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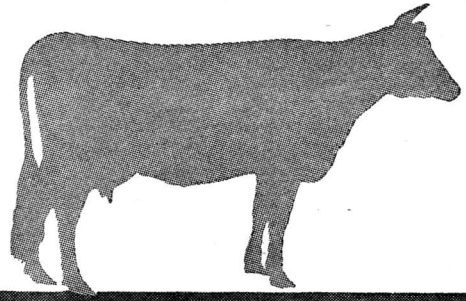

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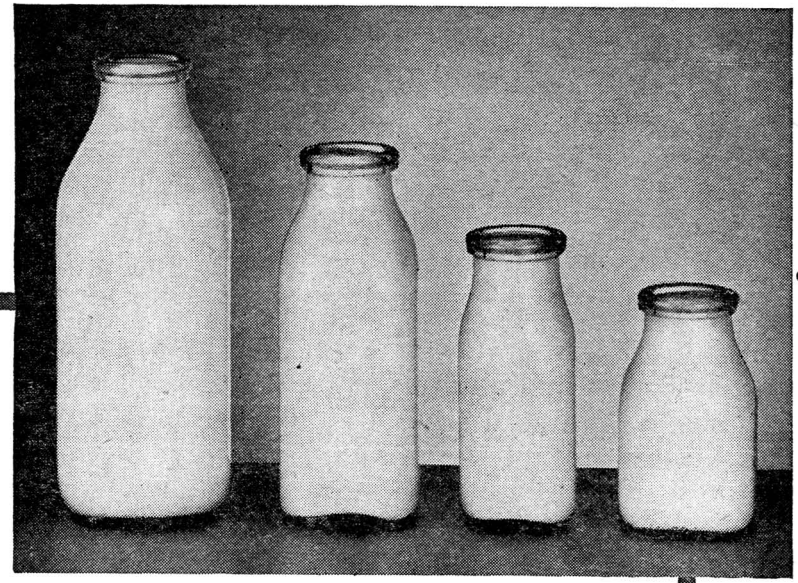

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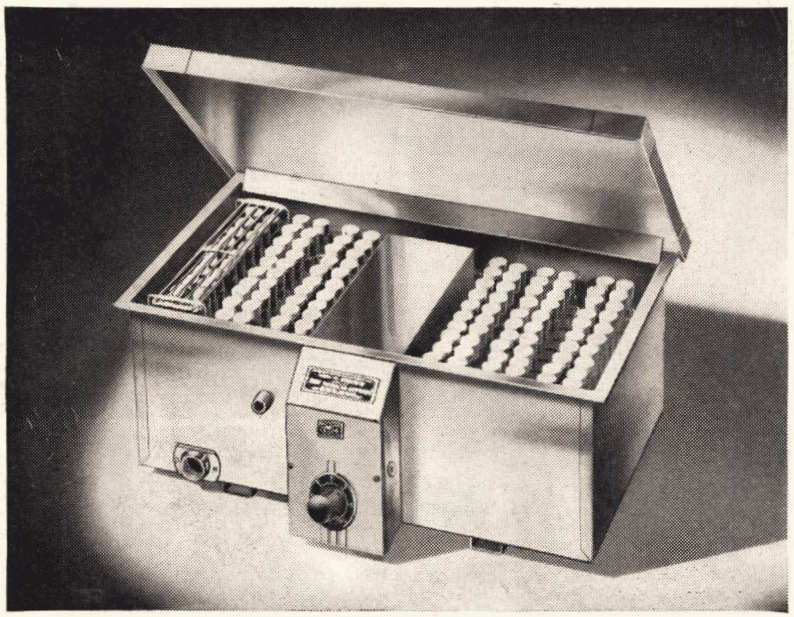

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JOURNAL OF DAIRY SCIENCE

VOLUME XXVIII NOVEMBER, 1945 NUMBER 11

THE EFFECT OF PITOCIN ON MILK LIPASE*

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The author (1) is publishing data indicating that milk lipase appears to hydrolyze tributyrin and the other volatile triglycerides at a much more rapid rate than it does the longer chain triglycerides. There also appears to be some association of the oestrogenic cycle in open cows and milk lipase activity (1). A review of previous literature is presented in that paper. Unpublished data have been obtained which show a relationship between pitocin and bovine mammary gland tissue.

In this study the data were obtained with two fundamental objectives. The first was to learn if pitocin also affected milk lipase. If there was such a relationship, the second objective was to study the effects of the hormone on some of the triglycerides found in milk fat to learn if all were affected in a similar manner.

PROCEDURE

The method of drying and defatting the milk with acetone and ether, and carrying out of the analyses has been described (1). The pitocin used was a Parke-Davis product containing ten International Units per ml. of solution. This was added to approximately one-half of the samples, the others serving as controls. Approximately one-half of each group was incubated at once. The other half was incubated 24 hours before extraction. It had been determined previously that when pitocin was added at the rate of two drops per gram of dry-defatted milk sample made from milk which had previously been boiled to kill enzyme action that there was no increase in titration even at the end of a 72-hour period.

The data collected when tributyrin was the substrate are presented in table 1. The pitocin acts consistently as an activator except for the milk of cow 4 which by organoleptic test developed the strongest rancidity of any samples used. Table 2 shows the effect of pitocin on the other substrates tried.

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The two series with triacetin with normal milk acted in a manner similar to tributyrin. When ethyl-oleate was the substrate, the results varied from series to series. This seemed true also when butter oil was the substrate.

A study of the data in tables 1 and 2 shows that the effects of the pitocin used with the same batch of milk will vary with the substrate. For instance the addition of this hormone to the milk of cow 2 on 12/13/43 activated the tributyrinase but showed no effect on the tricaproninase activity. Its addition to the herd milk of 1/11/44 caused an increase in the hydrolysis of ethyl-

TABLE 1

The effect of pitocin as an activator on the tributyrinase of milk

Source and date	Samples		Pitocin added per gm. dried milk	Ave. difference in titration per gm. dried milk	Kind of milk used
	Non-incubated	Incubated 24 hrs.			
	number	*number*	*drops*	*ml. 0.1 N NaOH*	
Herd milk					
1/3/44	9	10	0	0.00	Normal
	10	10	1	0.17	
Cow 2					
12/13/43	2	2	0	0.15	Normal
	3	3	1	0.29	
2/15/44	3	3	0	0.16	Normal
	3	3	2	0.20	
10/27/43	9	9	0	0.45	Unknown
	10	10	2	0.55	
12/31/42	4	5	0	0.55	Developed rancidity
	2	3	1	0.80	
Cow 3					
3/21/44	2	2	0	0.42	Developed rancidity
	2	3	5	1.09	
3/23/44	3	3	0	0.47	Developed rancidity
	5	3	5	0.51	
3/24/44	3	3	0	0.36	Developed rancidity
	3	3	5	0.75	
Cow 4					
3/31/44	3	3	0	1.15	Developed rancidity
	3	3	5	1.10	
4/ 1/44	3	3	0	1.41	Developed rancidity
	3	3	5	0.97	

oleate but had no effect on tripalmitin. Under similar conditions the herd milk for 1/3/44 showed an increase in tributyrinase activity as contrasted to an actual inhibition of the lipase activity on sterilized butter oil. The milk of cow 3 for 3/21/44, which if allowed to stand undried would have developed rancidity, had its tributyrinase activity increased three times by the added pitocin. This was in contrast to only a slight increase in the lipase activity of the butter oil. The milk from cow 3 two days later showed a slight increase in activity with both tributyrin and butter oil. Cow 4 produced milk that became so rancid it would not churn. Her milk was consistent in that each time the hormone was added it inhibited lipase activity.

TABLE 2

The effect of pitocin as an activator of milk lipase on substrates other than tributyrin

Source and date	Length of incubation period	Samples		Substrate	Pitocin added per gm. dried milk	Ave. difference in titration per gm. dried milk	Kind of milk
		Incubated	Non-incubated				
	hrs.	*number*	*number*		*drops*	*ml. 0.1 N NaOH*	
Herd milk 10/18/43	24	5	11	Triacetin	0	0.07	Normal
		4	10		2	0.17	
Cow 2 2/15/44	24	3	3	Triacetin	0	0.22	Normal
		3	6		2	0.32	
Cow 2 12/13/43	24	2	2	Tricapronin	0	0.04	Normal
		3	3		2	0.03	
Cow 2 10/ 8/44	48	10	10	Ethyl-oleate	0	0.74	Unknown
		10	10		1	0.05	
Herd milk 12/27/43	24	10	10	Ethyl-oleate	0	−0.02	Normal
		11	9		1	0.03	
Herd milk 1/11/44	24	10	10	Ethyl-oleate	0	0.09	Normal
		10	10		1	0.24	
Herd milk 1/11/44	24	10	10	Tripalmitin*	0	0.07	Normal
		9	9		1	0.08	
Herd milk 1/ 3/44	24	6	6	Butter oil	0	0.08	Normal
		5	6		2	0.04	
Cow 3 3/ 6/44	48	7	6	Butter oil	0	0.50	Developed rancidity
		7	8		1	0.54	
Cow 3 3/21/44	24	3	3	Butter oil	0	0.09	Developed rancidity
		3	3		5	0.14	
Cow 3 3/23/44	24	3	3	Butter oil	0	0.12	Developed rancidity
		3	3		5	0.18	
Cow 3 3/22/44	24	3	3	Butter oil	0	0.09	Developed rancidity
Mixed 3/24/44		3	3		5	0.03	
Cow 4 3/31/44	24	6	6	Butter oil	0	0.07	Developed rancidity
		6	6		5	0.03	
Cow 4 4/ 1/44	24	3	3	Butter oil	0	0.11	Developed rancidity
		3	3		5	0.05	
Herd milk 5/22/44	72	5	5	Butter oil	0	0.08	Normal
		5	5		5	0.09	

* 3 per cent concentration of substrate in toluene instead of the usual 20 per cent substrate in ether.

The addition of the hormone appeared to exert a specific effect on the various substrates tried. Sometimes the hydrolysis was activated and sometimes it was inhibited. Although the amount of data was too small to obtain a complete picture, the addition of pitocin appeared to increase the hydrolysis of the short chain triglycerides and to retard that with the longer chain triglycerides and ethyl-oleate.

In order to study this somewhat further, additional series were started with butter oil as the substrate. Some of the samples were extracted with ether in order to obtain the total hydrolysis. Others were placed in distilling apparatus to compare the effect of the hormone on the hydrolysis of the

TABLE 3

The effect of pitocin on the lipolytic hydrolysis of volatile fatty acid as compared to total fatty acid of butter oil

Source and date	Length of incubation period	Samples		Pitocin added per gm. dried or 5 ml. undried milk	Ave. difference in titration per gm. dried or 5 ml. undried milk*		Remarks
		Non-incubated	Incubated				
	hrs.	*number*	*number*	*drops*	*ml. 0.1 N NaOH*		
Herd milk 5/2/44	72	5	5	0	0.043	.....	Normal milk
Mixed 5/9/44		4	5	5	0.074	.....	Dried
		5	5	0	.....	0.082	
		5	5	5	.....	0.087	
Herd milk 5/22/44	72	5	5	0	0.042	.....	
		5	4	5	0.151	.....	
		5	2	0	.....	0.082	Normal milk
		5	5	5	.....	0.300	Dried
Herd milk 5/16/44	24	2	1	0	0.042	.....	Normal milk
		2	1	1	0.045	.....	Undried
		3	3	0	.....	0.10	
		3	3	1	.....	0.12	

* Five ml. of undried milk is not equivalent to 1 gm. of the dried milk.

shorter chain substrates which would be collected in the distillate with its effect on the total hydrolysis which would consist largely of the longer chain triglycerides. The samples of herd milk for 5/16/44 were undried. These were kept in a refrigerator. At the beginning and at the end of the periods 425 ml. of water and 20-ml. aliquots of the milk were placed in distillation apparatus equipped with ground-glass joints. Approximately 400 ml. of distillate were collected. Five-ml. aliquots of the milk were dried with plaster of Paris and placed in the ether extractors to obtain the total hydrolysis. The results are presented in table 3.

In two of the series the hormone did increase the hydrolysis of the volatile fatty acids with only a comparatively slight increase in the total titration. In both, the changes which occurred in the total titrations were almost entirely due to the volatile fatty acids liberated. With the series for 5/22/44

this was not so evident. The volatile fatty acid content of the butter oil was less than 11 per cent of the total fatty acid present, yet in the normal sample this accounted for one-half of the hydrolysis which took place. When the hormone was added, the total lipase activity was increased four times with the volatile fatty acid content still accounting for half of the hydrolysis which occurred.

Data have been reported (1) indicating that when hydrolytic rancidity developed, the shorter chain fatty acids were selectively hydrolyzed. Since pitocin caused a further selective hydrolysis of the same triglycerides, it seemed possible that additions of pitocin to normal milk would produce rancidity. This was not true by organoleptic test in any of the series tried with the normal herd milk although it was detected in one trial for which the milk of cow 2 was used in April, 1944. This cow was producing normal milk but in her previous lactation had produced milk which developed rancidity. The milk normally gave readings from 0.10 to 0.20 ml. per gm. of dried milk which was somewhat higher than those obtained with the herd milk. In all of the series with normal milk, a characteristic flavor and odor did develop.

In five paired series the hormone was added to the milk of cow 3 which developed rancidity on standing. The object of these series was to learn if its addition caused a more rapid or a stronger development of rancidity. When the hormone was added at the rate of 1 ml. per 100 ml. of normal milk these also gave negative results. The judges, not knowing the identification of the samples, chose, with few exceptions those to which the hormone was added as the mildest in the series.

DISCUSSION OF RESULTS

The data show quite a consistent action of pitocin in activating the tributyrinase content of milk lipase. The results, when the longer chain triglycerides or sterilized butter oil were used as the substrates, were unpredictable with some series activated and some inhibited.

The results confirm those of previous studies (1) indicating that forces can act on milk lipase which will cause it to attack individual substrates in butter fat rather than all of those present, to produce a characteristic taste and odor. If this is true there may be other factors which cause the selective hydrolysis of other substrates to produce specific defects of other types.

SUMMARY

1. The addition of pitocin to samples of normal milk and that which developed rancidity was quite consistent in activating the tributyrinase found in both.

2. When the longer chain substrates or butter oil were used, the pitocin might either decrease or increase the amount of the hydrolysis.

REFERENCE

(1) KELLY, PHILIP L. The Determination of Lipase from Milk Extracted with Acetone and Ether. JOUR. DAIRY SCI., 27, No. 8: 675. 1944.

A DIACETINASE IN BOVINE MAMMARY GLAND TISSUE*

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Work at this station with dried, defatted mammary gland tissue indicated that when diacetin was used as a substrate rather than tributyrin in lipase studies the two substrates did not always react in the same manner. This paper is a report on the comparative activity of mammary gland tissue enzymes on diacetin and tributyrin.

METHOD

The method of drying the tissue with acetone and ether and the procedure for carrying out the experiments has been reported (1).

EXPERIMENTAL RESULTS

When the first portion of tissue from the bovine mammary gland 117 was dried with acetone and ether, a small amount of heat was applied with the

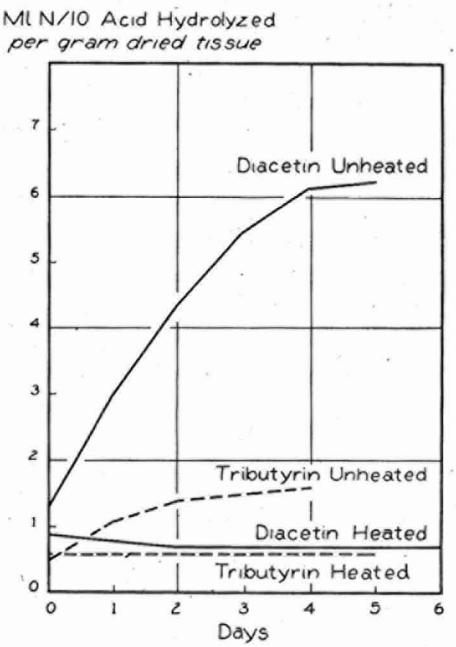

FIG. 1. The comparative enzyme action of the dried tissue of bovine gland 117 with diacetin and tributyrin as substrates.

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idea of facilitating the action of the solvents. When this material was used, however, it was discovered that while the action with diacetin was good, a large series in which tributyrin was the substrate failed to show any action. A second portion of this tissue was dried without heating and was found to be capable of hydrolyzing both substrates. The data for this comparative action are plotted in figure 1.

The unheated samples with diacetin gave a much more active hydrolysis than did those with tributyrin although similar comparisons made with the tissue of glands 113 and 116 did not give as great differences between the substrates, with tributyrin showing the greatest hydrolysis with gland 113.

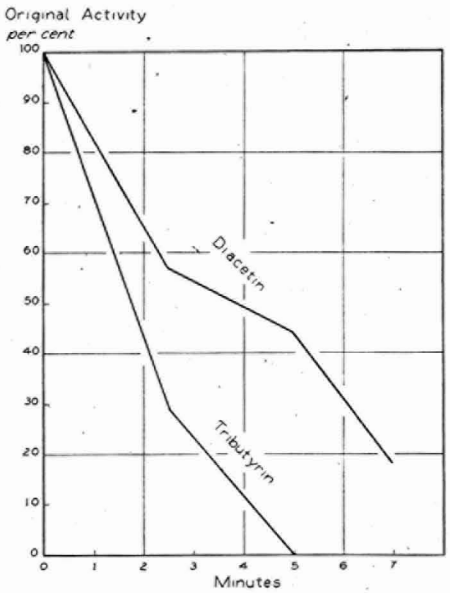

FIG. 2. The effect of boiling in acetone on the destruction of tributyrinase and diacetinase in mammary gland tissue.

Gland 113 was non-lactating while the other two were in active lactation although this may not have accounted for the difference. When the diacetin samples were heated in a water bath to over 80° C. the enzyme action was stopped.

Acetone was added to samples of the dried tissue of gland 117. These were boiled for varying periods in order to find the amount of destruction which would be caused to the speed of hydrolysis of the two substrates. The data are shown in figure 2.

It was possible to heat the tissue to a point at which the tributyrinase was completely destroyed and yet allow a portion of the diacetin activity to continue. This would again indicate that the enzymes attacking the substrates were different, the one being more susceptible to heat than the other.

Pitocin was added to samples containing both substrates to learn if there would be an increased speed of hydrolysis. The hormone increased the hydrolysis with the tributyrin but failed with the diacetin.

DISCUSSION AND SUMMARY

The data obtained indicate that the enzymes which attack diacetin and tributyrin are different even though the structure of the two compounds is somewhat similar.

Since diacetin is not known to be a constituent of blood, the presence of an enzyme in mammary gland tissue which will hydrolize it may not be important. Probably the very active enzyme which was found actually hydrolizes some other compound which is quite similar to diacetin in structure.

REFERENCE

(1) KELLY, PHILIP L. The Lipolytic Activity of Bovine Mammary Gland Tissue. JOUR. DAIRY SCI., 26: 385–399. 1943.

MILK LIPASE ACTIVITY: A METHOD FOR ITS DETERMINATION, AND ITS RELATIONSHIP TO THE ESTRUAL CYCLE*

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Several methods have been outlined for the study of the lipase action that causes rancidity in milk. One of these presented by Rice and Markley (21), and Palmer (18) uses raw milk with a suitable substrate and preservative. Another in common use (6, 7, 9, 22) consists of milk and substrate only, but kept at low temperatures. This allows lipase action but inhibits bacterial growth. In a third, Mattick and Kay (17), Reder (20), and Peterson *et al.* (19) have used a buffer to increase lipase activity and thus shorten the incubation time sufficiently to eliminate the need for a preservative. The preservatives in common use are toluene, formalin, glycerine and the saturation of the substrate with sugar. The usual substrates in the more recent papers have been tributyrin and butterfat. The velocity of lipolytic hydrolysis is usually measured by the changes in free fatty acid content. Several methods have been used to determine the amounts of free fatty acid present. Reder (20) and Peterson *et al.* (19) have titrated the entire sample against sodium hydroxide on the assumption that all of the change in titration is due to lipase activity. Use of the acid degree, a common means of comparison, is determined by churning the fat, melting the butter oil and determining the amount of free fatty acid. Another method developed by Mattick and Kay (17) uses a substrate, such as tributyrin, which contains volatile fatty acids. Their results were obtained by titration of the distillate. Although there were only small amounts of volatile fatty acids in the milk, it was assumed that the action of milk lipase on these would be similar to that of the longer chain fatty acids of which milk fat is largely composed. Tarussuk (24), on the basis of earlier work with Palmer, used surface tension measurements to determine the changes in milk caused by lipolysis.

Fundamentally, even though the problem of rancid milk control is important, knowledge of what causes this defect is still inadequate. The development of rancidity has long been attributed to the hydrolizing action of milk lipase on the triglycerides of milk fat. Of the acids set free, the most important one causing rancidity in butterfat is supposed to be butyric acid. However, the origin of the enzymes which liberate these acids, the number involved, and the way in which they are activated and inhibited are unknown. Without this information, methods may be worked out which are only indirectly related to the development of rancidity.

