than 48 hr. A similar deterioration of quality was observed when the milk was cooled to only 44–50° F. before bottling, although subsequent storage was at 36–43° F. M. L. Speck

180. Visual conditions on the farm as an indication of the keeping quality of milk. D. A. McKenzie and D. A. Bowie. Dept. of Agr., Univ. of Leeds. Proc. Soc. Applied Bact., **1946,** 1: 35–36. 1946.

A study was made of the bacteria present in

milk and on the equipment of farms having adequate and inadequate facilities for the normal production of high quality milk. The "poor type" farms selected for study consistently produced good milk, and the "good type" farms consistently produced poor milk, as judged by the resazurin reduction tests and keeping quality tests. Milk and swabbings of equipment from the "poor type" farms showed a predominance of micrococci and gram-negative, alkali-producing rods which had little effect on the tests used. Similar samples from the "good type" farms showed a predominance of streptococci and gram-negative, acid-producing rods which have marked influence on the tests used. These differences were believed to be caused by the greater opportunity for contamination offered on the "good type" farms by the more elaborate equipment used. M. L. Speck

181. Types of farm milk coolers. R. C. SHIPMAN, United Coop. Lab., Ithaca, N. Y. Ann. Rpt. N. Y. State Assoc. Milk Sanit., **21:** 165–183. 1948.

This discussion applies to cooling milk in cans. This method is most generally used on small and average-sized dairy farms. Thermocouples were placed at various positions in the milk and in the water in the cooling tank. Cooling was relatively slow in milk placed in unagitated water, especially the top milk in the can if the cooler were only partially filled. Agitation of the water for an hour gave prompt cooling below 50° F., providing the water level was sufficiently high. A. C. Dahlberg

182. Spray cooling apparatus for milk cans. D. DONNELLY. (assigned to Universal Milking Machine Co.). U. S. Patent 2,455,162. 15 claims. Nov. 30, 1948. Official Gaz. U. S. Pat. Office, **616,** 5: 1354. 1948.

Freshly-drawn milk in cans is cooled by placing the cans in an insulated tank containing water cooled by submerged expansion coils. A pump circulates the water to a pan which delivers it to the neck portions of the milk cans. R. Whitaker

Also see abs. no. 148, 149, 150, 152, 153, 154, 161, 167, 182, 187.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

183. Studien über den Mechanismus der Milchbildung. (Studies concerning the mechanism of milk secretion.) English summary. W. FRITZ. Die Milchwissenschaft, **3,** 3: 65–75; **3,** 4: 97–102. 1948.

After obtaining evidence against the 2-phase milk secretion theory, the author investigated the 1-phase theory and was able to determine the rate of milk secretion in a lactating animal. Milk cows during their 2nd to 3rd month of lactation showed the highest rate of milk secretion per hr. during the 4th hr. from time of previous milking, whereas cows during their 7th to 10th mo. showed the highest rate during the 7th hr. Assuming the maximum milk-holding capacity of the secretory glands and the maximum pressure exerted upon the glands to be constants, the secretory glands held 42% of the milk obtained in one milking.

The rate of milk secretion per lactation period with 2 or 3 milkings daily when plotted on a graph follows the curve of a quarter ellipse. Immediately after each milking the rate of secretion starts from zero, no matter what the rate was at the onset of milking. Total milk yield per lactation period was highest with 4 milkings daily, as compared with 2, 3 or 5 milkings, provided that the animal is healthy, milk secretion is not disturbed, abundant feed is available and a normal lactation period of 300 d. is practiced. Evidence was obtained that 4 hr. is the minimum filling time of the secretory glands. Milking only twice daily reduces total milk yield per lactation period by 30 and 34% as compared with 3 and 4 daily milkings, respectively. For optimum milk yield per lactation period, 4 milkings daily should be practiced for the first 5 mo., followed by 3 milkings for the next 4.1 mo., and reduced to 2 milkings from then on unless the lactation period is to terminate in 10 mo., in which case only 1 milking daily should be carried out for the last 27 d.