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[1] Now at South Dakota State College.

Several lipolytic enzymes may eventually be found in milk. These might originate in the milk itself, come from the blood serum, or come from mammary gland tissue. Some milk constituents are derived directly from blood serum. While no work on bovine blood lipase is known, Cherry and Crandall (1) have used determinations of serum lipase to establish pancreatic injury in humans, since a large portion appears to be of pancreatic origin. However, there are other sources of blood lipase. Goldstein and Roe (5) have shown that human blood lipase hydrolizes tributyrin much more rapidly than it does ethyl-butyrate, benzyl-butyrate, triolein, or olive oil. They explain this by suggesting that the tributyrin is acted upon by more than one enzyme while the others are not. The number of lipases and esterases in mammary gland tissue is unknown though Kelly (10) has separated mammary gland tributyrinase from a diacetinase by means of heat. If there are several enzymes in blood serum and mammary gland tissue, traces of these might be found in milk. Perhaps only a few of these would cause definite defects, but they might act on different substrates under different conditions. When rancidity develops, some of these might act abnormally on only a few of the triglycerides in milk fat.

The literature is meager regarding the number of lipolytic enzymes found in milk. Mattick and Kay (17) have shown that the phosphatase in milk appears to bear no relationship to the tributyrinase content. Harrington and Krukovsky (9) have suggested the presence of two lipases, one of which is destroyed by formalin. This observation has been confirmed by several workers including the author, who, however, has not found the same relationship to exist for mammary gland lipase (11). Peterson *et al.* (19) suggest the possibility that the use of buffers may prevent some of the lipase from acting because it is not in the optimal range of hydrogen ion concentration. They also note the instability of lipase action. They used samples of milk from ten to fifteen hours old in order to obtain comparable results. Tarussuk (25) shows somewhat similar data although he attributes the drop in lipase activity which he found after the first ten hours to an accumulation of the products of hydrolysis and a change in the pH. Although adequate proof is not available, these papers suggest the presence of several lipolytic enzymes.

Very little is known regarding the relationship between such physiological factors as hormones and the lipase activity of mammary gland tissue, serum lipase and milk lipase, or whether any single group of these is affected independently from the others. With one exception, all of the literature dealing with a possible relationship between these lipases and the estrogenic hormones is concerned with the lipase activity of the milk. Koestler, Roadhouse, and Loertscher (14) observed one cow producing milk during the period she had cystic ovaries. When the cysts were ruptured, the milk became normal three days later and remained so until cysts again developed.

With the removal of these the milk again returned to normal. Csiszar (2) reported on two nymphomaniac cows (and two others with an inflammation of the uterus) which produced milk that developed rancidity. He also mentions one cow whose milk developed strong rancidity until she aborted, after which the milk again became normal.

Maass (16) observed that the milk of cows near the end of their lactation periods was more apt than that from others to develop hydrolytic rancidity. This has been confirmed many times since, though it has been disputed by Mattick and Kay (17). More recently Krienke (15), using organoleptic tests as the basis for his measurement, has reported that cows are apt to produce rancid milk at the beginning and end of their lactation periods with a period between during which the defect is not present. Some were very persistent while others produced it only occasionally and at irregular intervals.

In a previous paper the author (11) has published data to show the presence of lipase activity in mammary gland tissue if it had previously been developed by pregnancy. Tissue from the udders of non-pregnant heifers showed no activity. The author (12) is also publishing data to show that the hormone, pitocin, definitely affects the activity of milk lipase, with a small amount of additional data to show that a relationship also appears to exist between milk lipase and stilbestrol and the anterior pituitary hormones. All of this evidence indicates a relationship between the estrogenic hormone(s) and milk lipase.

Unpublished data and a preliminary report (13) show that the hormone, pitocin, also has the ability to activate the tributyrinase in mammary gland tissue in *in vitro* experiments. No additional work has been conducted with other hormones.

There were two objectives in the work reported herein. The first was to develop a method which could be used with pure substrates to show even slight amounts of lipase activity. The assumption has usually been made that all of the triglycerides of milk fat are attacked with somewhat similar velocities of hydrolysis. The correctness of this could be studied. The second objective was to study the relationship between the estrual cycle and milk lipase.

EXPERIMENTAL METHOD

The samples analyzed were taken from the mixed milk of the University herd which consists of forty-five Holsteins, Jerseys and Guernseys. This was considered typical of milk with normal lipase content. Cows 2, 3, 4 and 12 were family cows, all Jerseys, whose owners had complained because of the quality of milk which they produced. All were producing milk which developed rancidity by organoleptic test. Cow 2 was producing rancid milk when she was purchased in December, 1942, and continued to produce this defective milk until she freshened in January, 1943. Subsequently her milk was normal until she was sold sixteen months later. Cows 87, 100, 104, 108

and 114 were purebred Jerseys in the University herd. Cows 10, 34 and 39 were Holsteins in the University herd. Cow 46, also a Holstein in the University herd, had milked 8 months with her first calf and judged by behavior and external appearance was suffering from nymphomania. The stage of lactation of these cows will be mentioned in describing the lipase activity of their milk.

The milk was dried by a method similar to that of Willstatter and Waldschmidt-Leitz (26). One volume was extracted by mixing at room temperature twice with acetone, once with equal parts of acetone and ether, and twice with ether, each time using five volumes of solvent. A very soft, fine precipitate was obtained which kept indefinitely. Since it contained only 5 or 6 per cent moisture, the dried milk and substrate were used alone without preservative. It had previously been determined that milk heated to kill enzyme action before drying could not hydrolize the substrate even after prolonged periods of incubation.

Tributyrin, diacetin, triacetin, tricaproin, ethyl-oleate, trimyristin, triplamitin, and sterilized butter oil were employed as substrates. The diacetin was used to obtain data to compare with that previously obtained with mammary gland tissue. The triacetin and tricaproin were used because of their similarity to tributyrin.

Ethyl-oleate was used because it might give an indication of the action of triolein which was not available. All of these triglycerides were Eastman Kodak Company products. The sterilized butter oil was used since it consists largely of the longer chain fatty acids. It was obtained by melting and centrifuging butter made in the University creamery.

At first, definite weights, usually 4 gm. samples of the dried milk, were saturated with the pure substrate. Later similar results were obtained when the substrate was used as a 20 per cent solution in ether. The ether was then evaporated at room temperature, leaving a dry powdery sample. When this solution was used with normal milk, the samples usually contained 2 gm. of the dried milk. With the milk which developed rancidity one gram was sufficient.

Approximately half of the samples were extracted at once to serve as controls. The others were incubated at 37° C., usually for 24 hours, before extraction. Alcohol was added to the extracts which were titrated against 0.1 N sodium hydroxide with phenolphthalein as the indicator.

EXPERIMENTAL RESULTS

Table 1 shows typical data for the tributyrinase activity of the dried, defatted milk. Samples which by organoleptic test did not develop rancidity had the lower readings.

There was some overlapping in the titration ranges (from 0.36 to 0.60 ml. per gm. of milk) though in only two instances did normal milk show titra-

TABLE 1

The tributyrinase activity of dried, defatted milk

Source and date	Size of sample	Samples				Ave. difference per gm. sample	Kind of milk
		Non-incubated	Ave. titration	Incubated 24 hrs.	Ave. titration		
	gm.	*number*	*ml. 0.1 N NaOH*	*number*	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*	
Herd milk 10/ 4/43	1	5	0.49	5	0.57	0.08	Normal
1/ 3/44	1	9	0.44	10	0.44	0.00	Normal
Cow 46 5/22/44	2	2	0.46	3	0.74	0.14	Normal by organoleptic test from nymphomaniac cow milked 8½ months
Cow. 2 12/31/42	2	4	0.49	5	1.59	0.55	Developed rancidity
1/ 1/43	4	1	0.88	2	3.36	0.62	Developed rancidity
10/27/43	1	9	0.85	9	1.30	0.45	No organoleptic test made
11/11/43	1	7	0.31	3	0.47	0.16	Normal
12/13/43	2	2	0.46	2	0.76	0.15	Normal
12/15/43	2	3	0.43	3	0.75	0.16	Normal
4/24/44	1	3	0.41	4	0.52	0.11	Normal
Cow 3 2/17/44	2	4	0.50	4	2.12	0.81	Developed rancidity
2/18/44	1	4	0.39	4	0.79	0.40	Developed rancidity
2/25/44	1	5	0.44	5	1.01	0.57	Developed rancidity
3/16/44	1	2	0.48	2	1.23	0.75	Developed rancidity
3/21/44	1	2	0.38	2	0.80	0.42	Developed rancidity
3/23/44	1	3	0.40	3	0.87	0.47	Developed rancidity
3/24/44	1	3	0.48	3	0.84	0.36	Developed rancidity
Cow 4 3/31/44	1	3	0.48	3	1.63	1.15	Developed rancidity
4/ 1/44	1	3	0.41	3	1.82	1.41	Developed rancidity
Cow 104 7/27/44	1	3	0.49	3	2.52	2.03	Developed rancidity
7/29/44	1	2	0.36	3	0.83	0.47	Developed rancidity
7/30/44	1	3	0.41	3	0.65	0.24	No organoleptic test made
Cow 108 11/30/44	1	5	0.47	5	0.84	0.37	Normal
12/ 5/44	1	3	0.45	4	0.96	0.51	Developed rancidity
12/ 7/44	1	3	0.50	3	0.97	0.47	Somewhat oxidized
12/ 9/44	1	3	0.48	4	1.07	0.59	Somewhat oxidized
12/11/44	1	3	0.48	3	0.95	0.47	Oxidized and rancid
Cow 114 10/14/44	2	5	0.54	5	1.73	0.60	Developed rancidity
Cow 34 11/29/44	1	4	0.52	5	1.09	0.57	Normal
12/ 1/44	1	3	0.50	3	1.08	0.68	Normal

tions higher than 0.37 ml. These were for cow 34, which had freshened and was producing milk testing 3.2 per cent fat. This would indicate a low total solids content in comparison to the Jersey milk previously used. Since the test is based on a gram of total solids irrespective of the solids content of the milk, lower testing milk might require a higher lipase activity per gram in order to give an organoleptic test for rancidity.

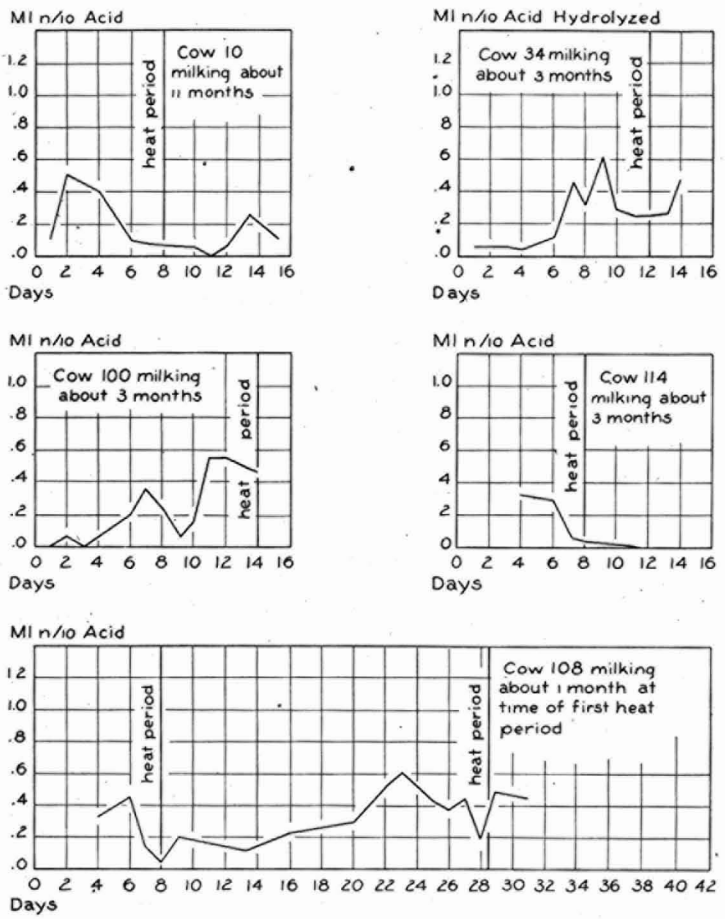

FIG. 1. The effect of stage of the estrous cycle in open cows on the lipase activity of the milk.

Cow 2 produced rancid milk of high lipase activity until she finished her lactation in January, 1943. She was not tested until 10/27/43 when her milk again was high in lipase activity. However, at this time she was open and later data show this could have been due to a stage in her oestrus cycle.

The next readings which were uniformly low in lipase activity were made after the cow was with calf.

Cows 3, 4 and 104 were all at the end of their lactation periods. Their milk varied in lipase activity from day to day. The greatest variation occurred with that of cow 104 whose lipase activity dropped from 2.03 to 0.24 ml. within three days.

The data obtained from cows 10, 34, 100, 108 and 114 are shown in figure 1. From 2 to 5 analyses were made from each milk sample. The results of 420 analyses were used. These were all open cows. The milk showed marked variations with the greatest lipase activity reached a few days before

TABLE 2

Effect of holding milk 24 hours before drying on the lipase activity of the dried, defatted milk

Source and date		Samples				Ave. increase per gm. sample
		Non-incubated	Ave. titration	Incubated 24 hrs.	Ave. titration	
		Sample dried immediately				
		number	*ml. 0.1 N NaOH*	*number*	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*
Cow 3	3/16/44	2	0.48	2	1.23	0.75
	3/23/44	3	0.40	3	0.87	0.47
	3/24/44	3	0.48	3	0.84	0.36
Cow 108	11/30/44	5	0.47	5	0.84	0.37
		Sample held 24 hrs. before drying				
Cow 3	3/16/44	2	0.47	2	1.54	1.07
	3/23/44	3	0.41	3	1.25	0.84
	3/24/44	3	0.50	3	0.96	0.46
Cow 108	11/30/44	3	0.45	4	0.94	0.49

the onset of the appearance of heat. This was followed by a drop just before the heat period, followed by a slight increase in activity at or immediately after. The two series continued several days into the next cycle both indicated comparatively low activity, although the milk of cow 34 two days after the heat period showed high activity. Unfortunately this series was not carried farther.

The milk from cow 108 sometimes showed an oxidized rather than a rancid flavor associated with high tributyrinase activity. This was the only instance in this series in which this defect was observed.

Table 2 includes the data illustrating the effect of holding the milk in a refrigerator 24 hours before drying. In every instance there was a definite increase in the tributyrinase activity at the end of the holding period. This might indicate there was some unstable compound in the milk acting as a temporary inhibiting agent. Since ascorbic acid is such an unstable com-

TABLE 3

The tricaproinase activity of dried defatted milk

Source and date	Size of sample	Samples				Ave. difference per gm. sample	Kind of milk
		Non-incubated	Ave. titration	Incubated 24 hrs.*	Ave. titration		
	gm.	*number*	*ml. 0.1 N NaOH*	*number*	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*	
Cow 87 2/ 2/44	1	3	0.81	3	0.91	0.10	Normal milk
Cow 2 12/13/43	2	2	1.64	2	1.69	0.04	Normal milk
2/15/44	2	3	2.03	3	2.12	0.05	Normal milk
Cow 3 2/25/44	1	3	1.83	3	2.00	0.16	Developed rancidity

pound, samples of the dried milk from cow 4 on 4/6/44 were incubated with tributyrin for 24 hours, one-half of the samples having been saturated with ascorbic acid. The milk used had been allowed to stand in a refrigerator 24 hours previous to drying. At the end of the incubation period there was no difference between those which had the added ascorbic acid and those which did not.

Table 3 shows the tricaproinase activity. While there are not as many samples analyzed as in table 1, the data indicate an activity similar to that for tributyrin though without the very marked increases in the samples which showed definite rancidity. This was also shown when samples taken from the same batch of dried milk and used with the two substrates were compared. For instance, some of the dried milk from cow 3 on 2/25/44 when used with tributyrin gave an average increase over the blanks of 0.57 ml. compared to only 0.16 ml. when tricaproin was the substrate. The normal milk for cow 2 on 2/15/44 also showed a higher titration with tributyrin.

Table 4 includes similar data for diacetinase. There seems to be no relationship between the amount of activity and the development of rancidity in milk.

Table 5 includes similar data for triacetinase activity. The ranges for normal milk and that which developed rancidity appear to be somewhat similar to those for tributyrin, though the differences are somewhat less and there was overlapping between the two groups.

Table 6 shows the results when ethyl-oleate was the substrate. There seems to be no relationship between the degree of rancidity of the milk and the amount of ethyl-oleate hydrolyzed.

The data in table 7 showing the results when tripalmitin was the substrate are included even though there are not a sufficient normal series to make a definite comparison. However from the data which were obtained the variation between the milks does not appear to be large and a good possibility for overlapping of values is indicated.

Trimyristin was the substrate in five series including 33 separate analyses. The dried, defatted milk used with this substrate did not show the ability to hydrolize it though the milk did hydrolize other substrates when used in the other series. As with tripalmitin, toluene was the solvent, but this should not have made any noticeable difference, since it is commonly used in such experiments and previous work with tributyrin had shown it was interchangeable with ether when used with that substrate.

Table 8 shows the comparable action with sterilized butter oil as the substrate. A definite difference appears though there was slight overlapping and the differences are not large.

The data from butter oil and the various pure triglycerides indicate that normal milk lipase attacks both long and short chain triglycerides with the amount of short chain triglyceride hydrolized per gram of milk showing defi-

TABLE 4

The diacetinase activity of dried, defatted milk

Source and date	Size of sample	Samples				Ave. difference per gm. sample	Kind of milk
		Non-incubated	Ave. titration	Incubated 24 hrs.	Ave. titration		
	gm.	*number*	*ml. 0.1 N NaOH*	*number*	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*	
Herd milk 4/19/44	2	2	0.65	3	1.40	0.38	Normal
5/ 2/44	2	3	0.79	3	1.21	0.21	Normal
5/ 9/44	1	3	0.59	3	0.64	0.05	Normal
Cow 2 12/30/42	4	2	0.80	3	0.75	−0.01	Developed rancidity
12/31/42	4	2	0.55	2	0.68	0.03	Developed rancidity
Cow 3 3/21/44	1	3	0.54	2	1.07	0.53	Developed rancidity
2/23/44	1	6	0.54	5	1.10	0.56	Developed rancidity
Cow 4 4/ 4/44	1	3	0.57	3	1.31	0.74	Developed rancidity

TABLE 5

The triacetinase activity of dried, defatted milk

Source and date	Size of sample	Samples				Ave. difference per gm. sample	Kind of milk
		Non-incubated	Ave. titration	Incubated 24 hrs.	Ave. titration		
	gm.	*number*	*ml. 0.1 N NaOH*	*number*	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*	
Herd milk 10/11/43	1	6	1.45	3	1.65	0.20	Normal
10/18/43	1	5	1.18	11	1.25	0.07	Normal
Cow 2 11/19/43	1	6	0.32	9	0.57	0.25	Normal
2/15/44	1	3	0.91	3	1.13	0.22	Normal
Cow 3 2/28/44	1	5	0.72	5	0.90	0.18	Developed rancidity
3/21/44	1	3	0.52	3	0.87	0.35	Developed rancidity
3/23/44	1	3	0.57	3	0.97	0.40	Developed rancidity
Cow 4 4/ 4/44	1	3	0.60	2	1.26	0.66	Developed rancidity

TABLE 6

The lipase activity of dried, defatted milk with ethyl-oleate as the substrate

Source and date	Size of sample	Samples				Ave. difference per gm. sample	Kind of milk
		Non-incubated	Ave. titration	Incubated 24 hrs.	Ave. titration		
	gm.	*number*	*ml. 0.1 N NaOH*	*number*	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*	
Herd milk 12/27/43	1	10	1.11	10	1.09	−0.02	Normal
1/11/44	1	9	7.05	9	7.14	0.09	Normal
Cow 87 2/ 2/44	1	3	1.69	4	1.75	0.06	Normal
Cow 3 2/17/44	2	4	3.88	4	4.27	0.20	Developed rancidity
2/28/44	1	5	2.83	5	2.99	0.16	Developed rancidity
3/21/44	1	3	1.71	3	1.71	0.00	Developed rancidity
3/23/44	1	2	1.59	2	1.50	−0.09	Developed rancidity
Cow 4 3/31/44	1	4	1.67	4	1.68	0.01	Developed rancidity

TABLE 7

The tripalmitinase activity of dried, defatted milk

Source and date	Size of sample	Samples				Ave. difference per gm. sample	Kind of milk
		Non-incubated	Ave. titration	Incubated 24 hrs.	Ave. titration		
	gm.	*number*	*ml. 0.1 N NaOH*	*number*	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*	
Herd milk 1/11/44	1	10	0.35	9	0.42	0.07	Normal milk*
Cow 3 3/ 8/44	1	3	0.39	3	0.46	0.07	Developed rancidity
Cow 4 3/31/44	1	3	0.36	3	0.54	0.18	Developed rancidity
4/ 4/44	1	3	0.48	5	0.59	0.11	Developed rancidity
Cow 12 9/22/44	1	3	0.41	3	0.49	0.08	Developed rancidity

* 3 per cent concentration of substrate instead of the usual 20 per cent. However, on the basis of the work of Peterson, Johnson and Price (19), the results should still show fairly good comparisons.

TABLE 8

The lipase activity of dried, defatted milk with butter oil as the substrate

Source and date	Size of sample	Samples				Ave. difference per gm. sample	Kind of milk
		Non-incubated	Ave. titration	Incubated	Ave. titration		
	gm.	*number*	*ml. 0.1 N NaOH*	*No.*	*hrs.*	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*	
Herd milk 1/ 3/44	2	6	0.70	6	24	0.86	0.08	Normal
Herd milk 5/ 2/44	2	5	0.50	5	72	0.67	0.08	Normal
Mixed 5/ 9/44								
Herd milk 5/22/44	2	5	0.64	2	72	0.80	0.08	Normal
Cow 3 3/ 6/44	1	7	0.42	7	48	0.92	0.50	Developed rancidity; stood 48 hrs. before drying
Cow 3 3/21/44	1	3	0.42	3	24	0.51	0.09	Developed rancidity
Cow 3 3/23/44	1	3	0.45	3	24	0.57	0.12	Developed rancidity
Cow 3 3/22/44	1	3	0.42	3	24	0.51	0.09	Developed rancidity
Mixed 3/24/44								
Cow 4 3/31/44	1	6	0.49	6	24	0.56	0.07	Developed rancidity
Cow 4 4/ 1/44	1	3	0.45	3	24	0.56	0.11	Developed rancidity
Cow 4 4/ 4/44	1	3	0.82	3	24	1.81	0.99	Gravity separated cream. which developed rancidity

TABLE 9

A comparison of the volatile and total fatty acid liberated by lipase action

Source and date	Length of incubation period	Ave. increase in volatile fatty acid per 5 ml. undried or per gm. dried sample	Ave. increase in total fatty acid per 5 ml. undried or per gm. dried sample	Volatile fatty acid in comparison to total fatty acid	Type of sample used	Kind of milk used
	hrs.	*ml. 0.1 N NaOH*	*ml. 0.1 N NaOH*	*per cent*		
Herd milk 4/ 6/44	24	0.039	0.048	81	Undried milk	Normal
Herd milk 5/16/44	24	0.042	0.092	46	Undried milk	Normal
Herd milk 5/22/44	72	0.042	0.082	51	Dried milk	Normal
Herd milk 5/ 2/44	72	0.043	0.082	52	Dried milk	Normal
Mixed 5/ 9/44						
Cow 108 11/27/44	24	0.038	0.070	54	Undried milk	Normal
Cow 4 4/ 5/44	24	0.110	0.340	32	Undried milk	Developed rancidity
Cow 4 4/ 4/44	24	0.310	0.990	31	Dried cream	Developed rancidity

nitely higher titration values than when pure butter oil was the substrate. Since, according to Davies (3), the volatile fatty acid content constitutes only 5.2 to 11.6 per cent of the total fatty acids in butter fat this would indicate a somewhat selective hydrolysis which would be especially true in milk developing rancidity.

Since these trends were consistent with the method used, it was thought worth while to check the method by using undried samples as well as those made of dried milk. When the undried milk was used, it was kept in a refrigerator for various periods. Five-ml. aliquots were dried with plaster of Paris and extracted with ether. At the same time 20-ml. aliquots were placed in distilling apparatus equipped with ground-glass connections to learn the proportion of volatile fatty acids liberated by hydrolysis in comparison with the total fatty acid. These results appear in table 9. While the amount of data obtained, especially with the normal milk lipase, is small,

TABLE 10

A comparison of the tributyrinase content of the cream and skim milk samples

Source and date	Ave. increase in titration per gm. dried cream used	Ave. increase in titration per gm. dried skim milk used
	ml. 0.1 N NaOH	*ml. 0.1 N NaOH*
Cow 4 4/1/44	2.60	0.71
Cow 4 4/5/44*	7.46	1.51
Cow 4 4/6/44	4.79	2.40

* Milk was held in the refrigerator 24 hours before drying.

the results are consistent. In carrying out the distillations approximately 350 ml. of the sample and water were collected, which would not account for 100 per cent of the volatile free fatty acids present. Yet with normal milk, the volatile fatty acids accounted for from 46 to 81 per cent of the fatty acid hydrolized. The percentage of volatile to total acid was not as high in the milk from cow 4 which developed extreme rancidity on standing, but here too the milk lipase was selective in the type of triglyceride attacked.

Samples of milk from cow 4 were allowed to cream in the refrigerator. The cream and skim milk were separated, dried, defatted and analyzed for tributyrinase activity. The data follow in table 10. There was a definite increase in the lipase activity of the cream.

DISCUSSION OF RESULTS

The method was developed to study lipase action in milk with pure substrates. It is recognized that there are both advantages and disadvantages to the method.

One important advantage is that the fresh milk can be dried in a few minutes and stored for later analysis. The dried, defatted milk is in a satis-

factory form for use with pure substrates. Water-soluble fatty acids are not lost from the final analysis. The method is suitable for carrying out comparatively large numbers of analyses at the same time. The lipase activity did not change significantly after several months storage.

One disadvantage is that the physical condition of the sample is not the same as it would be in undried milk. With dried milk the substrates are hydrolyzed without their solubility becoming a factor as it is in the natural state. Previous work has indicated this may be important (11).

The results with the various pure substrates indicate that the more important differences between normal and rancid milk were in the tributyrinase content. Slightly smaller increases were shown for the other volatile substrates. The results with the trimyristin were somewhat surprising since no lipase action was indicated. When the tripalmitin and butter oil are considered together they did not appear to be as sensitive indicators of rancidity as was the tributyrin. The differences were sufficiently small as to permit an overlapping of values between normal and rancid samples.

The data in table 9 showing the amount of volatile substrate in comparison to the total substrate hydrolyzed also indicates a selective hydrolysis with the shorter chain acids hydrolyzed to the greater extent. However, the data also show two series of samples of rancid milk or cream which showed a smaller percentage of volatile to total fatty acid than did those series containing normal milk. While there was still a selective hydrolysis with the volatile acids accounting for approximately one-third of the total hydrolysis, the non-volatile fatty acids had also shown a large increase.

The data in table 2, showing that milk held at a low temperature for a twenty-four-hour period increases lipolytic activity, suggest that some compound in milk acts temporarily as an inhibitor. This is in contrast to the data of Peterson and associates (19) and Tarussuk (25) all of whom obtained reduced activity when milk was allowed to stand. There is no satisfactory explanation for these differences except that in the methods reported, all acetone- and ether-soluble compounds have been removed. It is clear, however, that milk in lipase analyses should be handled within very specific time limits.

Roahen and Sommer (22) have shown that gravity-separated cream contains greater lipase activity than does machine-separated cream. They attributed this to the greater loss of lipase in the separator slime. Sharp and de Tomasi (23) show the greater lipase content of the cream as compared to whole and skim milk, but they do not offer an explanation. The data in this paper on the dried, defatted skim milk and cream agree with that previously obtained. Since the fat has been eliminated from these samples, it seems possible that, as the fat globules rise accompanied by Brownian movement, they adsorb the lipase on their surface so that when the milk has creamed there is a concentration of lipase in the cream layer.

With the substrates, diacetin and ethyl-oleate, there appeared to be no relationship between the type of milk used and the resulting hydrolysis. Previous work by the author (10) has shown that in mammary gland tissue the enzyme attacking diacetin was not the same as that which hydrolized tributyrin. The data obtained in this paper add supporting evidence that ethyl-oleate might be hydrolized by different enzymes.

When samples of milk were collected at frequent intervals from five open cows, the data show a relationship between the stage of the estrus cycle of the cow, and the lipase activity of the milk which she produces. This indicates a close association in activity between some of the estrogenic hormones and the activation of milk lipase.

This would partially supplement the observation of Krienke (15) that cows may produce milk which develops rancidity during the early stages of their lactation periods. While the activity of all of the milks went up just before the heat period, they did not all go sufficiently high to allow detection by organoleptic test. This was true for the milk of the Holstein cow 34.

SUMMARY

1. A new method for lipase analysis has been reported.

2. Tributyrin appeared to be a more sensitive indicator of milk which developed rancidity than did tricaproin, triacetin, tripalmitin, or sterilized butter oil.

3. The method was developed primarily for the study of milk lipase activity with pure substrate. Enough data have not been taken under different conditions to set absolute limits to indicate rancidity by organoleptic test. Most of the data obtained were from milk which had a high total solids content. One series of samples from a cow producing milk with a low butterfat content was slightly higher in activity per gram of dried, defatted milk powder than some others which had shown rancidity on organoleptic test and yet they produced milk in which the defect could not be detected. It is possible that these border-line cases could have been eliminated had total solids as well as the titration per gram of dried, defatted milk been considered.

4. The milk lipase appeared to show some selectivity in its hydrolysis, acting more rapidly on the volatile than on the non-volatile substrates.

5. When the milk was kept for an additional 24 hours before drying, there was a marked increase in tributyrinase activity.

6. Cream which was obtained by gravity separation contained much higher lipase activity than did the skim milk from the same sample.

7. There appeared to be no relationship between the lipolytic action which causes hydrolytic rancidity and that which attacks the substrates diacetin and ethyl-oleate.

8. When samples of milk were collected at frequent intervals from open

cows, the data showed a definite relationship between the estrus cycle and the lipase activity.

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VITAMIN A AND CAROTENE CONTENT OF THE BLOOD PLASMA OF DAIRY CALVES FROM BIRTH UP TO FOUR MONTHS OF AGE*

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Compared with the wild state, the present methods of raising dairy calves have considerably altered the quality and quantity of foods which a calf receives up to four months of age. This change has reduced very materially the vitamin A intake. In the wild state the calf remained with its dam and thus received a considerable quantity of whole milk during this period of development. Under some of our present procedures of hand feeding a maximum of only about ten pounds per day is fed, and then for only a month to six weeks. In the wild state the calf usually was born in the spring of the year when the milk which it received was high in vitamin A. Under present day conditions winter milk contains only one-half to one-third the vitamin A of summer milk (5). Furthermore, when the calf was born in the spring of the year in the wild state, the green grass eaten was high in carotene, whereas field cured hay retains only five to ten per cent of its original carotene content.

In order to throw some light on this problem Nelson and co-workers (7) obtained blood samples of beef and dairy calves up to four months of age. The dairy calves were raised by the usual modern methods and the beef calves were permitted to run with the dams. Both groups were on winter feed so that the comparison did not exactly simulate average present day methods of raising calves as compared with the wild state. The results showed that the vitamin A content of the blood plasma of the beef calves was considerably higher than for the dairy calves, which was probably due to the larger intake of whole milk.

The reduced vitamin A intake of dairy calves may account in part for the losses due to pneumonia and scours in this age group. Krauss and co-workers (3) at the Ohio Agricultural Experiment Station reported a decreased incidence of pneumonia in calves which received 15,000 units of vitamin A concentrate daily. Gullickson and Fitch (2) in an experiment involving 72 calves reported less trouble from digestive disturbances was encountered in young calves that were fed cod liver oil than in calves not receiving the vitamin supplement. Some of the non-supplemented calves died. Phillips (8) reported that the administration of high vitamin A potency shark liver oil and certain members of the B complex eliminated

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diarrhea and lowered the mortality resulting from pneumonia. Nelson (7) recommended the feeding of fish liver oil where there was difficulty in raising calves.

The vitamin A requirements of calves from birth to four months of age has not been established. Converse and Meigs (1) reported results which indicated a higher vitamin A requirement for this age group than for older calves.

In order to obtain further information vitamin A and carotene determinations were made each two weeks on calves up to four months of age raised in the dairy herd of the University of Maryland. The effect of feeding lespedeza hay high in carotene was also studied.

EXPERIMENTAL

Blood samples of the Holstein, Ayrshire and Guernsey breeds were drawn every two weeks from calves up to four months of age. Plasma, vitamin A and carotene determinations were made according to an adapta-

TABLE 1

Daily feeding schedule for dairy calves

Age of calf	Whole milk, lbs.	Dry calf starter, lbs.	Grain, lbs.	Hay, lbs.
Ayrshire and Holstein-Friesian				
1–4 days	With cow	.....	.....	.....
4–7 days	8	.....	.....	.....
2nd week	9	¼	.....	.....
3rd week	10	½	.....	Free access
4th week	9	1	.....	Free access
5th week	7	1½	.....	Free access
6th week	6	2	.....	Free access
7th week	4	3	.....	Free access
8th–10th weeks	.....	3½–4	.....	Free access
11th–14th weeks	.....	4	.....	Free access
15th–16th weeks	.....	4–0*	0–4†	Free access
17th–24th weeks	.....	.....	4–5	Free access
Guernsey				
1–4 days	With cow	.....	.....	.....
4–7 days	5	.....	.....	.....
2nd week	6	⅛	.....	.....
3rd week	7	¼	.....	Free access
4th week	7	½	.....	Free access
5th week	6	1	.....	Free access
6th week	6	1½	.....	Free access
7th week	5	1½	.....	Free access
8th week	4	2	.....	Free access
9th week	3	2½	.....	Free access
10th week	3	3	.....	Free access
11th–14th weeks	.....	3½–4	.....	Free access
15th–16th weeks	.....	4–0*	0–4†	Free access
16th–24th weeks	.....	.....	4	Free access

* Gradually decreased.
† Gradually increased.

tion of a previously published method (4). The calves were fed according to the schedule given in table 1. Number 1 clover and timothy hay was fed as a roughage. Milk from the Holstein herd was fed to all calves during the milk feeding period.

In addition, one group of calves was fed according to the schedule in table 1 for the first 90 days after which they were placed on a vitamin A deficient ration to study the rate of depletion as indicated by blood-plasma analysis. A similar group of three calves were fed lespedeza hay in place of clover and timothy hay. The clover and timothy hay contained 15 micro-

TABLE 2

Vitamin A and carotene content of blood plasma of calves of the various breeds

Age, days	Holstein	Ayrshire	Guernsey
	Micrograms vitamin A per 100 ml. plasma		
8–21	(18)* 10.8 ± 0.62	(10) 9.0 ± 1.19	(10) 11.0 ± 1.08
22–35	(17) 7.7 ± 0.79	(10) 7.2 ± 0.52	(10) 9.8 ± 0.80
36–49	(19) 8.2 ± 0.40	(11) 8.1 ± 0.51	(9) 9.8 ± 1.05
50–63	(15) 9.9 ± 0.76	(10) 9.2 ± 0.50	(9) 9.7 ± 0.51
64–77	(19) 10.2 ± 0.58	(11) 8.9 ± 0.79	(10) 11.1 ± 0.81
78–91	(17) 10.1 ± 0.73	(11) 9.9 ± 0.79	(9) 12.8 ± 0.87
92–105	(20) 10.9 ± 0.57	(10) 10.3 ± 0.91	(9) 13.1 ± 1.11
106–119	(19) 10.2 ± 0.63	(11) 11.7 ± 0.94	(10) 14.0 ± 1.74
120–133	(19) 10.5 ± 0.53	(8) 10.8 ± 0.80	(8) 13.0 ± 0.96
	Micrograms carotene per 100 ml. plasma		
8–21	(20) 17.7 ± 1.8	(10) 19.6 ± 2.0	(9) 40.8 ± 5.0
22–35	(19) 23.3 ± 2.9	(11) 16.5 ± 1.9	(10) 35.4 ± 4.7
36–49	(20) 34.4 ± 3.7	(12) 30.8 ± 3.8	(10) 42.9 ± 6.4
50–63	(16) 52.6 ± 4.4	(10) 46.5 ± 8.1	(9) 68.9 ± 10.6
64–77	(19) 52.6 ± 4.5	(12) 42.0 ± 5.0	(10) 82.8 ± 9.3
78–91	(20) 57.9 ± 4.1	(12) 45.5 ± 6.2	(9) 80.9 ± 7.1
92–105	(21) 62.8 ± 5.3	(12) 52.9 ± 8.0	(10) 79.4 ± 10.2
106–119	(20) 68.5 ± 5.1	(12) 63.6 ± 6.6	(9) 100.0 ± 13.7
120–133	(18) 70.5 ± 5.8	(9) 81.1 ± 11.3	(8) 97.6 ± 12.7

* Figures in parenthesis indicate number of determinations.

grams of carotene per gram of hay while the lespedeza contained 50 micrograms per gram.

RESULTS AND DISCUSSION

The results of the analysis of the blood plasma for vitamin A and carotene for the calves raised in the herd are shown in table 2. The data have been averaged according to breeds for each two weeks and the standard error calculated. The blood plasma values for the Holstein and Ayrshire calves are in close agreement. The plasma, carotene and vitamin A values for the Guernsey calves are slightly higher. The higher values may be due to the fact that the Guernsey calves received whole milk for a longer period of time than the Holstein and Ayrshire calves, or the correction factor applied to the antimony trichloride vitamin A value for the carotene content

of the blood plasma may be in error. While beta carotene was used as a standard for correction, this procedure is open to criticism. However, it

TABLE 3

Vitamin A in micrograms per 100 ml. of blood plasma of calves fed a mixture of clover and timothy or lespedeza hay

Clover and timothy

468		469		470	
Age	Vitamin A	Age	Vitamin A	Age	Vitamin A
41	7.5	39	9.6	25	19.7
52	7.4	54	7.2	39	13.2
70	7.7	68	5.7	54	10.4
83	10.4	81	7.5	68	12.9
97	5.7	95	6.3	81	10.2
112	2.4	110	2.4	95	4.5
126	0.6	124	0.3	110	7.2
				126	7.8

* Placed on carotene-free ration at 90 days of age.

471		472		473	
Age	Vitamin A	Age	Vitamin A	Age	Vitamin A
25	9.0	26	6.3	16	9.0
39	6.0	41	6.0	23	5.4
54	6.6	55	6.3	38	5.4
68	4.8	68	12.3	52	12.9
81	7.5	82	9.0	65	14.1
95	3.0	97	12.6	79	10.5
110	4.8	111	5.4	94	11.7
124	5.1	124	5.1	108	7.8
				129	3.0

* Placed on carotene-free ration at 90 days of age.

Lespedeza

463		464		466	
Age	Vitamin A	Age	Vitamin A	Age	Vitamin A
36	10.5	32	12.6	34	13.4
50	12.3	39	15.9	60	12.3
57	21.6	49	21.9	71	23.4
76	27.3	55	26.7	84	22.5
107	16.2	64	31.2	98	10.2
120	10.5	77	27.8	112	3.6
128	13.5	91	21.0	126	8.1
		105	5.1		
		120	6.6		

* Placed on carotene-free ration at 90 days of age.

seems quite unlikely that the vitamin A values for the Guernsey calves for this age group are lower than for the Holstein and Ayrshire calves.

It should be pointed out and emphasized that the vitamin A values are in the deficient range when compared to those obtained from calves four months to one year of age in data reported from this laboratory (6). While it should be noted that there are individual variations in calves kept on the same carotene intake, such low values as noted in this group of calves would lead to some expression of deficiency symptoms in older calves during the winter months (6). Values of 15 micrograms of vitamin A per 100 ml. of plasma or above should be maintained to prevent deficiency symptoms in older calves.

The data obtained with the two groups of calves fed lespedeza and clover and timothy hays are shown in table 3. Where clover and timothy hay was fed low-plasma vitamin A values similar to those as shown in table 2 resulted. When the calves were placed on a carotene free ration at 90 days of age, the vitamin A values dropped sharply. Where lespedeza was fed in place of the clover and timothy hay, the plasma vitamin A values were markedly higher. These higher values presumably were due to the higher carotene intake from the lespedeza hay. When the three calves were placed on the carotene free ration at 90 days of age, the vitamin A content of the blood plasma decreased at a slower rate and not to the extent as noted for the calves fed clover and timothy. This would indicate a much greater storage of vitamin A in the liver for the calves fed lespedeza hay.

From the practical feeding standpoint where limited amounts of whole milk are fed these data indicate that some vitamin A supplementation might be beneficial for calves for the first four to six weeks of age, especially where some difficulties are encountered in raising calves. Where the roughage is of poor quality some supplementation to three months of age is indicated. These data, also, indicate that lespedeza hay because of its high carotene content should prove to be a good hay for calves. Some further attention should be given to the use of high carotene roughages for young calves which would obviate the feeding of vitamin A supplements, except for the first 30 days.

SUMMARY

1. The vitamin A content of the blood plasma of calves of the Holstein, Ayrshire and Guernsey breeds from birth up to four months of age varies from 7.2 to 14 micrograms per 100 ml.

2. These values are in the deficient range when compared with those for vitamin A deficient calves at one year of age.

3. Some vitamin A supplementation is indicated where difficulty in raising calves is encountered.

4. The feeding of high-carotene lespedeza hay produced higher plasma vitamin A values in young calves than No. 1 mixed clover and timothy hay.