A decrease in feed consumed by an animal results in a directly proportional decrease in milk yield if the cow is milked 3 times daily; at 4 milkings daily the decrease in milk is more pronounced and at 2 milkings less pronounced than at 3 daily milkings, indicating in the last case that substances are withdrawn from the animal body which may weaken the latter and impair its health.

The article presents (a) derivations of formulas for calculating rate of milk secretion at different stages of lactation, (b) milk yield variation with frequency of milking, (c) effect of subnormal feeding and of irregular milking intervals upon milk yield. I. Peters

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

184. Milk in human nutrition. C. P. SEGARD. Wisconsin Alumni Research Foundation. Milk Dealer, **38,** 3: 114–118. Dec., 1948.

The four great discoveries in the field of hu-

man health within the past century are: (a) discovery of anesthetics, (b) discovery of antiseptics, (c) cause and control of epidemics and (d) the vital importance of nutrition in the prevention of disease.

Milk contains the four basic groups of nutritionally important substances, namely carbohydrates, proteins, fats and minerals and vitamins. The most important mineral element needed by the human body is calcium. The growing child needs fully one-fourth of a teaspoonful of calcium daily, and the adult about one-third less. Milk is the only food taken in average serving quantities that can supply this amount. Iodine, copper, iron, manganese, magnesium and others also are needed and are present in milk. Milk sugar or lactose, which accounts for about 30% of the energy value of milk, is in readily assimilable form. As each new vitamin has been discovered, its presence in milk has been shown. The cream, or milk fat, is not only an important energy food but it is the only fat, with the exception of fish oils, that contains four vitamins. Milk may be fortified with vitamins, and the product most recently introduced is fat-free milk fortified with vitamins A and D. C. J. Babcock

Also see abs. no. 178.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

185. Detergents for dairy plants and methods of their evaluation. H. G. HARDING AND H. A. TREBLER. Natl. Dairy Research Labs., Inc., Baltimore, Md. Food Tech., **1,** 3: 478–493. July, 1947.

The important requirements of a good dairy cleaner are enumerated and the general characteristics of several washing compounds are discussed. Alkaline detergent mixtures for dairy plants should contain sufficient polyphosphate to prevent precipitation of the calcium, magnesium and iron salts from the water and milk residues, sufficient wetting agent to promote contact between the soil on the equipment and the wash solution and sufficient aklalinity to dissolve denatured protein residue. The most desirable composition of the dairy cleanser will vary with a number of factors, particularly with the type and amount of soil to be removed from the equipment and with the cost of the different chemicals. A method is given for calculating the composition of detergents for specific uses. Graphs showing the influence of hardness (from soil and water) on the composition and cost of recommended cleaning solutions are given.

Tests for pH, alkalinity, surface tension and turbidity-preventing power under simulated plant conditions are recommended to indicate performance quality of detergents.

E. R. Garrison

186. Modified non-ionic synthetic detergents and quaternary ammonium compounds as cleaner sanitizers in food and dairy operations. G. J. HUCKER, N. Y. Agr. Expt. Sta., Geneva. Ann. Rpt. N. Y. State Assoc. Milk Sanit., **21:** 35–52. 1948.

Studies for the past few years at Geneva have been focused on the development of a mixture of compounds that would efficiently clean and sterilize in one operation with cold water. Only the non-ionic synthetic detergents with quaternary ammonium compounds offer possibilities of success. Non-ionic detergents without agitation removed about 50–60% of the soil from aluminum or glass; with agitation about 70% was removed, but when modified by the addition of special compounds, 100% of the soil was removed. When the soil was dried on the hard surface, then removal was difficult.

Selected quaternary ammonium compounds were effective sterilizers without odor. They were effective in the cleansing solution against the usual test bacteria, including *Pseudomonas fluorescens* which often has been found so difficult to kill. The unmodified non-ionic detergents and quaternaries were not effective, however. The modified non-ionic detergent-quaternary solution was used as a cleaner-sterilizer for milking machines. "Dairymen were instructed at the premises to draw three gallons of the mixtures through the machine once daily and also once daily to use a brush on all rubber parts." The milking machines were clean and the milk gave low total and thermoduric bacterial counts. A. C. Dahlberg

187. Cleaning and sterilizing milk tank trucks. J. R. PERRY, Sealtest Inc., New York. Ann. Rpt. N. Y. State Assoc. Milk Sanit., **21:** 95–110. 1948.