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THE "SMEAR" OF BRICK CHEESE AND ITS RELATION TO FLAVOR DEVELOPMENT*

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Brick cheese is a semi-hard variety having the characteristic body of a soft Cheddar cheese and a flavor somewhat resembling that of Limburger. Its curing especially resembles that of Limburger for it is ripened about two weeks in a moist room at approximately 60° F. and 85 per cent relative humidity. During this period of two weeks there develops upon the surfaces of the cheese a characteristic, fat-like, yellow or orange-colored growth of microorganisms, commonly called "smear," that is essential for the development of the typical flavor (10, 24).

This investigation was begun in 1939 to study the general nature of the surface "smear" on Brick cheese and its relationship to some of the changes in the ripening cheese.

Although no literature has been found that discusses the surface smear of Brick cheese, studies of several related varieties, such as Limburger, Tilsit, and Backsteinkäse (12, 15, 22), have shown that the surface growths on these cheese usually contain large numbers of yeasts, micrococci, and bacilli; that *Oidium lactis* may be present; that yellow and red pigmented rods are commonly found; and that the pigmented types dominate the smears in the later stages of development.

Gratz and St. Szanyi (9) decided that the organisms on the surface play no part in the chemical changes in the interior of hard cheese and that there was no diffusion of enzymes from the surface to the center of the cheese.[1] Quite opposed to this view is that of Kelly (12) who found, in studying the ripening of Limburger cheese, that yeast-like and red-pigment producing, rod-shaped bacteria are chiefly responsible for the surface changes and that, ". . . through their enzymes they have a major part in the breaking down of the cheese mass from the firm, rubbery texture of green cheese to the soft, buttery consistency of the ripened cheese."

Foster, Garey and Frazier (7) studied the bacterial flora of the interior of Brick cheese and found that *Streptococcus lactis* is the predominant organism when it constitutes part or all of the starter used in making the

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[1] Since this paper was written G. Koestler (Landw. Jahrb. Schweiz., 57: 499–519, 1943) has reported studies on the decomposition of protein in ripening cheese. He found that the surface coating on Greyerz and Tilsit had no direct effect on protein degradation in the cheese itself but that it did establish a lower acidity. This encouraged extensive decomposition and formation of highly dispersed substances, which diffused into the cheese giving it odors characteristic of the decomposition products.

cheese. They stated: "The most important organism in bringing about the changes in body within the cheese during ripening is *L. casei,* and this organism also contributes to the flavor."

During the making of Brick cheese and for a period of 6 to 10 days thereafter, the most important chemical change is probably the production of lactic acid from lactose. Foster, Garey and Frazier (6) showed that the lactose in Brick cheese may disappear after the first day when *Streptococcus lactis* starter is used; that it remains longer when *Streptococcus thermophilus* starter is used; and that a slight amount of lactose may persist in the rind throughout the ripening period, probably because lactics are inhibited by the salt in the surface layers. This rapid production of lactic acid limits the nature of subsequent bacterial changes. The acid reacts with the calcium salts and with the proteins in the milk and curd (3, 19, 21). Van Slyke and Bosworth (20) studied some of the first chemical changes in Cheddar cheese by measuring the changing solubility of the proteins in 5 per cent salt solution and in water. It can probably be assumed that the changes in protein solubility that precede the development of flavor in Cheddar cheese are similar to changes occurring in the solubility of the proteins of Brick cheese before characteristic flavor appears.

The phases of ripening that finally produce characteristic flavor and body in ripened cheese are associated with a series of slow reactions which usually have been studied by determining the progressive formation of simpler nitrogenous compounds from the original cheese proteins.

The possibility that chemical changes in the lactates and fat may produce flavor in cheddar cheese was suggested by Suzuki, Hastings and Hart (18) and has been emphasized by others (4, 5). Some investigators have stated that lipase may have a beneficial effect in ripening (4, 13); others have called attention to defects caused by lipolytic enzymes (11).

METHODS

Manufacture. Experimental cheese was made from holder-pasteurized milk by methods described by Wilson and Price (23) and Spicer and Price (17). The cheese was salted in 22 per cent sodium chloride brine at 60° F. for 48 hours, then drained free of excess brine, and placed upon wooden shelves in the curing cellar at 58° to 60° F. and 85 per cent to 90 per cent relative humidity. Here the cheese was washed in the usual manner every other day in a weak (10 per cent) sodium chloride solution to keep it moist, and to prevent excessive mold growth. After 10 to 20 days it was taken from the curing cellar, washed in lukewarm water to remove the smear, dried for 24 hours, paraffined, and placed in a room at 40° to 45° F. for the remainder of the curing period. The data and figures reported in this discussion were obtained by intensive study of one typical lot of cheese.

Examination of the surface smear. Samples were always taken before

the cheese was washed so that there was at least a twenty-four hour growth of smear at sampling time.

Contact slides for microscopic examination were made by pressing clean glass slides against the surface of the cheese and distributing the adhering material uniformly over each slide with the aid of a loopful of sterile water. After the slides had dried and the fat had been removed with xylol, they were fixed with alcohol and stained by methylene blue and by the Gram method.

For culture studies, pieces of rind (approximately 1 mm. thick) were removed from the top side of the cheese with a sterile spatula and placed on

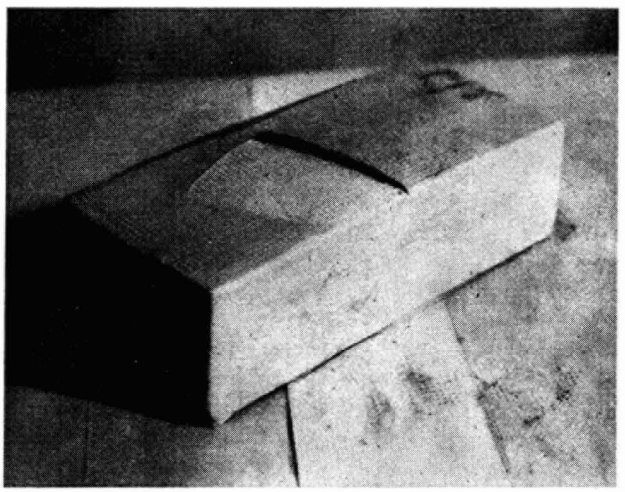

FIG. 1. The smear at two weeks of age when it exhibits its most luxuriant growth.

a sterile glass slide. Each piece of rind was trimmed down to one square centimeter and placed in 100 ml. of sterile water at 100° F.; 0.5 gram of sodium citrate was added and the mixture was shaken vigorously until the rinds were thoroughly dispersed. Further dilutions were then made as desired.

Tryptone-glucose-extract-milk agar (hereafter in this report called TGEM agar) was used for estimating the number and types of microorganisms in the rind and for isolating the dominant types of organisms. Plates were incubated at room temperature and examined after forty-eight and ninety-six hours.

Measuring changes in ripening. Hydrogen ion concentrations were measured by the quinhydrone method, using a Leeds-Northrup portable potentiometer, a saturated calomel half-cell, and a gold electrode.

The methods described and discussed by Allen (1) were used to determine the changes in the proteins of the cheese that are indicated by the formation of water-soluble and alcohol-soluble nitrogen-containing compounds.

Sectioning the cheese for analysis. A three-inch cross section was cut from the middle of the Brick cheese; from this portion three layers, parallel to the rind, were removed for analysis. One layer, hereafter called the "rind," consisting of the rind and the cheese beneath it, measured one-eighth of an inch in thickness. The second layer, which will be termed the "midsection," consisted of the quarter-inch section midway between the surface and exact center of the cheese. The third layer, called the "center," was the inch by inch-and-one-half section surrounding the center of the cheese.

BACTERIOLOGICAL OBSERVATIONS

The contact slides from the fresh cheese showed a variable flora; but one or two days after salting, yeast-like organisms began to appear on the cheese

TABLE 1

Percentages of dominant organisms in the surface flora of a typical lot of Brick cheese

Typical groups of organisms*	Form	Days after salting					
		2	4	6	8	10	12
		%	%	%	%	%	%
1	Coccus	5.5	11.1	‡	4.3	1.4	7.5
2	Rod	0.1	10.7	12.3	17.2	28.4	37.6
3	Rod	0.4	27.4	31.2	42.8	20.5	7.5
4	Coccus	11.0	15.6	11.3	10.0	18.3	22.6
5	Coccus	40.0	12.5	8.5	11.4	9.4	7.5
6	Coccus	22.0	1.4	20.8	11.5	1.7	15.0
7	Coccus	‡	5.6	0.5	1.4	1.5	‡
Others †	.....	21.0	15.7	15.4	1.4	18.8	2.2

* These dominant groups were distinguished from each other by colony morphology, color, microscopic appearance, and growth in litmus milk.

† This group consisted chiefly of spore-forming, spreader types.

‡ Less than 0.1%.

in appreciable numbers. These organisms constituted the major portion of the surface growth until the third or fourth day after salting; after that, cocci and small, rod-shaped organisms began to appear in significant numbers.

Plate counts of organisms (table 1) in the smear were not very satisfactory. They were highly variable from day to day, partly because of the difficulty in sampling properly the rapidly changing surface flora and partly because of nonuniformity of the smear. Numbers of organisms increased to a maximum on the sixth day after salting when the total approximated 1,540,000,000 per square centimeter; twelve days after salting the total

count had decreased to 130,000,000. The yeast-like organisms observed microscopically failed to grow on the medium used; this may have been true of other organisms in the smear. During the first few days of curing, plate examinations showed micrococci in large numbers, many of them capsulated; later, the rod-shaped, pigmented organisms of the *Bacterium linens* type became prominent; groups 2 and 3 in table 1 were of this type.

Influence of salt and acidity on growth of typical organisms isolated from the smear. Cheese makers commonly control smear development by amount of salting and frequency of washing; these treatments affect the salt content and reaction of the cheese surface. The possible significance of high salt and acidity on the development of organisms of the smear are indicated in table 2. These data were obtained by observing the growth

TABLE 2

*Influence of sodium chloride and acidity on the growth of organisms isolated from the surface of Brick cheese**

Culture number†	Amount of salt in medium‡					Acidity of medium‡		
	0	1%	5%	10%	15%	pH 5.0	pH 6.0	pH 7.0
1	++++	++	+	−	−	++	++++	++++
2	++++	+++	+	−	−	+	++	+++
3	++++	++++	+++	−	−	+	+++	+++
4	++++	+++	+++	+	+	++	+++	++++
5	++++	+++	+++	+	+	+	+	++
6	++++	++	++	+	−	++	+++	++++
7	++++	++++	+	−	−	+	++	+++

* Extent of bacterial growth is indicated as follows: − = none; + = slight; ++ = fair; +++ = good; ++++ = abundant.

† These pure cultures were numbered to correspond with the numbered groups (Table 1) from which they were selected.

‡ Cultures were grown on tryptone-glucose-extract-milk agar, the salt content and acidity of which had been adjusted with sodium chloride, hydrochloric acid or sodium hydroxide.

of pure cultures of the typical organisms on TGEM agar containing varying amounts of sodium chloride or with different pH values. It is to be expected that the growth of organisms on TGEM agar with varied salt contents and different acidities may not duplicate the effect of such differences on the surface of the cheese itself. The artificial medium can only be expected to indicate the trends of the effects of these variations. All organisms tended to grow better as salt content and hydrogen ion concentration were decreased.

It is significant that salt content and acidity of the Brick cheese surface tend to become more favorable for the growth of microorganisms while the cheese is being held and washed in the curing cellar.

MEASUREMENTS OF CHANGES DURING RIPENING

Sharp and McInerney (16) have shown that ". . . salt solubility of the protein is merely a reflection of the changes in hydrogen ion concentration

of the extracting solution or cheese. . . ." In this study the extreme differences in the salt contents of extracting solutions were too small to be of significance. The outer and inner sections of the cheese showed the greatest differences in salt immediately after salting. At that time the salt concentration of the water solutions prepared for analysis from the rind section contained approximately 0.2 per cent sodium chloride while solutions prepared from the center portion contained approximately 0.08 per cent. This difference in salt content, which represented the extreme variation in this study, did not affect significantly either the water solubility or alcohol solubility of the cheese proteins (14). It also has been shown (14) that variations in the pH of a 0.12 per cent salt solution within the limits of 5.4 to 6.5 have no measurable effect on the water solubility or alcohol solubility of the nitrogen compounds of the cheese. Observations in this study were made on suspensions which were regularly at pH 6.0.

EFFECT OF NORMAL ACTION OF THE SMEAR

Acidity. The acid measurements from the three layers of a typical Brick cheese are shown in figure 2. Minimum pH was attained in all sec-

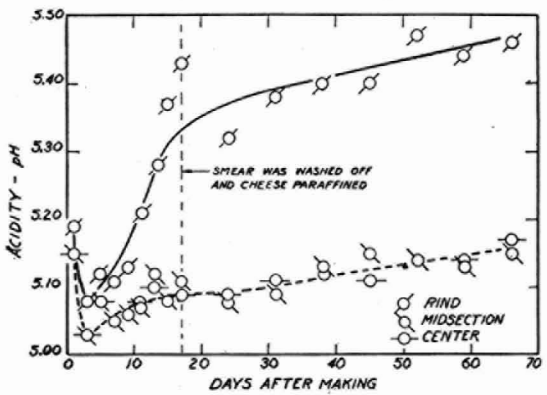

FIG. 2. Changes in acidity (pH) of the three sections of Brick cheese during normal ripening.

tions 3 days after making; the general trend thereafter was upward. This upward trend in the midsection and center layers was much slower than that of the rind layer. It is significant that the pH value of the rind at 17 days of age was distinctly more alkaline than the pH one week later after the cheese had been washed and paraffined. Most interesting was the wide difference in pH between the rind and inner layers.

The increase in pH of the inner sections is characteristic of the acidity changes which occur in the normal ripening of hard cheese like Cheddar (2).

The increase in pH of the rind section can be explained by the changing conditions on the surface. Some microorganisms in the smear decompose

lactates, an oxidative process that eventually forms carbonates; others, acting on the protein, produce or liberate compounds like basic amines and ammonia that are probably responsible in part for the odor of the smear. Then, of course, the washing itself tends to remove some of the acid compounds and buffers from the surface, thus producing conditions more favorable for further bacterial action.

Water-soluble nitrogen. The data in figure 3 show that during the ripening of the Brick cheese there was a gradual increase in the water-solubility of the nitrogenous compounds in all layers of the cheese. With the possible exception of the period between the 10th and 17th days, the changes in the water-soluble, nitrogenous compounds in the rind section corresponded fairly closely to those occurring at the same time in the interior

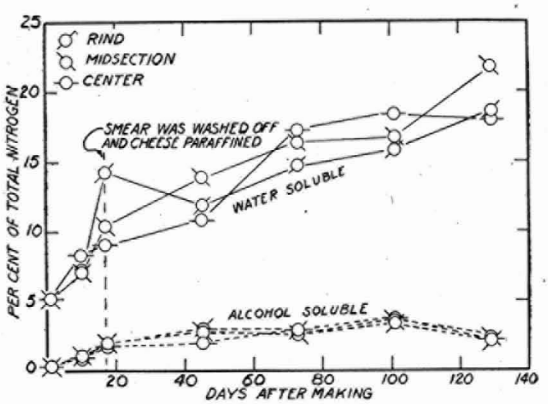

FIG. 3. Percentages of the total nitrogen in Brick cheese soluble in water and 80% alcohol during normal ripening.

sections. Perhaps the slightly higher percentages of these water-soluble compounds in the rind layer between the 10th and 17th days were actually caused by the presence of the luxuriant growth of smear; but the difference disappeared after the 17th day when the smear was removed and the cheese paraffined. Subsequent changes in percentages of water-soluble nitrogen seem to show that ripening might have occurred a little more slowly in the rind than it did in the inner sections, perhaps because of drying effects or more rapid loss of nitrogen in the form of ammonia.

Alcohol-soluble nitrogen in the water extract. Figure 3 shows the changes in solubility in 80 per cent alcohol of the water-soluble nitrogenous compounds in Brick cheese during the course of ripening. The data indicate that development of a normal surface smear did not increase this alcohol-soluble nitrogen fraction in the rind of the cheese. Such an increase in this nitrogen fraction would be expected if the cheese were ripened essentially by the action of the smear.

The similarity of the changes in water- and alcohol-solubility of the nitrogenous compounds throughout the cheese indicates that ripening of Brick cheese follows the same ripening pattern as that of the hard cheese discussed by Gratz and St. Szanyi (9).

EFFECT OF PROLONGED ACTION OF THE SMEAR

The surface flora on normal Brick cheese reaches the peak of its visible growth at approximately two weeks after salting. If nothing is done to prevent it, the smear tends to dry and form a sticky or paste-like coating. Growth practically ceases when the cheese is held at 45° F. or less. Some manufacturers wrap Brick cheese in metal foil shortly after the smear

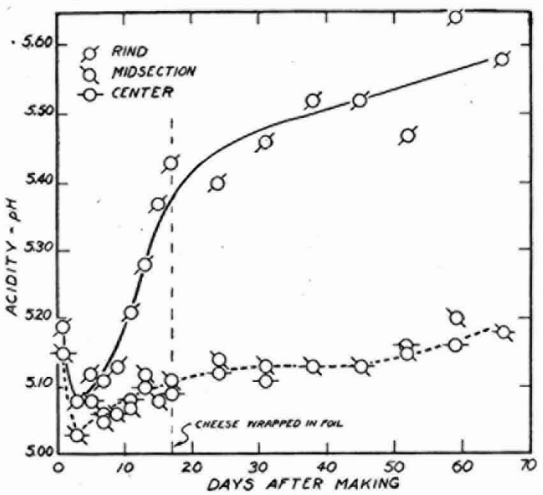

FIG. 4. Changes in acidity (pH) of the three sections of Brick cheese during ripening while wrapped and held at 58 to 60° F.

development reaches its maximum in order to preserve the characteristics of the luxuriant, active smear for several weeks. The foil tends to exclude air thus encouraging the growth of organisms with lower oxygen requirements. This is evident generally in the absence of all but traces of molds and in the development of a pronounced Limburger-like odor. This method of maintaining the maximum smear effect was used in these experiments. The cheese was cured in the normal manner during the first 17 days; it was then wrapped in parchment paper and an outer wrapper of metal foil. The wrapped cheese was held for the remainder of the experiment in the wet-curing room at 58° to 60° F. Parchment paper was applied underneath the foil wrapper because it maintained conditions more favorable for the normal aerobic organisms of the smear than a direct application of foil. The over-wrap of foil tended to limit oxygen and to retain moisture.

Figures 4 and 5 show the changes in acidity, water-soluble nitrogen, and alcohol-soluble nitrogen in the water extract of cheese wrapped and held at 58° to 60° F. Cheese from the same vat of curd that were given the normal curing treatment of washing, paraffining, and holding at 40° to 45° F., supplied the data for figures 2 and 3.

Acidity. Prolonging the action of the smear by wrapping and curing at 58°–60° F. produced a wider spread in pH between the rind and inner sections than did the normal curing process. This is shown by comparing figures 4 and 2 which are based on the data from the paired lots of cheese. The inner sections of the specially cured cheese and the normal lot showed practically identical increases in pH. These acidity changes were similar to those which occur in Cheddar cheese.

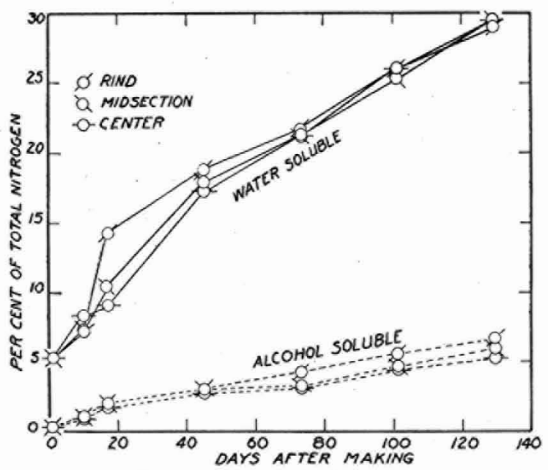

FIG. 5. Percentages of the total nitrogen in Brick cheese soluble in water and 80% alcohol during ripening while wrapped and held at 58 to 60° F.

Water-soluble nitrogen. The effect of the prolonged activity of the smear on the water-solubility of nitrogen compounds is shown in figure 5. Comparison of figure 5 with figure 3 shows that during the 129 days of aging the percentage of nitrogen in the water soluble form tended to be higher in the cheese subjected to the special treatment. It is significant, however, that these differences obtained *throughout* the cheese and *did not* progress from the rind inward. The differences between the normal cure and the special treatment can be attributed more to differences in curing temperatures than to exaggerated effects of surface flora activity. Freeman and Dahle (8), for example, recently have shown that the rate of proteolysis in Cheddar cheese ripening can be increased 40 to 100 per cent by raising the curing temperature from 45° to 63° F. Figure 5 illustrates increases well within the limits suggested by Freeman and Dahle for the temperature effect.

Alcohol-soluble nitrogen in the water extract. The result of prolonged activity of smear upon solubility in 80 per cent alcohol of the water soluble nitrogenous compounds in cheese is shown in figure 5. Comparison of figure 5 with figure 3 shows that after 129 days of curing under conditions of maximum and normal smear activity, there were no significant differences evident in the alcohol-soluble nitrogen fractions that could not be attributed to the temperatures of curing. The data indicate no significant tendency toward more decomposition of the nitrogen compounds in the rind of the cheese than in the center.

DISCUSSION

The presence of the heavy growth of microorganisms on the surfaces of Brick cheese during the first two or three weeks of curing is essential for the development of characteristic flavor. It has been pointed out (12) that organisms on the outside of the cheese may elaborate enzymes which gradually penetrate the cheese and bring about in the proteins the chemical changes associated with ripening. A second explanation of flavor development in Brick cheese would be that the characteristic and penetrating odor of the smear is absorbed by the cheese and is diffused through it. The results of the experiments reported in this paper seem to encourage this second explanation.

If differences in amounts of water-soluble and alcohol-soluble nitrogenous compounds in the cheese were caused by direct action of the microorganisms in the smear or by enzymes elaborated by them then it would seem that the three cheese layers studied should have revealed analytical evidence of ripening progressing from the surface to the center. Such progressive changes were not apparent in the percentages of total nitrogen in the water-soluble form nor in measurements of nitrogen in compounds soluble in water and 80 per cent alcohol.

The rapid increases in pH of the rind and simultaneous development of characteristic odors on the cheese surfaces, during both the normal and prolonged exposures to smear action, are indicative of the activity of the smear. If the pH of the inner layers had also increased like that of the rind after a reasonable lapse of time it might be assumed that ripening agents in the smear were penetrating the cheese—but this did not occur; the wide spread between the pH of the rind and that of the inner layers persisted throughout the ripening period.

The role of the surface flora in Brick cheese ripening seems to be limited to the surface layers. The characteristic pungent flavor which distinguishes this cheese from Cheddar probably is not formed by biological agents acting within the cheese but is produced in the surface layer or rind during the early stages of ripening. This pungent flavor gradually diffuses through the remainder of the ripening process.

CONCLUSIONS

1. The smear on the surface of Brick cheese appears only when the growth of several types of microorganisms occurs; yeast-like organisms develop first in large numbers and are succeeded by micrococci and by rod-shaped organisms of the *Bacterium linens* type. The growth of typical smear organisms on tryptone-glucose-extract-milk agar is somewhat inhibited by the low pH and high salt concentration characteristic of the surface of newly salted cheese.

2. The smear definitely reduces the acidity of the surface layer of cheese; this decrease is greatest during the period when the smear is developing luxuriantly.

3. The surface or rind sections of Brick cheese show no marked changes in water- and alcohol-soluble nitrogen-containing compounds that are not also apparent simultaneously in the inner sections. Under the conditions of these experiments there was no measurable, analytical evidence of ripening "from the outside" although the gradual diffusion of the characteristic surface flavor was very obvious.

4. Curing Brick cheese at 58° to 60° F. under conditions which favor greater activity of the smear gives the cheese more flavor and causes a faster production of water- and alcohol-soluble nitrogen, but even under these conditions the changes as measured by analytical methods appear simultaneously in all sections of the cheese.

5. It seems probable that the slight odor of extensive protein decomposition which distinguishes the flavor of normal Brick cheese is produced by the microorganisms growing on the surface of the cheese and that the characteristic odor itself is absorbed by the cheese.

6. These experiments suggest that the flavor of Brick cheese, and related smear-ripened types, may be controlled by regulating the development, activity and preservation of the smear and its characteristic odors.

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OUTLINE OF A NEW TECHNIQUE FOR DIGESTION TRIAL PROCEDURE

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At the Virginia Agricultural Experiment Station, during the last 30 years, a great deal of feeding research has been conducted with dairy cows, to determine digestibility and nutrient balances. In conducting such trials the research man knows that only by constant vigilance can reliable data be obtained. Relaxed vigilance may result in errors which cannot be corrected and elimination of data during a period in which an error occurs is likely to introduce another error of similar or even greater magnitude. Therefore, in this type of work at this station it has been found necessary to maintain a twenty-four hour supervision by a trained chemist, of the whole procedure of collecting and sampling the excrements.

Further, considerable attention has been given to the possibility of loss of nitrogen from the solid and liquid excrements when these are kept for twenty-four hours before taking samples for chemical determinations. Warm weather, warm rooms, unsterilized containers, volatilization and chemical changes may cause losses which may be very significant in evaluating the results. Therefore, in the procedure described below the sampling of all material for chemical analysis follows each voiding and the analytical samples are treated so that losses will not occur. The room in which the test stalls are located is equipped as a laboratory with chemical balances and other apparatus necessary to take all the samples for analysis immediately after each voiding.

GENERAL PROCEDURE

The test cows (usually two in number) are placed in digestion stalls about an hour prior to the start of the trial in order to get them accustomed to the surroundings. The trial continues over a five-day period, and the experimental day runs from 8 A.M. to 8 A.M. on the following day.

Previously weighed and sampled feeds are fed twice daily. Waste feed is collected at the end of the trial, weighed, sampled, and the samples stored in airtight containers for subsequent analysis. Each stall is equipped with a metered drinking trough.

One collector for each cow is on duty at all times and usually works on a twelve-hour shift. A chemist is present all the time and works on an eight-hour shift.

Each voiding of dung and urine is collected separately in a large (12-

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quart) dishpan and weighed immediately on a solution balance. The dung is mixed with a hand trowel and the urine is mixed with a stirring rod.

Twenty-four-hour Dung Samples for Nitrogen Determinations. After the dung is thoroughly mixed, a representative sample is weighed into a 40 × 80 mm. weighing bottle on a trip balance until an approximately 0.5 per cent sample is obtained, and then it is weighed accurately on an analytical balance. The contents are transferred to a weighed 32-ounce museum jar containing 200 ml. sulfuric acid (30 ml. conc. sulfuric acid per 1000 ml. distilled water). The empty weighing bottle is then weighed before being cleaned. A 0.5 per cent sample from each subsequent voiding during this twenty-four-hour period is likewise transferred to this jar. The sum of the weights of these samples gives the total amount (A) of dung for this twenty-four-hour period. At the end of the trial each of these samples is transferred to a Waring blender, ground for five minutes, quantitatively transferred with distilled water back to the original jar, and weighed (B). While being thoroughly mixed with a motor stirrer, duplicate samples of approximately 50 ml. are transferred, by means of an inverted pipette, to 40 × 80 mm. weighing bottles which have been weighed previously. These bottles and contents are weighed and the contents (C) transferred to Kjeldahl flasks for subsequent nitrogen determinations.

CALCULATIONS

A = Net weight of dung in jar (24-hour sample).

B = Net weight of jar contents (A + 200 ml. sulfuric acid and transfer water).

C = Net weight of (B) taken for nitrogen determination.

X = Weight of dung (A) taken for nitrogen determination.

$$\text{Then } X = \frac{A}{B} C.$$

Five-day Composite Dung Samples for Dry Matter, Proximate Constituents, Calcium and Phosphorus. A one per cent sample is taken for each voiding for the five-day period (in the same manner as described above for dung) and is transferred to a weighed enamel pan approximately 2 × 10 × 15 in. in size. At the end of the trial this material is dried in the steam drying box, weighed, ground, and subsequently analyzed for dry matter, proximate constituents, calcium and phosphorus.

Twenty-four-hour Urine Samples for Nitrogen Determination. After mixing the sample thoroughly, approximately a one per cent sample is transferred, by means of a pipette, to a 40 × 80 mm. weighing bottle and weighed in the same manner as described under dung. This sample is transferred to a weighed 16-ounce museum jar containing 25 ml. dil. sulfuric acid (1 + 4). At the end of the trial each of the five 24-hour samples is stirred with a motor stirrer and duplicate samples of approximately 5 ml. are transferred, by

means of a pipette, to small weighing bottles which have been weighed. These bottles and contents are weighed and the contents are quantitatively transferred with distilled water to Kjeldahl flasks for subsequent nitrogen determination. Calculations are made as for dung.

Five-day Composite Urine Samples for Calcium and Phosphorus Determinations. Another one per cent sample for each voiding for the five-day period is taken in the same manner as described above for urine, and transferred to a 32-ounce museum jar containing 5 ml. of formaldehyde. At the end of the trial the contents of this jar are quantitatively transferred with distilled water to a volumetric flask, made up to volume, and, while being stirred with a motor stirrer, appropriate aliquots are taken for calcium and phosphorus determinations.

ADVANTAGES

The chief advantages of this method over the old 24-hour composite sample method are as follows:

1. By providing a collector for each cow and a chemist at all times during the trial, proper collecting and sampling are assured.
2. Sampling each voiding separately gives a much better chance of obtaining a representative sample.
3. Taking care of samples immediately after being voided and keeping them under sulfuric acid prevents bacterial action and loss of ammonia.
4. Grinding the samples with a Waring blender results in a very fine homogeneous suspension, thereby insuring better sampling conditions.
5. The estimates of the variance and standard deviation in the per cent nitrogen as an indication of comparative accuracy of laboratory sampling by the old and new methods are as follows:

	Urine	*Dung*
New method—Estimate of variance $(\sigma)^2$	$=0.000119$	0.000032
Old method—Estimate of variance $(\sigma)^2$	$=0.000139$	0.000097
New method—Estimate of standard deviation (σ)	0.014	0.006
Old method—Estimate of standard deviation (σ)	0.019	0.009

In comparing the variance of per cent nitrogen in dung obtained by the old and new methods it was found that the variability of the new method for sampling dung when compared with that of the old gave a probability of less than 0.5 per cent and that the standard deviation was reduced from 0.009 to 0.006 by using the new method. The differences were so small for urine that they could be explained by chance fluctuations.

A PRACTICAL METHOD OF COLORING SEMEN FOR IDENTIFICATION PURPOSES

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In the operational aspects of artificial insemination of dairy cattle not infrequently the wrong sample of semen is used. This most frequently occurs when the semen of one breed is used to breed cows of another breed. This may not be serious in the grade herd but it may be serious in the pure-bred herd. A means of reducing this type of operational error would be of practical value.

Since certain vital dyes are apparently tolerated by the animal body without apparent harm a series of stains and dyes were tested for their effect upon bull spermatozoa in storage.

METHODS

The method used in these observations was the storage time of active sperm in yolk-buffer (1) in comparison with yolk buffer plus a suitable and sufficient concentration of a dye to distinctly color the diluent. Fresh semen was diluted 1:5 with the fresh egg yolk buffer prepared after the method of Phillips and Lardy (1). In all cases each gelatin capsule containing the Na_2HPO_4 and KH_2PO_4 was dropped into 50 cc. of redistilled H_2O, the buffer boiled gently for 3 minutes, cooled and an equal volume of fresh egg yolk added. Semen was then diluted with yolk buffer as indicated. Five per cent solutions of methylene blue, Nile blue sulfate, thionin, Janus green, alizarin sulphonate, neutral red and Sudan III were used. Enough of each dye was added dropwise to color the sample. It was impossible to dissolve the Sudan III in aqueous solution and a suspension of this dye was used. The diluted semen samples were stored in the refrigerator at approximately 5° C. Daily microscopic observations were then made on each treated sample until differential motility ratings were established. Motility rating was scored from 5 + for the best semen down to 0 for the completely inactive sample. In most cases observations were extended to 144 hours.

It is readily seen by the summary data of five experiments in table 1 that Nile blue sulfate, neutral red and Sudan III when added to the yolk buffer semen were without appreciable detrimental effect upon motility for several days. Neutral red and Sudan III were especially remarkable in this respect. Sudan III–treated samples were superior to yolk buffer alone in maintaining a high degree of vigor and motility. It appeared that the Sudan III through its affinity for free fat and its solution in the fat droplets

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TABLE 1

Summary data on the effect of added dyes upon sperm motility in yolk-buffer storage

Dye stuff	Hours							Color of semen sample
	0–5	24	48	72	96	120	144	
Methylene blue	5+	3+	3+	fm*	fm	fm	0	Light green
Nile blue sulfate	5+	5+	4+	4+	3+	2+	1+	Light green
Thionin	5+	5+	5+	2+	2+	1+	1+	Violet
Neutral red	5+	5+	4+	4–5+	4+	4+	4+	Red
Janus green	5+	5+	5+	3–4+	2	fm	0	Green
Sudan III	5+	5+	5+	5+	4+	4+	4+	Light orange
Yolk buffer (only)	5+	5+	5+	5+	4+	4+	3+	Yellow

* fm = few motile.

actually improved the storage medium. This dye was inferior in that its insolubility prevented the development of only an average tinge to the diluent. Nile blue sulfate in concentrations necessary to just tinge the storage pabulum was satisfactory but did not equal the other two dyes in any single test. Methylene blue, Janus green, thionin and alizarin sulphonate were not satisfactory. Janus green could be used for a limited time if necessary.

At the time these observations were made actual breeding tests could not be used as the final and more desirable evaluation. Since the storage data with the added dyes approximates the length of storage time with the undyed yolk-buffer it would seem that the use of "painted" semen would be equally satisfactory for impregnation purposes.

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VOLUMETRIC DETERMINATION OF MOISTURE IN DAIRY PRODUCTS

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The increased use of continuous systems for dairy product processing requires the use of faster analytical methods. This is particularly true of the determination of moisture in powdered milk where the toluol distillation method requires over an hour for completion.

A faster method being used to a great extent in some other industries is that proposed by Karl Fischer (3). This method makes use of the quantitative reaction between water and a solution of iodine, pyridine and sulfur dioxide in absolute methanol. Fischer applied his method to various organic liquids and to some solids. Since he used a visual end point, he could not successfully apply his method to colored substances.

Almy, Griffin, and Wilcox (1) showed that it was possible to obtain a potentiometric end point using platinum and tungsten electrodes which were periodically dipped in fused sodium nitrite. They found it necessary to add an excess of Karl Fischer reagent and back titrate with a standard solution of water in absolute methanol in order to get a sharp and reproducible end point. McKinney and Hall (7) present a complete electrometric apparatus using two platinum wires to indicate potential changes.

Richter (9) analyzed Edam cheese (obtaining low values) and margarine, using a visual end point. Kaufman and Funke (5) state that the method is applicable to butter dissolved in chloroform.

Johnson (4) recently has shown that such products as dehydrated vegetables required soaking 0.5 hour or longer in methanol prior to the addition of Fischer reagent. He also points out that ascorbic acid interferes, but that the amount present introduces only a very small error. He obtained good agreement between moisture obtained by the vacuum oven method and that obtained by the volumetric method on several dry foods.

Smith, Bryant, and Mitchell (10) report a study of the chemical reactions involved and state that Karl Fischer's reagent is believed to be a mixture of two binary compounds $C_5H_5NI_2$ and $C_5H_5NSO_2$.

EXPERIMENTAL

Preliminary trials with the various types of electrometric apparatus previously reported led to the glass-platinum system suggested by Lykken and Tuemmler (6). The glass electrode is used as a reference electrode, platinum wire is used as an indicating electrode, and the Beckman pH Meter, Laboratory Model, is used to detect the end point. When this system was tried with the Karl Fischer reagent, it was found to give sharper end points

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than any of the previously reported systems. It was also found that the end point was just as sharp when the sample was titrated directly with the Fischer reagent as it was when an excess of methanol was added and the end point determined by back titration.

When titrations were made directly with the reagent, the end point was taken as the point at which the needle remained stationary for 10 to 15 seconds after the voltage change.

The reagent was prepared according to the method of Almy, Griffin and Wilcox (1). Their apparatus was used as described but with the addition of a third burette system for dispensing absolute methanol. The Karl Fischer reagent available commercially has been used with equally satisfactory results, but is slightly more expensive.

Standardization of the reagent was carried out according to Wernimont and Hopkinson (11).

In all cases, the dairy products were weighed directly into a 100-ml. beaker, 20.0 ml. of absolute methanol added and the sample placed immediately in the titration assembly. The size of the various samples was chosen so that the sample contained about 100 mg. of water, except in the case of butter oil where a 20-gm. sample was used.

When back titration is used, moistures were calculated as follows:

$$\% \ H_2O = \frac{100\left(\begin{matrix}\text{ml. of Fischer reagent} - \text{ml. of std.} \\ \text{methanol solution} \times R\end{matrix}\right) \times FI_2}{\text{weight of sample}}$$

$$\text{Where } R = \frac{\text{ml. of Fischer reagent}}{\text{ml. of std. methanol solution}}$$

$$\text{and } FI_2 = \frac{\text{wt. of water (in 20.0 ml. abs. methanol)}}{\text{ml. of Fischer reagent} - \text{ml. of std. methanol solution} \times R}$$

When direct titration was used, the factor of the Fischer reagent is determined by adding a known weight of water (about 100 mg.) to 20.0 ml. of absolute methanol and titrating this solution with the Fischer reagent. Moistures were then calculated as follows:

$$\% \text{ water} = \frac{100 \times \text{ml. of Fischer reagent} \times FI_2}{\text{wt. of sample}}$$

$$\text{Where } FI_2 = \frac{\text{wt. of water (in 20.0 ml. absolute methanol)}}{\text{ml. of Fischer reagent}}$$

When analyzing dry milk, it is essential that the powder be thoroughly dispersed in the methanol before adding the Fischer reagent.

The toluol distillation method (2) was used as a basis for comparison with the Karl Fischer reagent on dry milk. Butter moistures were determined by the Mojonnier method (8) as were trials with other dairy products.

RESULTS

Table 1 shows the results of the determination of moisture in two samples of dry milk solids not fat. The first set were made by adding an excess of

reagent and back titrating with standard methanol. The second set were made by direct titration with the reagent. The average of the moisture tests made by back titration was 0.155 per cent higher than the toluol test; those made by direct titration averaged 0.01 per cent higher than the toluol test. The results obtained by back titration ranged from 0.12 per cent to 0.19 per cent higher than the toluol determination; while those made by direct titration ranged from 0.03 per cent lower to 0.09 per cent higher.

Table 2 shows the analysis of 26 samples of dry milk solids made by the toluol distillation method and by the volumetric method. These results were all secured by adding an excess of reagent and back titrating with a standard

TABLE 1

Comparison of direct titration and back titration for detection of end point

	Moisture found	Moisture above toluol
	per cent	*per cent*
Sample A (Dry milk solids)	3.54	0.12
(Toluene moisture = 3.42%)	3.61	0.19
Excess reagent added and	3.60	0.18
end point reached by back	3.54	0.12
titration with standard	3.61	0.19
methanol.	3.56	0.14
	3.55	0.13
	3.61	0.19
Sample B (Dry milk solids)	4.04	0.08
(Toluene moisture = 3.96%)	3.96	0.00
Sample titrated directly	3.97	0.01
with reagent.	3.97	0.01
	3.93	− 0.03
	4.05	0.09

solution of water in absolute methanol. The results of the volumetric method range from 0.13 per cent lower to 0.34 per cent higher, and average 0.076 per cent higher than the toluol method.

Table 3 shows the analysis of dairy products low in moisture content and those high in moisture content as determined by the Mojonnier method and by the volumetric method.