The problem of cleaning and sterilizing may be grouped as (a) cleaning and sterilizing facilities and equipment, (b) cleaning and sterilizing procedure, (c) sanitary control, (d) construction of tanks and (e) design of tanks. Details of the cleaning and sterilizing procedures are presented. Sealtest has developed several aids in the cleaning operation. These include water mechanically controlled at 115° F., light, flexible hot water hose, control of the water pressure, an automatic hose water-shut-off valve, and a

special washing solution-fed brush. A portable cleaning solution tank has been developed to maintain the solution at 115° F. and under constant pressure. A special tank with rotary brush has been developed for washing sanitary fittings and pipes. A special milk tank truck has been constructed which should simplify cleaning.

A. C. Dahlberg

188. Restaurant sanitation. GEORGE WEST, Rochester, N. Y., Health Bureau., Rochester, N. Y. *Ann. Rpt. N. Y. State Assoc. Milk Sanit.*, **21:** 157–164. 1948.

The significance of food in outbreaks of disease is shown by statistics of the U. S. Publ. Health Service from 1938–1945, inclusive, showing in the United States that there were 12,102 individual cases of illness due to milk, 111,839 due to water, and 57,591 due to food. Over half of the food-borne illness occurred from public eating places. The author discussed the problem of regulations prescribing proper sanitation and clean food for public eating places.

A. C. Dahlberg

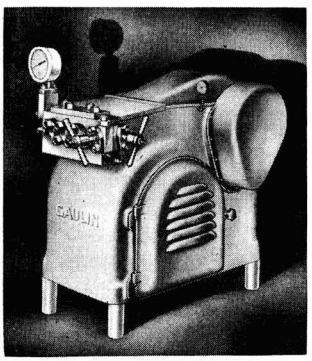
Also see abs. no. 151, 157, 158, 163, 166, 180.

MISCELLANEOUS

189. Symposium on food technology. Ind. Eng. Chem., **40,** 12: 2241–2257. Dec., 1948.

Four excellent review papers are presented on different phases of food technology as follows: Sterilization of Foods, J. M. Jackson and H. A. Benjamin, Amer. Can Co., Maywood, Ill., pp. 2241–2246; Processing Edible Fats, Warren H. Goss, Pillsbury Mills, Inc., Minneapolis 2, Minn., pp. 2247–2251; Frozen Food Industry, Clifford F. Evers, Natl. Assoc. of Frozen Food Packers, Wash., D. C., pp. 2251–2253; Measurement of Food Acceptance, E. C. Crocker and L. B. Sjöström, Arthur D. Little, Inc., Cambridge, Mass., and G. B. Tallman Mass. Inst. of Tech., Cambridge, Mass., pp. 2254–2257.

B. H. Webb



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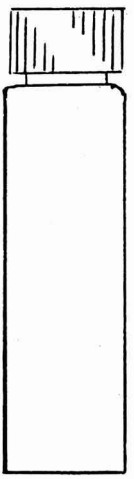

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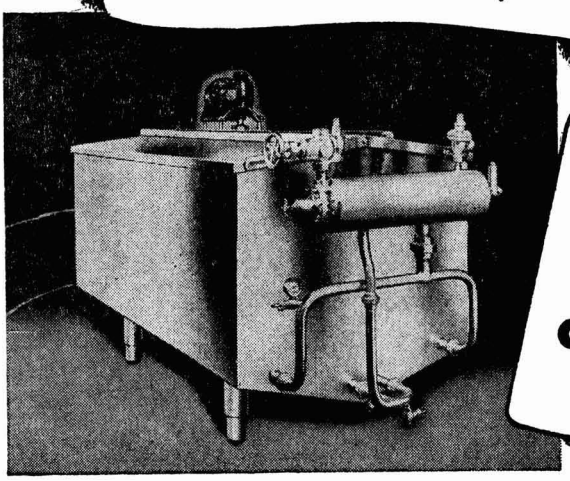

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