DISCUSSION

Direct titration offers several advantages over back titration for determining moistures with the Karl Fischer reagent. Standardization is simpler and a standard solution of water in methanol is not required. However, results are more erratic when using direct titration than when adding an excess of Fischer reagent and back titrating. The reason for this probably lies in the fact that the Fischer reagent is not stable. Thus, while titrating directly, the small excess required to produce an end point is rapidly destroyed, reversing the end point. That this change is going on even when

TABLE 2

Comparison of the toluol method and the volumetric method for determining moisture in dry milk solids

Sample	Toluol	Volumetric	Difference between volumetric and toluol
Spray dried non-fat solids	2.60	2.94	0.34
	2.66	2.85	0.19
"	2.62	2.74	0.12
	2.60	2.71	0.11
"	2.70	2.81	0.11
	2.70	2.70	0.00
"	2.76	2.94	0.18
	2.76	2.81	0.05
"	3.58	3.80	0.22
	3.64	3.80	0.16
"	3.40	3.49	0.09
	3.40	3.57	0.17
"	2.66	2.83	0.17
	2.70	2.82	0.12
"	3.70	3.76	0.06
	3.72	3.78	0.06
"	3.40	3.49	0.09
	3.36	3.51	0.15
"	2.46	2.62	0.16
	2.40	2.54	0.14
"	3.44	3.51	0.07
	3.46	3.53	0.07
"	3.60	3.66	0.06
	3.64	3.70	0.06
"	3.74	3.82	0.08
	3.76	3.83	0.07
"	3.66	3.76	0.10
	3.62	3.54	0.08
"	3.36	3.59	0.23
	3.40	3.66	0.26
"	4.04	3.98	−0.06
	4.06	4.03	−0.03
"	3.68	3.74	0.06
	3.68	3.70	0.02
"	3.48	3.57	0.09
	3.44	3.65	0.21
"	2.60	2.60	0.00
	2.58	2.66	0.08
"	3.96	4.04	0.08
	4.00	4.05	0.05
Non-fat solids roller dried	2.80	2.91	0.11
	2.80	3.04	0.24
"	3.90	3.84	0.06
	3.84	3.76	−0.08
"	3.82	3.92	0.10
	3.80	3.82	0.02
Whole milk powder	2.32	2.57	0.25
	2.34	2.56	0.22
"	5.40	5.27	−0.13
	5.40	5.36	−0.04
"	2.80	2.90	0.10
	2.82	2.93	0.11
Average	3.324	3.400	0.076

TABLE 3

Comparison of the Mojonnier method and the volumetric method for total solids in dairy products

	Product	Mojonnier	Volumetric
A. Products high in moisture content	Evaporated milk	25.96	23.0
		25.99	28.5
	" "	26.21	25.5
		25.15	28.6
	Skim condensed milk	30.11	26.4
		29.99	29.0
	Whole milk	13.25	13.8
		13.21	11.3
B. Products low in moisture content	Butter	15.91	15.76
		15.82	15.95
	"	16.49	16.48
		16.43	16.67
	"	15.66	15.68
		15.65	15.70
	" oil	0.15	0.16
		0.15	0.15
	" "	0.09	0.09
		0.09	0.09
	Sweetened condensed milk	70.92	71.5
		71.13	72.1

an excess of Fischer reagent is added and the solution back titrated is shown in the results given in table 1. Here, the samples titrated directly present a closer agreement with the toluol method, while those samples to which an excess of reagent were added show a higher moisture value. This may be due either to the rapid reaction of the Fischer reagent with moisture in the air or to reactions occurring within the solution which destroy its effective ingredients.

Table 1 also shows that either direct titration or back titration yields results which are satisfactorily reproducible for routine analysis.

Because conditions can be more easily controlled when back titration is used, the results given in table 2 were obtained in this manner. Those results listed which are lower than the toluol are probably due either to incomplete dispersion of the dry milk solids or to the presence of lumps in the sample. Those results listed which are more than 0.2 per cent higher than the toluol method, are probably due to prolonged titration allowing more opportunity for absorption of moisture from the air. It should be pointed out also, that the errors in the toluol method tend to be low owing to incomplete removal of all moisture during distillation, while the errors in the volumetric method tend to be high owing to the instability of the reagent.

The results shown in table 3 indicate that the volumetric method is unsatisfactory for dairy products containing more than 20 per cent moisture. This may be explained on the basis of: 1) the small size of sample permitted;

2) errors in determination of the end point; 3) interfering substances such as vitamin C and; 4) instability of the reagent after it has been added to the sample. While the amount of water in the sample is recovered with an error of only ± 3 mg., the effect of this error on percentage of water present becomes comparatively greater as the percentage of water in the sample increases.

Excellent results are secured when the volumetric method is used for determining the moisture content of butter oil. In this case, the amount of the sample is large, the amount of water is small, there are apparently no interfering substances present and the small errors in determining the end point are of little importance. Dry milk solids likewise give good results with the volumetric method except in this case vitamin C is usually present in small amounts. Butter gives satisfactory results; but, the Kohman moisture test is probably better for routine work. The volumetric method, however, would be of value in studying the distribution of moisture in butter because of its adaptability to micro-techniques. Sweetened condensed milk can be tested for moisture volumetrically with satisfactory results. In this instance, back titration has been found to be more reliable than direct titration, and it is necessary to exercise close control over the technique of making the determination.

The chief disadvantage of the reagent is its instability. Standardization daily or before use is essential. An attempt was made to prepare a solution of the reagent, store it for 8 months, and restore its lost strength by adding more iodine. Although this improved the stability, it resulted in a broad end point which was unsatisfactory. The cost of the reagent for a single analysis is about 20¢ but the complete test requires only a few minutes.

CONCLUSIONS

The Karl Fischer reagent was applied to the analysis of moisture in dairy products and found to be satisfactory for butter oil, butter, dry milk solids and sweetened condensed milk and it is not satisfactory for products containing more than 20 per cent moisture.

Direct titration with the Karl Fischer reagent was found to be satisfactory when a glass-platinum electrode assembly was used with a Beckman pH meter.

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VARIATION IN FAT, ASCORBIC ACID, AND RIBOFLAVIN CONTENT OF GOAT'S MILK*

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Goat's milk is very generally considered a food for infants and invalids, probably because many believe that its small fat globules and soft curd facilitate its digestion by persons with delicate digestive tracts. However, the present curtailed supply of cow's milk and milk products in Massachusetts has stimulated the use of goat's milk instead of cow's milk, particularly in families of foreign birth or ancestry. Coincident with the increase in the number of goats and in the number of consumers of goat's milk, there has been a constantly increasing demand for information regarding its nutritive value. A demand is also developing for additional information concerning the influence of feed, breed and management conditions upon the vitamin content of goat's milk. Accordingly this study was undertaken to accumulate additional data regarding the nutritive value of goat's milk produced in Massachusetts.

EXPERIMENTAL

Thirty-nine samples of goat's milk were supplied from various localities throughout the state. Samples 1 and 2 were pasteurized, but all the others were raw. Most of the samples were shipped to the laboratory carefully packed in cracked ice. The temperature of the milk on arrival was between 34° and 40° F. However, in three instances the ice had melted and the temperature of the milk was 50° F. A few samples were delivered at the laboratory by the producer.

Since data were desired regarding average goat's milk produced in this state, it was intended that the samples should represent a composite of milk produced by each dairy. However, approximately 60 per cent of the samples represent a single milking of one animal. Two samples were composites of milk from 65 goats, one sample was representative of milk from 50 goats, two samples were from 19 goats, and two were produced by herds of eight goats. Consequently it is assumed that the average results obtained from the assays of the thirty-nine samples are representative of the goat's milk consumed in this area.

The samples were produced by four breeds of goats, French Alpine, Nubian, Saanen, and Toggenburg, and a Saanen-Nubian cross. Some of the goats were grades, but the majority were pure bred. All were normal, healthy animals in good physical condition. Their ages varied from 1 to 12

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years. However, with few exceptions the animals were young and well matured. The stage of lactation varied from extremes of 10 days to 36 months. A number of the goats had just freshened and about the same number had been producing milk for a year or more, so the average stage of lactation was about 5 to 6 months. In general the goats were fed a ration of hay and a commercial concentrate feed. The variety and quality of hay is indicated in table 1. Several brands of commercial feed were used but, with three exceptions which are noted in table 1, all the concentrates were reported as supplying 16 per cent of protein. While nearly all dairy cows in this state receive corn or grass silage, none of the goats that produced milk used in this study received any silage. The goats that produced samples 28 and 36 were fed carnation clippings from a near-by greenhouse. Carrots were fed occasionally to the goat that produced sample 37, and the goats that produced samples 4, 5, and 27 were fed beet pulp. Many types of herbage were consumed by the goats that were allowed pasture, and ranged in nutritive value from browse of blueberry, birch, oak, maple, and sweet fern (sample 37) to good quality Ladino clover, timothy, and red top pasture.

ASSAY PROCEDURES

As soon as the samples were received, each was mixed, sampled, and tested for fat by the Babcock method (4). The rapid assay method reported by Sharp (5) was used to determine the ascorbic acid content. The method described by Holmes and Jones (1) was followed in the riboflavin determinations.

RESULTS

Fat. The fat content of the twenty-five samples of milk from the goats confined to the barn, varied from 2.2 per cent for sample 19 to 6.3 per cent for sample 15 with an average of 4.2 per cent. The 14 samples of milk from the goats allowed access to pasture ranged from 3.1 per cent for sample 30 to 6.5 per cent for 39 and averaged 4.5 per cent.

Reduced ascorbic acid. Eight or ten of the samples of milk from the goats that were confined indoors, contained an unusually small amount of reduced ascorbic acid. The values obtained ranged from 0.5 mg. for sample 2 (one of the samples of pasteurized milk) to 31.5 mg. for sample 17 and averaged 15.1 mg. per liter. The 14 samples of milk from goats that were given pasture in addition to hay and grain, contained more reduced ascorbic acid than those of the stall-fed goats. The smallest value was 12.5 mg. for sample 30 and the largest value was 27.5 mg. for sample 37 with an average value of 20.0 mg. per liter.

Since the first thirty-three samples arrived late in the day, it was not possible to assay them at that time and they were held overnight in a large refrigerator at 40° F. Thus the ascorbic acid was determined when the milk was two days old. The remaining six samples, 4, 5, 6, 22, 23, and 24, were

TABLE 1

Source and results of assays of goat's milk

Sample No.	No. of goats	Breed*	Age	Stage of lactation	Type of hay†	Type of pasture	Fat	Ascorbic acid	Riboflavin
					Stall-fed				
			yrs.	*months*			%	*mg./l.*	*mg./l.*
1	65	N.T.S.	5.0	12.0	Alfalfa and clover	None	3.7	2.0	1.24
2	65	N.T.S.	5.0	12.0	" " "	"	3.7	0.5	1.28
3	50	N.T.S.	5.0	12.0	" " "	"	3.5	1.5	1.19
4	19	S.A.T.	5.0	10.0	" " beet pulp	"	4.9	20.0	0.96
5	19	S.T.A.	5.0	10.0	" " " "	"	4.9	19.0	1.39
6	8	T.	3.5	4.0	Canadian clover and timothy	"	4.3	10.5	0.80
7	5	N.T.	5.0	5.0	2nd cutting mixed	"	3.0	21.5	.....
8	4	N.T.	4.0	6.0	Alfalfa, 2nd cutting	"	5.3	4.5	.....
9	4	N.	3.5	2.0	Early home-produced clover	"	5.1	21.5	.....
10	3	S.T.	6.0	8.0	Late cut mixed, not very good	"	4.3	16.5	1.09
11	2	T.	3.0	8.0	Alfalfa	"	4.2	14.5	2.35
Average for 11 herds			4.5	8.1	.....	.....	4.3	12.0	1.29
12	1	N.	3.0	2.0	Alfalfa, 2nd cutting	"	5.2	7.5	0.81
13	1	T.	5.0	12.0	" " "	"	5.1	3.0	1.02
14	1	N.	3.0	1.0	" " "	"	3.1	8.5	.....
15	1	N.	5.0	12.0	" " "	"	6.3	6.7	1.67
16	1	T.	4.0	1.0	2nd cut timothy, clover, red top	"	3.8	23.0	.....
17	1	N.	2.0	11.0	2nd cutting mixed	"	4.3	31.5	1.65
18	1	T.	3.0	11.0	" " "	"	2.3	12.5	1.17
19	1	T.	5.0	1.0	" " "	"	2.2	23.0	1.15
20	1	T.	10.0	1.0	" " "	"	3.3	17.5	1.68
21	1	N.	3.0	2.5	1st cutting clover	"	4.8	19.0	1.22
22	1	N.	5.0	14.0	Timothy and alfalfa	"	5.5	28.5	1.33
23	1	T.	4.0	8.0	Alfalfa and timothy	"	4.4	22.3	1.04
24	1	N.	2.0	0.5	Timothy and alfalfa	"	5.3	28.3	0.77
25	.....	.....	.....	.....	.....	"	3.7	13.5	0.99
Average for 14 individuals			4.2	5.9	.....	.....	4.2	17.5	1.21
Average for 25 samples			4.3	6.9	.....	.....	4.2	15.1	1.24

TABLE 1—(*Continued*)

Source and results of assays of goat's milk

Sample No.	No. of goats	Breed*	Age	Stage of lactation	Type of hay†	Type of pasture	Fat	Ascorbic acid	Riboflavin
Stall-fed plus pasture									
			yrs.	*months*			%	*mg./l.*	*mg./l.*
26	8	T.	5.0	6.0	Ordinary hay	Native grass	4.8	17.0	0.86
27	4	S.	6.0	6.0	2nd and 3rd cutting alfalfa	Ladino clover, timothy, red top	4.2	22.5	0.85
28	3	T.N.S.	4.0	8.0	2nd cutting and carnation clippings	Scrub wood lot leaves and buds	4.1	20.0	1.23
29	2	T.	9.0	6.0	Mixed	Natural grass	4.5	20.5	0.76
Average for 4 herds			6.0	6.5			4.4	20.0	0.93
30	1	T.	6.0	1.5	Timothy	Native grass	3.1	12.5	1.05
31	1	A.	3.0	1.0	"	" "	6.1	27.0	.....
32	1	S.	5.0	10.0	"	" "	4.0	20.5	.....
33	1	S.	2.0	1.0	"	" "	3.8	20.8	.....
34	1	T.	9.0	0.5	Poor grade timothy	Usual grass—no brush	3.5	.....	1.12
35	1	S.	4.5	0.5	Poor timothy	Witch grass	4.9	17.5	0.98
36	1	S.–N.	4.0	2.0	2nd cutting and carnation clippings	Scrub wood lot leaves and buds	4.3	16.5	.....
37	1	S.	2.0	2.0	Poor grade alfalfa	No grass—blueberry, birch, oak, and maple browse	4.4	27.5	1.01
38	1	S.	5.0	0.8	Timothy, clover early cut	Witch grass and Ladino clover	5.1	24.3	1.16
39	1	S.	10.0	1.0	Very poor timothy	Grasses in the yard	6.5	13.5	1.22
Average for 10 individuals			5.1	2.0			4.6	20.0	1.09
Average for 14 samples			5.3	3.3			4.5	20.0	1.02
Average for 39 samples			4.7	5.6			4.3	16.8	1.17

* N = Nubian; T = Toggenburg; S = Saanen; A = French Alpine.

† All the goats received grain (amount unreported) containing 16 per cent protein, except that the goats which produced samples 26, 38, and 6 received grain containing 18 per cent, 13 per cent, and 13 per cent protein, respectively.

titrated the day they arrived. Samples 22, 23, and 24 were titrated during the day that they were produced and should be representative of fresh milk from individual goats.

Riboflavin. Twenty samples of milk produced by stall-fed goats were assayed for riboflavin and showed considerable variation. The minimum and maximum values were 0.77 mg. and 2.35 mg. for samples 24 and 11 respectively, and the average value for the 20 samples was 1.24 mg. per liter. This value is identical with that previously obtained (2) for 18 samples of commercial winter milk produced by stall-fed goats, namely, 1.25 mg. per liter. The values obtained for milk from the goats that had access to pasture were quite variable and averaged 1.02 mg. per liter; the smallest value was 0.76 mg. for sample 29 and the largest was 1.23 mg. for sample 28. In any case it is apparent that the riboflavin content of the milk from the stall-fed goats was appreciably higher than that of the goats that had access to pasture of various sorts. This observation is in agreement with an earlier observation (3) that the riboflavin content of cow's milk showed a similar decrease, *i.e.*, from 1.43 mg. to 1.26 mg. per liter, when a mixed herd of cows was changed from a ration of hay, silage, and grain to a pasture of young, rapidly growing green grass. The riboflavin values obtained in this study as well as those of the previous report (2) indicate that goat's milk does not contain as much riboflavin as cow's milk.

While the data accumulated in this study are insufficient to permit an accurate comparison of the milk produced by the different breeds of goats, it is of interest to consider the relative value of the milks. Excluding samples 1, 2, 3, 4, 5, 7, 8, 10, 28, and 36, which represent a composite of milk produced by two or three breeds, there are 8 samples of milk from Nubian, 12 samples from Toggenburg, and 7 samples from Saanen. The average values for these breeds were: for Nubian, fat, 5.0 per cent, ascorbic acid, 18.9 mg., and riboflavin, 1.24 mg. per liter; for Toggenburg, fat, 3.8 per cent, ascorbic acid, 16.0 mg., and riboflavin, 1.18 mg. per liter; and for Saanen, fat, 4.7 per cent, ascorbic acid, 20.9 mg., and riboflavin, 1.04 mg. per liter. The fat content of Toggenburg milk was somewhat less than that of the other two breeds. The ascorbic acid and riboflavin content of the milk varied for the three breeds of goats and tended to be lower than for average cow's milk. Since the rations, age and stage of lactation of the different breeds of goats were not controlled, these values may not represent breed differences and probably should be considered merely as indications of the nutritive value of milk produced by the three breeds of goats.

SUMMARY

Thirty-nine samples of goat's milk produced in various localities in Massachusetts were examined for their fat, ascorbic acid, and riboflavin content. The milk was produced by four breeds of goats. They were from 1 to

12 years of age, and from the tenth day to the thirty-sixth month of lactation. All were fed a grain ration and some type of hay. Fourteen samples of milk were from goats that also received grass or other herbage. The milk of the stall-fed goats contained 4.2 per cent of fat, 15.1 mg. of ascorbic acid, and 1.24 mg. of riboflavin per liter. The other samples of milk averaged 4.5 per cent of fat, 20 mg. of ascorbic acid, and 1.02 mg. of riboflavin per liter. In general the riboflavin was lower and the fat and ascorbic acid content were not materially different from cow's milk. The significance of difference of the values obtained for the milk of the four breeds was discussed.

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SEASONAL VARIATIONS IN THE BLOOD PLASMA CAROTENE AND VITAMIN A OF ADULT DAIRY CATTLE

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Several investigators have reported on the carotene and vitamin A found in the blood of adult dairy cattle: Boyer, Phillips, and Smith (2), Braun (4), Davis and Madsen (5), and Duel, Hallman, Johnston, and Mattson (6). Most of these data, however, have been reported from experiments of short duration in which controlled experimental conditions were employed to measure the effects of definite variables. Braun (4) has reported monthly determinations over a period of a year on 49 cows in four dietary groups. One of these groups was carried for the entire year on non-irrigated, natural grass pasture of Strawberry Canyon, California, without vitamin A supplement. The other dietary groups either received a vitamin A supplement or were fed an unnatural type of vitamin A deficient ration. Conditions in this California area are quite different from those generally found in dairy sections of the North Central States.

In view of the growing interest in vitamin A in the nutrition of farm animals, particularly dairy cattle, it was decided to follow by monthly determinations the vitamin A and carotene in the blood of dairy cows through a full year. It was later decided to continue as many of these cows as possible through a second year. Determinations were also made for 11 consecutive months on a group of 16 bulls in service in the Northern Ohio Breeders Cooperative Association. The conditions under which these animals were maintained were quite typical of those found on many dairy farms throughout the North Central States. The results of this study are presented herein.

EXPERIMENTAL

Experimental animals. The experimental animals consisted of 24 cows in The Ohio State University Dairy Herd and 16 bulls in the artificial insemination stud of the Northern Ohio Breeders Cooperative Association. Six animals each of the Jersey, Guernsey, Ayrshire, and Holstein breeds made up the cow group; whereas the bull group consisted of 7 Holsteins, 5 Guernseys and 4 Jerseys. The entire group of 16 bulls were carried through the 11-month period. In the cow group, it was necessary to make two substitutions during the 2-year period for animals that were sold or otherwise disposed of.

Feeding of the animals. The experimental cows were fed and managed

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similarly to most dairy cows in the north central states. Each year they were turned on permanent bluegrass pasture on the first of May. During July and August, when these permanent pastures were short and dried up, some supplementary Sudan grass pasture was provided, together with barn hay feeding. The cows were taken completely off pasture during the latter part of October. Barn feeds consisted of alfalfa and mixed alfalfa hay and alfalfa and corn silages. The hay was somewhat variable in quality but would probably average better than that generally fed in Ohio. Concentrates were fed throughout the year.

The bulls were fed hay and a concentrate mixture. The amount of green feed and pasture provided was limited. No silage was fed.

It was assumed that the blood carotene values would be indicative of the carotene intake and no carotene determinations were made on the feeds consumed. The cows used in the study were all fed and managed in a similar manner except for such changes as are common at drying off and freshening.

Analytical techniques. Approximately 30 ml. of venous blood from the cows were drawn by puncture of the jugular vein into a brown-glass bottle containing 0.5 ml. of 20 per cent potassium oxalate. The samples were shaken and immediately taken to the laboratory for analysis.

The cows were bled at monthly intervals for the 24-month period. Shortly after the study was begun, unexplainable low values were obtained on certain individuals. A check of the records showed that these animals were closely approaching parturition or had recently calved. Consequently, in reporting the data of this experiment, values of samples drawn within a month prior to, or following, parturition were excluded. Data covering the effects of parturition and beginning lactation on blood vitamin A and carotene are being published in a separate report.

In the case of the bulls which are kept on a farm near Tiffin, Ohio, 15-ml. oxalated samples were shipped to the laboratory in a refrigerated semen-shipping carton. Approximately 24 hours elapsed from the time these samples were drawn until they were analyzed.

Blood plasma was obtained by centrifugation and the vitamin A and carotene extracted from a 5-ml. portion by the method described by Kimble (7). Purified petroleum ether was used throughout the experiment. Total carotenoids were determined by the method of Kimble (7), using an Evelyn photoelectric colorimeter with a 440 filter. The instrument was previously standardized with β-carotene crystals in purified petroleum ether solution.

When the carotene concentration in the petroleum ether extract was sufficiently great to give a galvanometer reading of less than 30, the vitamin A was determined by the method of Boyer, Phillips, and Smith (2). The samples were not saponified prior to solution in alcohol. Correction was made for the pigments (chiefly xanthophyll) not precipitated from the alcohol-water mixture.

With carotene concentrations giving galvanometer readings of 30 or above, Kimble's (7) method of vitamin A determination was employed. The Evelyn photoelectric colorimeter with a 620 filter was used and was previously standardized with crystalline vitamin A alcohol in chloroform solution.

As pointed out by Boyer, Phillips, and Smith (2), carotene concentrations are so great in certain samples of cattle blood that nearly all of the color produced in the Carr-Price reaction may result from the carotene present, thus preventing accurate vitamin A determination. Throughout this study, when blood-carotene concentrations exceeded 300 micrograms per 100 ml., carotene was separated from the vitamin A before carrying out the Carr-Price reaction.

Ascorbic acid determinations were made on the blood plasma obtained from the cows. The method of Mindlin and Butler (8) was used for this determination.

RESULTS

The average vitamin A and carotene concentrations by months for each of the four breeds of dairy cows are presented graphically in figures 1, 2, 3, and 4, and that for all breeds is combined in figure 5.

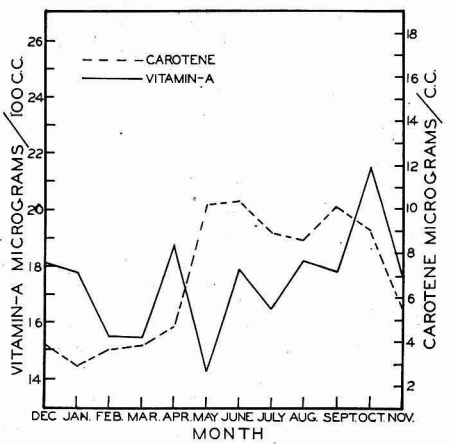

FIG. 1. Monthly variations in the blood-plasma vitamin A and carotene of Ayrshire cows.

Since the vitamin A and carotene followed the same general pattern each year, the data for the 2 years were combined for constructing these figures. That is to say, the peaks in carotene and vitamin A in the blood were reached in the same month each year, and, in general, the same was true for the low points, although not quite so uniformly for the separate breeds.

As stated previously the cows were turned on permanent bluegrass pasture on the first of May each year. The blood samples taken approximately

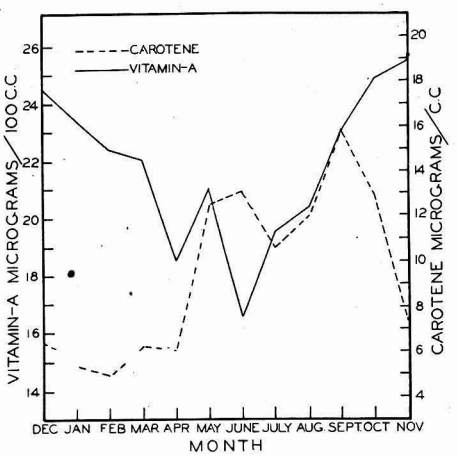

FIG. 2. Monthly variations in the blood-plasma vitamin A and carotene of Jersey cows.

2 weeks later showed a tremendous increase in carotene. A further small increase in carotene for the month of June is observed for all breeds except Holsteins. Carotene levels decreased from the June peak during July and August when the permanent pastures were short and dried up. With more favorable weather for pasture growth in September, the carotene reached the highest peak of the year. This occurred with striking uniformity in each breed and in each of the 2 years covered by this study (1943–1944).

The vitamin A values present a somewhat different picture. There seems to be a distinct breed difference in the response to the increased carotene

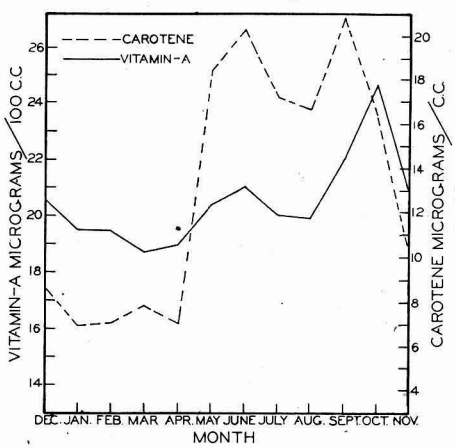

FIG. 3. Monthly variations in the blood-plasma vitamin A and carotene of Guernsey cows.

intake in the spring. For example, except for the Guernseys, there is a general downward trend in blood plasma vitamin A during May and June. This trend is marked in the case of Holsteins, and in both Holsteins and Jerseys the low point of the year is reached in June. The low point for vitamin A in Ayrshire blood plasma was in May.

Only in the case of the Guernseys was the increase in blood-plasma carotene accompanied by an increase in vitamin A during the lush spring-pasture season. The response, however, was not great. A possible explanation for this occurrence is offered by the work of Boyer *et al.* (1) in which they showed that a greater intake of carotene is required to maintain normal vitamin A levels in Guernsey calves than for other breeds studied.

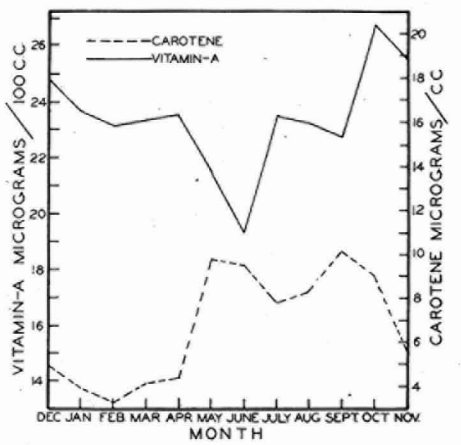

FIG. 4. Monthly variations in the blood-plasma vitamin A and carotene of Holstein cows.

As the pasture season progressed there was a general upward trend to the blood-plasma vitamin A values of all breeds. It is interesting to note that the peak in vitamin A was reached a month later than the peak in carotene for all breeds, except Jerseys, in which the vitamin A peak was reached 2 months following the blood-plasma carotene peak. Braun (4) has described a similar lag.

Figure 5, in which all the data obtained from all the cows over the 2-year period are combined, presents a general picture of what may be expected in the blood picture of well fed cows in the North Central States. Undoubtedly, a somewhat different picture might be expected in a group of cows having a lower carotene intake during the winter months. An average level of well over 600 micrograms of carotene per 100 ml. of blood plasma during January, February, March, and April indicates a substantial carotene intake during this period.

The ascorbic-acid determinations failed to show any definite seasonal

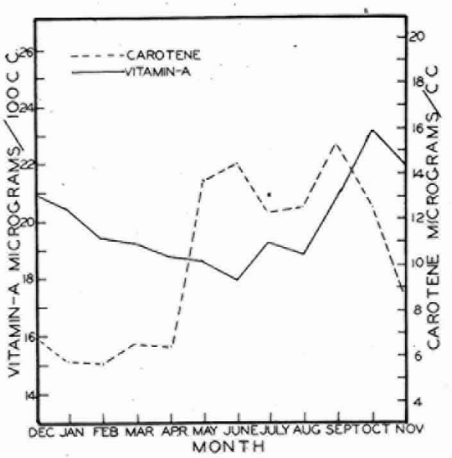

FIG. 5. Monthly variations in the blood-plasma vitamin A and carotene of 4 breeds of dairy cows. (Ayrshire, Jersey, Guernsey and Holstein combined.)

trends. Considerable variation was noted; however, as pointed out by Michigan workers (3), the causes of these variations are largely unknown. Values obtained varied from 0.2 to 0.7 mg. per 100 ml. and were in good agreement with those of others who have reported on the level of ascorbic acid in the blood plasma of adult cattle.

Figure 6 presents the data obtained from the bulls of the Northern Ohio Breeders Cooperative Association. The low level of carotene intake is reflected in the low level of blood-plasma carotene throughout the year. Even

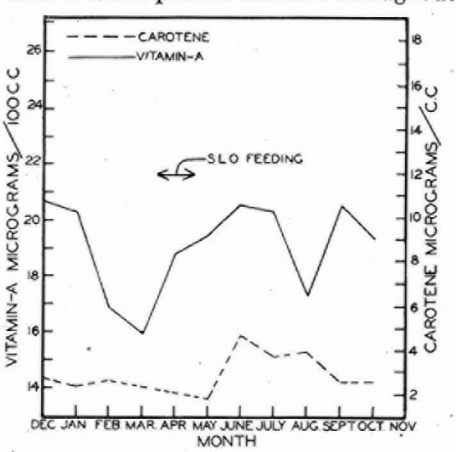

FIG. 6. Monthly variations in the blood-plasma vitamin A and carotene of 16 bulls maintained under the same system of feeding and management. (S. L. O. = shark liver oil feeding.)

when limited pasture was provided, the carotene level remained lower than that of the cows while on winter feed. The vitamin A also remained at a level lower than that found for the cows. The March determinations showed values so low (7 of the 16 bulls below 15 γ/100 ml.) that it was decided to provide a vitamin A supplement. These bulls were all being used to produce semen for use in artificial insemination, and the possibility of a lowered conception rate due to vitamin A deficiency was considered in making this decision. Consequently, approximately one fluid ounce of shark liver oil was fed daily beginning on March 15th and continuing for approximately one month.

The shark liver oil feeding produced an immediate response in blood vitamin A level but prevented an accurate measurement of the effects of increased carotene intake on the vitamin A level when some pasture was provided. It might be concluded that the increase in carotene intake did result in an immediate increase in blood vitamin A since the blood level increased from May to June simultaneously with the carotene increase. Although the shark liver oil feeding was discontinued approximately 2 months previous to the June blood determinations, a slight possibility of a carry-over effect remains.

DISCUSSION

Breed differences in ability to convert carotene to vitamin A are illustrated by the carotene lines in figures 1 to 4. The animals used for constructing these figures were fed the same roughages and pastured the same areas. The amounts consumed, though not accurately recorded, were on a roughly comparable basis. Listed in the order of increasing ability to convert carotene to vitamin A, the breeds are—Guernsey, Jersey, Ayrshire and Holstein. No difference of significance in this respect exists between the Ayrshire and Holstein, although when the level of vitamin A is considered it will be noted that a higher level of vitamin A is maintained in the Holsteins. Blood-carotene values are of little significance in evaluating the status of vitamin A nutrition in adult dairy cattle unless a number of other variables are also considered. Braun (4) has reported vitamin A : carotene ratios for animals on increasing carotene intake. It is quite evident from the data reported herein that considerable difference in such ratios would be obtained with decreasing carotene intakes abruptly following a period of high intake. A breed difference in the blood-plasma vitamin A response to the tremendous increase in carotene intake when cows are turned to pasture in the spring would also result in a breed difference in vitamin A : carotene ratio.

Breed differences in the level of vitamin A in the blood of cows maintained under comparable conditions are indicated in figures 1 to 4. Further evidence of these differences is presented in table 1 in which all the data for each breed have been statistically treated.

Although the number of breed representatives in each group is limited, the data strongly indicate breed differences in the normal level of vitamin A in the blood.

The highest value for blood-plasma vitamin A found in the 498 determinations was 35.5 micrograms per 100 ml. This is substantially lower than the maximum value of 65 micrograms per 100 ml. reported by Davis *et al.* (5). A possible explanation for the higher values reported by Davis *et al.* (5) may be found in the method of determination. The interference of

TABLE 1

Breed differences in the level of blood plasma vitamin A

Breed	Determinations	Mean vitamin A	Standard error
	Number	*γ/100 ml.*	
Ayrshire	121	17.7	± 0.43
Guernsey	128	20.5	± 0.37
Jersey	119	22.0	± 0.46
Holstein	130	23.1	± 0.36

carotene in vitamin A determinations has been pointed out (2). The method employed in this paper eliminates this source of error.

SUMMARY

Monthly blood-plasma carotene and vitamin A determinations on six mature cows each of the Ayrshire, Guernsey, Jersey, and Holstein breeds were made over a 2-year period. Monthly determinations were similarly made on 16 bulls over an 11-month period.

The data show wide fluctuations in blood plasma carotene, as might be expected. The different breeds respond similarly to changes in carotene intake but the extent of change in blood-plasma carotene varies greatly with the breed.

The blood-plasma vitamin A varies over rather narrow limits in comparison with blood-plasma carotene. The average monthly range, all breeds considered together, was from a low of 18 micrograms per 100 ml. in June to a high of 24 in October.

Breed differences in normal blood-plasma vitamin A are indicated.

Changes in blood-plasma vitamin A do not closely follow blood-plasma carotene changes but tend to lag behind. For example, maximum blood-plasma vitamin A values were found about one month after maximum blood-plasma carotene values.

ACKNOWLEDGMENT

We gratefully acknowledge the cooperation of Mr. Max Drake, Manager of the Northern Ohio Breeders Cooperative Association who provided the blood samples from the dairy bulls used in this study.

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DIGESTIBILITY OF COMMON LESPEDEZA HAY

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Common or Japanese lespedeza (*Lespedeza striata*) is one of the legumes most widely used for hay in the feeding of farm animals in Louisiana and in other regions of the South. It is also popular as a late-summer and early-fall pasture.

The feeding value and digestibility of hay from Korean lespedeza (*Lespedeza stipulacea*) have recently been reported by a few investigators (1, 5), but no studies have been noted on the digestibility of common lespedeza hay.

MATERIAL AND PROCEDURE

The hay used in this study was obtained from a farm approximately 30 miles north of Baton Rouge in East Feliciana Parish. The soil where the hay was grown is upland type of medium fertility. The field was fertilized with 400 lbs. of 4–12–4 fertilizer before oat planting in October 1943, and the common lespedeza was seeded in the oats in February 1944. The first cutting of hay was obtained during the blooming stage in late September 1944 and the second cutting during the early-seed stage in October 1944. The two lots of hay contained approximately 20 per cent foreign material, including grass, weeds, and oat stubble. The hay was chopped in a silage cutter to about 1-inch lengths. At each feeding a sample was obtained and at the end of the trial a composite sample representing the entire lot was taken for analyses.

Four dairy steers, ranging from 730 to 1000 pounds in weight, were used as experimental animals. Following a 10-day preliminary period during which a constant daily amount of hay was fed, a collection period of ten days was used. The steers were weighed on 3 successive days prior to and at the end of the collection period. Water was given twice daily and salt was available at all times. Each animal was kept in a box stall and brushed daily.

The feces were collected in a canvas collection bag as designed by Garrigus and Rusk with slight modifications (3), and were weighed prior to morning and afternoon feeding of hay. A 1/50 aliquot was taken from each day's fecal output for each steer and stored in a refrigerator at 0° C. until completion of the collection period. After mixing the feces, aliquots were taken for nitrogen determination, and the remainder was dried for several days on large trays in the laboratory over steam radiators. The excreta were then ground in a Wiley mill and sampled for analyses.

The A.O.A.C. methods (4) were used for analyses of the hay and feces for moisture, nitrogen, ether extract, crude fiber, ash, and nitrogen-free

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extract. Lignin and cellulose were determined by the method of Crampton and Maynard (2) and the usual methods were employed for the calculation of the digestion coefficients.

RESULTS AND DISCUSSION

Eleven to 16 pounds of hay were fed daily during the digestion trials. The steers refused very little feed and maintained their weights throughout the experiment.

TABLE 1

The composition, coefficients of digestibility, and digestible nutrients of common lespedeza hay at bloom and early-seed stages (dry basis)

	Dry matter	Crude protein	Crude fiber	N-free extract	Crude fat	Ash
	%	%	%	%	%	%
1. Bloom stage						
Composition	89.30	10.52	35.70	44.75	2.06	6.96
	Steer					
Coefficients of digestibility	96	48.82	49.40	63.78	43.07	.....
	97	48.64	44.22	64.05	40.76	.....
	98	46.99	29.38	59.66	24.10	.....
	99	46.08	44.90	66.17	38.39	.....
	Av.	47.63	41.98	63.42	36.58	.....
						T.D.N.
Digestible nutrients	.....	5.01	14.98	28.38	0.75	50.06
2. Early-seed stage						
Composition	84.98	8.55	38.26	46.59	1.72	4.88
	Steer					
Coefficients of digestibility	96	37.90	34.63	63.48	31.23	.....
	97	41.44	40.18	67.78	43.63	.....
	98	47.70	23.54	69.76	39.09	.....
	99	28.83	28.69	68.20	36.07	.....
	Av.	38.97	31.76	67.30	37.50	.....
						T.D.N.
Digestible nutrients	.....	3.33	12.15	31.35	0.65	48.28

The composition, coefficients of digestibility, and digestible nutrients of common lespedeza hay at the bloom and early-seed stages on the dry basis are presented in table 1. It can be noted that the early-cut hay (bloom stage) is the more desirable because of its higher percentages of crude protein, ash, and total digestible nutrients. The digestible nutrients were less in the more matured hay.

A comparison of the composition, coefficients of digestibility, total digestible nutrients and nutritive ratios of Korean and common lespedeza hay is given in table 2. In the bloom stage and early-seed stage the percentages of crude protein for the common lespedeza, 10.52 and 8.55, are much lower than those reported for the Korean lespedeza hay (1, 5) cut at similar stages of maturity, *i.e.*, 11.82 and 16.97 per cent and 13.28 and 19.27 per

TABLE 2

A comparison of the composition, coefficients of digestibility, total digestible nutrients and nutritive ratios of Korean and common lespedeza hay on the dry basis as reported by various investigators

Stage and kind of hay	Crude protein	Ether extract	Crude fiber	N-free extract	Lignin	Cellulose	Ash	
	%	%	%	%	%	%	%	
Early								
Korean (5)*	12.42	3.16	32.17	46.92	15.93	30.96	5.33	
" (1)	16.4	.....	.....	.....	.....	.....	.....	
Intermediate								
Korean (5)	11.84	2.37	34.09	46.22	19.97	34.50	5.48	
" (1)	16.9	.....	.....	.....	.....	.....	.....	
Common (present)	10.52	2.06	35.70	44.75	21.99	28.80	6.96	
Late								
Korean (5)	13.28	2.76	37.02	41.49	23.14	33.13	5.45	
" (1)	19.7	.....	.....	.....	.....	.....	.....	
Common (present)	8.55	1.72	38.26	46.59	22.43	30.83	4.88	
	Digestion coefficients						T.D.N.	Nutritive ratio 1:
Early								
Korean (5)	49.17	29.15	54.21	69.90	17.99	63.18	57.95	8.48
Intermediate								
Korean (5)	52.51	7.06	50.82	55.78	10.40	59.16	48.51	8.64
Common (present)	47.63	36.58	41.98	63.42	5.50	58.25	50.06	8.99
Late								
Korean (5)	41.03	10.18	49.92	46.03	10.60	56.34	43.66	7.01
Common (present)	38.97	37.50	31.76	67.30	3.28	50.13	48.28	13.49

* Numbers in parentheses refer to references.

cent, respectively. The other composition values compare favorably, with only a few exceptions. The lower protein content of the common lespedeza may be due partially to the foreign matter present in the hay and might result in a slightly higher lignin and cellulose content.

The total digestible nutrients for the two types of common lespedeza hay are similar to the values reported by other investigators for Korean lespedeza hay, although the nutritive ratios for the common lespedeza are wider than those for the Korean lespedeza (table 2).

The digestibility coefficients for lignin in the common lespedeza hay were 1/3 to 1/2 of those reported for the Korean hay. In their studies of the digestibility of Korean lespedeza hay Swanson and Herman (5) brought out the importance of the lignin content of a feed to its utilization. In this study the relationship between the lignin content and the T.D.N. values for the two types of common lespedeza also showed to some extent that as the lignin content increased the T.D.N. decreased.

SUMMARY

Two digestion trials using 4 dairy steers were made on common lespedeza hay, 80 per cent pure, cut during the bloom stage and early-seed stage.

The percentages of crude protein of the hay cut in the bloom stage and early-seed stage on the dry basis were 10.52 and 8.55, respectively. These figures are lower than those previously reported for Korean lespedeza hay. This may be due partially to the foreign matter present in the common lespedeza hay. The values found for the other nutrients compared favorably with those of Korean lespedeza hay. The digestibility of all the nutrients, with the exception of nitrogen-free extract, decreased in the late-cut hay.

The digestible protein and the total digestible nutrients for the dry matter in common lespedeza hay were found to be 5.01 per cent and 50.06 per cent, respectively, for the bloom stage, and 3.33 per cent and 48.28 per cent for the early-seed stage.

ACKNOWLEDGMENT

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DETERMINATION OF RIBOFLAVIN IN CHOCOLATE MILK AND THE COMPARATIVE PHOTOCHEMICAL LOSSES OF RIBOFLAVIN IN CHOCOLATE AND WHOLE MILK

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Several recent investigations have shown that the photochemical destruction of riboflavin in milk is quite rapid. Williams and Cheldelin (9) reported on factors which influence the destructive effects of light on riboflavin in liquid foods. Peterson, Haig and Shaw (5) found losses of riboflavin in pint glass bottles of whole milk exposed to direct sunlight of 28, 50, 66 and 72 per cent after 30, 90, 120 and 210 minutes of exposure respectively. Similar results were reported by Ziegler (10). Stamberg and Theophilus (7) report a loss of about 8 per cent in brown glass bottles or paper containers after a 4-hour exposure to sunlight, as compared to a loss of 40 to 50 per cent for milk in clear glass bottles. Holmes and Jones (4) studied the losses of riboflavin under various amounts of sunlight as measured by a recording pyroheliometer.

Considering the popularity of chocolate dairy drinks, it was proposed to study the effects of the added chocolate upon the riboflavin content of this beverage.

EXPERIMENTAL

The determination of riboflavin in chocolate milks by fluorometric methods is complicated by the presence of pigments introduced with the added chocolate mix. For this work several methods now in use for the determination of riboflavin have been modified as follows:

Procedure I. The procedure of Hand (2) was used without modification except that a Pfaltz and Bauer Model A Fluorophotometer was used. Standard riboflavin curves (fig. 1) were made from the photolyzed acetone filtrates in a manner similar to that described under Procedure II.

Procedure II. The method of Hodson and Norris (3) was modified as follows for use with milk: 50 ml. of approximately 0.1 N H_2SO_4 were mixed with 2 ml. of 2.5 M sodium acetate. To this was added 10 ml. of sample to be tested. This mixture was then filtered through a No. 1 Whatman filter paper into a dark container. Flasks, painted on the outside with black enamel, appeared to be satisfactory. An aliquot of the clear filtrate was placed in the cuvette of a Model A Pfaltz and Bauer Fluorophotometer for measurement of its fluorescence. The instrument was checked before each reading with a Pfaltz and Bauer glass riboflavin standard and the light intensity adjusted to the correct value by changing the setting of the iris

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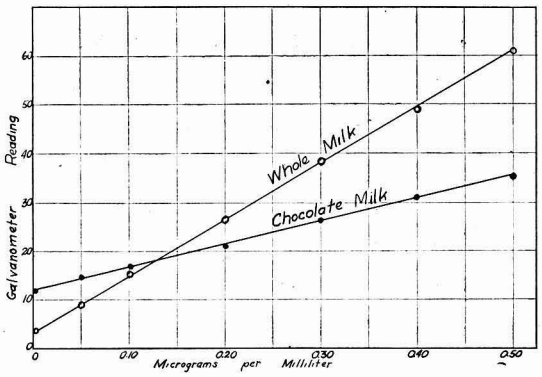

FIG. 1. Standard riboflavin curves prepared in photolyzed acetone filtrates.

diaphragm or the variable resistance in the galvanometer circuit. Standard riboflavin solutions were prepared by weighing 100 mg. of pure riboflavin (General Biochemicals, Inc.) into a 1000-ml. volumetric flask, dissolving in 5 per cent ethyl alcohol and making to volume with the same solution. This solution was diluted 1 to 10 with distilled water to give a solution containing 10 micrograms of riboflavin per milliliter. Standard riboflavin curves were made by adding aliquots of this solution to milk or chocolate milk filtrates which were prepared as described for fluorometric analysis and photolyzed by placing them in sunlight until they gave a minimum fluorometric reading. A range of solutions varying from 0.05 to 0.50 micrograms of riboflavin per milliliter was used. Fluorescence of these solutions was measured in the fluorometer in order to provide a standard curve (fig. 2) for use with milk samples. The fluorescent reading of the unknown was then translated to micrograms per milliliter by reference to this curve. Multiplication of this figure by the factor 6.13 (which allows for the volume

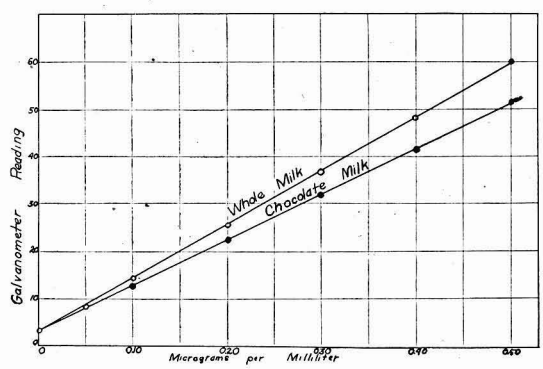

FIG. 2. Standard riboflavin curves prepared in photolyzed sulfuric acid filtrates.

of fat, 4 per cent, and casein, 3 per cent, after the manner of Hand (2) gives the micrograms of riboflavin present in one milliliter of original milk. The factors for chocolate milks vary somewhat, depending upon their respective fat content.

Procedure III. The method of Conner and Straub (1) was modified as follows: Milk filtrates were prepared as previously described, using 0.1 N H_2SO_4. The riboflavin in these filtrates were then absorbed on supersorb and eluted with 20 per cent pyridine in 2 per cent acetic acid. Fluorescence of these solutions were read in the fluorophotometer using the glass standard as a control. Standard curves for milk and for chocolate milk were prepared by passing standard solutions of riboflavin in photolyzed milk filtrates through the tower, eluting the absorbed riboflavin and determining their fluorescence as previously described. These curves, however, did not differ significantly from each other and are not shown.

Procedure IV. Bacteriological assays were made according to the procedure of Snell and Strong (6) and Strong and Carpenter (8) using Procedure II of the latter work for the preparation of the sample.

TABLE 1

Comparison of different procedures for assay of riboflavin

Procedure	Riboflavin micrograms per ml.	
	Whole milk	Chocolate milk
I. Acetone ppt.	1.85	2.38
II. H_2SO_4 ppt.	1.88	1.85
III. Supersorb abs.	1.80	1.78
IV. Microbiological	1.92	1.86

Sampling procedure. For the study of the comparative losses of riboflavin in whole and in chocolate milk the following procedure was followed: A sample of homogenized whole milk procured from The Ohio State University Dairy was used as a control each day. One sample of chocolate milk was obtained from the University Dairy. Samples of four other chocolate dairy drinks which are sold commercially in Columbus, Ohio, were obtained for comparison with these samples. These shall be designated as samples 1, 2, 3, and 4.

RESULTS

Comparison of procedures: In table I are recorded comparative values for the four different procedures.

Excellent agreement of results were obtained by all four procedures when whole milk was assayed. Equally good agreement among the latter three procedures was obtained for chocolate milk, but procedure I gave results which were too high.

Comparative photochemical losses of whole milk and chocolate milk. Be-

TABLE 2

Typical riboflavin contents of whole milk and chocolate milk upon exposure to sunlight, using procedure II

Time of exposure to sunlight	Riboflavin, micrograms per ml.					
	Whole milk	Chocolate milks				
		OSU	1	2	3	4
00	1.75	1.77	1.53	2.03	1.78	1.75
30 minutes	1.38	1.74	1.49	2.00	1.75	1.75
60 minutes	1.15	1.71	1.53	1.95	1.68	1.76
120 minutes	0.66	1.68	1.41	1.92	1.65	1.69
180 minutes	0.45	1.68	1.43	1.83	1.71	1.53
240 minutes	0.36	1.57	1.43	1.76	1.57	1.45

cause of its greater simplicity and rapidity, procedure II was chosen for the study of the comparative losses of riboflavin in chocolate and whole milks. Results of this study are shown in table 2.

This table records photochemical losses of riboflavin in whole milk similar to that reported in the literature and previously reviewed. The losses in chocolate milk, however, are comparatively negligible. Losses from the individual chocolate milks varied from 6.5 per cent to 17.2 per cent. Comparative riboflavin retentions for the whole milk sample and the average chocolate milks are shown graphically by figure 3.

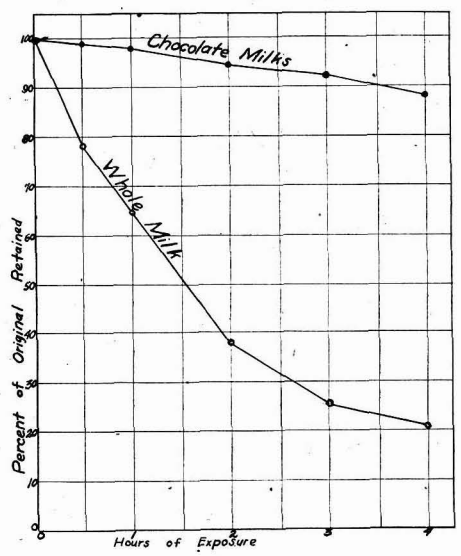

FIG. 3. Riboflavin retention in whole and chocolate milks upon exposure to sunlight.

DISCUSSION

The acetone precipitation method apparently fails for chocolate milk because it yields filtrates which are strongly pigmented. The influence of these pigmented filtrates upon the fluorescence of riboflavin is shown in figure 1. The slope of the standard curve prepared from photolyzed chocolate milk filtrates is much less than that of the corresponding curve prepared from similar whole milk filtrates, indicating that quenching of the fluorescence due to riboflavin occurs in the chocolate milk filtrates prepared by acetone extraction. The reason for the failure of this curve to give approximately correct values in spite of the low slope is not clear.

The slope of the standard curve prepared from photolyzed sulfuric acid filtrates of chocolate milk is slightly lower than that of the curve from the corresponding whole milk. However, this slope is steep enough to allow reasonable sensitivity for the method. Curves of the different chocolate milk filtrates investigated vary slightly from each other. Therefore, for the best results standard curves should be prepared for each brand of chocolate milk.

As might be expected, procedure III eliminates the effect of pigments in the chocolate mix. Standard curves prepared from photolyzed sulfuric acid filtrates, passed through the tower and eluted with the pyridine acid solution, were practically the same for chocolate and whole milk and for a sulfuric acid solution of riboflavin. Likewise the presence of pigments in chocolate milk apparently has no effect upon its microbiological assay.

The actual riboflavin content of chocolate milk is apparently only slightly lower than the corresponding unphotolyzed whole milk. Actual calculations based upon the increase of volume in the case of the University samples upon the addition of chocolate mix are slightly lower than those found. These may indicate that the chocolate mix contains some small amount of riboflavin. In any case chocolate milk as manufactured compares favorably to whole milk in riboflavin content.

The much slower photochemical loss of riboflavin in chocolate milk is probably due to the extra protection of the added pigment.

SUMMARY AND CONCLUSIONS

A rapid fluorometric method for determining riboflavin which is applicable to chocolate as well as whole milk is described. The method produces results which compare closely with those from other fluorometric methods and with the microbiological method.

Chocolate milks lose riboflavin very slowly when exposed to sunlight, the average loss of riboflavin after four hours for five different brands being about 12 per cent as compared to a loss of 80 per cent for whole milk.

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JOURNAL OF DAIRY SCIENCE

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ABSTRACTS OF LITERATURE

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MILK AND MILK PRODUCTS

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ABSTRACTS OF LITERATURE

BOOK REVIEW

365. Annual Review of Biochemistry. Vol. 14, 856 pages including author and subject index. Annual Reviews, Inc., Stanford University, P. O., California. $5.00.

This is the 14th of an annual series of reviews on selected subjects in the field of biochemistry by authors upon invitation of a reviewing board. It is a review of particular value and service to the research worker. None the less certain sections are of immediate importance to those engaged in other branches of the dairy industry. In general, each review attempts to include research on the subject since a previous review, and to present a brief appraisal of the various works in the light of available or correlating information.

This volume contains 28 reviews. Several cover selected phases in nutrition. These are: the metabolism of: fat, proteins and amino acids, minerals, sulfur and phosphorus; water-soluble and fat-soluble vitamins. The latter two sections include methods of assay, animal and human requirements, or applications. An interesting review of nutrition surveys with a critical appraisal of currently recommended dietary standards is presented. Other chapters include the chemistry of: carbohydrates, lipids, amino acids and proteins, nucleic acids and nucleoproteins, steroids, malignant tissue, hormones, bacteria, triterpenes and antibiotic substances. Other phases reviewed are: biological oxidations and reductions, carbon-nitrogen bond hydrolyzing enzymes, nonproteolytic, non-oxidative enzymes, animal pigments, detoxification mechanisms, nitrogenous fixation of plants, biological nitrogen fixation and mineral nutrition of plants. K.G.W.

BUTTER

366. The Keeping Quality of Tinned Butter. E. G. Port, Dairy Res. Sec. Jour. Council Sci. Indus. Res. Australia, *18*, No. 1: 53–61. Feb., 1945.

The essentially perishable nature of butter could not be modified enough by the methods attempted to make it suitable as a canned product for use in the Armed Services. Defects found were: rancid, cheesy and putrid flavors due to bacterial activity, tallowiness, and physical changes due to melting.

When storage was at summer room temperatures, bacterial defects could be controlled to a great extent by use of boric acid or by vacuum working plus salt in concentrations of 2.5 to 3 per cent and over. Even then tallowy flavors resulted from fat oxidation.

An increase of 15° F. in the pasteurizing temperature was not helpful, nor was reduction of the air content by vacuum processing. Copper, even as little as 0.06 to 0.17 p.p.m., was important, being correlated with peroxide and aldehyde values and final grades after three months of holding.

Ethyl gallate (0.02%) had marked antioxidant properties both in pure butter fat and in canned butter, but off-flavors still developed in the butter. Tallowiness was absent, however. W.C.F.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

367. A Colorimetric Method for the Estimation of Reducing Groups in Milk Powders. R. A. CHAPMAN AND W. D. MCFARLANE, Macdonald College, Quebec. Canad. Jour. Res., *23,* Sec. B, No. 3: 91. 1945.

"A method has been developed for the detection and estimation of certain reducing groups in milk powders. The method is based on the reduction of potassium ferri-cyanide at 70° C. and pH 5 and the colorimetric estimation of ferric ferro-cyanide. Titration with 2,6-dichlorophenol indophenol or potassium iodate failed to show any difference between fresh and stale powders. In contrast the ferri-cyanide test indicates much higher values in fresh powders than the titration methods, and it also reveals a marked increase in samples that have developed stale, musty odors and flavors. Lactalbumin and casein show reducing activity that increases on heating. Of the amino acids tested, only tryptophane gives a positive reaction. It is concluded that reducing groups are present in the protein molecule and become accessible on denaturation." Roller process whole milk powder or milk heated to high temperatures gave high values. O.R.I.

DISEASE

368. The Identity of Udder Streptococci in Seven Kentucky Dairy Herds. H. B. MORRISON AND F. E. HULL, Univ. of Kentucky, Jour. Bact., *49,* No. 5: 523. May, 1945. Proc. of Local Branches.

Milk samples were obtained during 1943 and 1944 from seven Kentucky dairy herds. Of 335 cows, 176 (52.6%) were giving off streptococci in their milk. From 139 cows, 144 cultures were identified by their biochemical characteristics and the Lancefield technique. Of these, 102 (70.8%) were *Strep. agalactiae,* 41 (28.5%) were *Strep. uberis,* and 1 (0.17%) were *Strep. dysgalactiae.*

These percentages agree in general with other reports. However, *Strep. agalactiae* was the major infectious agent in only three of the seven herds. In each of these three herds, which included the two largest herds in the study, more than 90% of the cultures were *Strep. agalactiae. Strep. uberis*

infection predominated in the other four herds. From one of these herds no cultures of *Strep. agalactiae* were identified.

The authors believe it to be more than mere coincidence that the three herds in this study having a high percentage of infections from *Strep. agalactiae* had only average or poorer quality of labor for milking and other herd care, whereas the herds with a low percentage of *Strep. agalactiae* infection as compared with *Strep. uberis* had much better than average quality of labor. D.P.G.

369. A Cheese-Borne Outbreak of Typhoid Fever. A. R. FOLLEY, Ministry of Health, Quebec, AND E. POISSON, Medical Officer, Victoriaville, P. Q. Canad. Jour. Public Health, *36,* No. 3: 116. 1945.

Details given of this outbreak which was traced to the consumption of infected, freshly made, Cheddar cheese. (Abstractor's Note—The maturing of all hard-pressed cheese for 90 days at not less than 45° F. for the remaining period is now required in Canada.) O.R.I.

370. Effect of Infused Penicillin in the Bovine Mammary Gland. F. J. WEIRETHER, D. E. JASPER, AND W. E. PETERSEN, Univ. of Minn. Proc. Soc. Expt. Biol. and Med., *59,* No. 2: 282. June, 1945.

Studies were made on the rate of decline and the irritating effects of the sodium salt of penicillin when mammary gland infusions were made via the teat canal. Aqueous solutions of penicillin containing 5,000 Oxford units per ml. had slight irritating effects on the normal bovine mammary gland as measured by physical examination of the gland, body temperature and changes in the character and quantity of milk. The largest dose infused was 40 ml. The introduction of penicillin in large volumes of water resulted in an increase in body temperature, tremors, and changes in the character of the milk. The largest volume infused was 950 ml. It was thought that these effects may have been due to the pyrogenic properties in the water used. In glands infused with small volumes of water containing 5,000 Oxford units per ml., the rate of decline of pencillin per ml. of milk was greatest during the first 10 hours, after which the decline was at a slower rate. The milk contained 0.5 or more units of pencillin per ml. 24 hours after the infusion of 30,000 or more units. Except in coliform infected glands the disappearance rate from pathologic glands was similar to that of normal glands. When large volumes of water were used the water was rapidly absorbed leaving most of the penicillin in the gland. R.P.R.

371. Non-permeability of the Lactating Bovine Mammary Gland to Penicillin. H. W. SEELEY, JR., E. O. ANDERSON, W. N. PLASTRIDGE, AND PATRICIA PEARSON, Storrs Agr. Expt. Sta. Science, *102,* No. 2637: 44. July, 1945.

The milk from a Jersey heifer, free of mastitis, was tested for penicillin

activity following intravenous injections of the calcium salt of penicillin. In the first trial 80,000 Oxford units of pencillin in 8 ml. of 0.95% saline were introduced into the jugular vein. In a second trial a total of 500,000 units were given in 2 portions of 15 ml. each about one minute apart. Prior to the injection the milk was removed from the udder. During the 6-hour period following the injection the heifer was milked for 3 to 4 minutes at intervals of a half-hour, and in the subsequent 6-hour period at intervals of an hour. A final sample was obtained 24 hours after injections. Penicillin activity was not observed in any of the milk samples obtained in the 24-hour period following injection. The samples secured during the first 6-hour period in the second trial were pooled and the cream and skimmed milk tested separately for penicillin. No penicillin activity was observed. Attention was called to the fact that these observations did not lend supporting evidence to the value of the intravenous injection of penicillin for the treatment of chronic bovine mastitis. R.P.R.

372. **Recent Advances In Mastitis Control.** C. S. BRYAN, J. W. CUNKELMAN, AND F. W. YOUNG. Mich. Agr. Expt. Sta. Quarterly Bul., Vol. *27*, No. 4. Article 27–47. 3 pages. May, 1945.

A general statement embodying up-to-date information on the problem. It stresses sanitary milking procedures and herd management practices, early and accurate diagnosis, elimination by slaughter or complete segregation from the herd of infected cows with badly damaged udders, and early and proper treatment. J.G.A.

FEEDS AND FEEDING

373. **Legume Silage for Dairy Cows.** S. A. HINTON, C. E. WYLIE, AND G. A. SHUEY. Tenn. Agr. Expt. Sta. Circular 89. 2 pages. May, 1945.

Describes a trial in which two ten-ton silos were filled, one with corn and soybeans, the other with soybeans to which phosphoric acid was added, at the rate of 18 pounds of 70 per cent acid per ton of green beans. Silage was of splendid quality in both cases, and proved to be very palatable to dairy cows. There was no significant difference in milk production of the cows on the two kinds of silage, although those fed the phosphoric acid-soybean silage gained less weight and produced slightly less milk and butterfat. J.G.A.

374. **Investigations of Methods of Preservation of Grass.** F. D. COLLINS AND F. B. SHORLAND, Anim. Res. Div., Dept. of Agr., Wellington, New Zeal. New Zeal. Jour. Sci. and Technol., *26A*, No. 6: 372–381. April, 1945.

To preserve grass for later chemical analysis boiling in alcohol for 20

minutes is recommended. Next best is treatment with boiling water followed by storage in an airtight container. To preserve by drying, air at 120° C. is used until the moisture content of the grass reaches 10 per cent after which the drying is finished at 60–70° C. The moisture content should be brought below 4 per cent and kept there. W.C.F.

ICE CREAM

375. Freeze Fruit Using Little or No Sugar. DONALD K. TRESSLER, Consultant, Westport, Conn. Ice Cream Field, *46*, No. 4: 14. 1945.

Cranberries and blueberries may be frozen without sugar or sirup, but better results are obtained with blueberries if frozen with sugar. Sliced apples do not require sugar, but they should be either blanched by steaming for 90 to 120 seconds and then rapidly cooled, or they should be treated with a 0.2% solution of sulfur dioxide or equivalent for 10 minutes and then kept cool for 8 hours before freezing.

Only a small amount of sugar is required for freezing the following: raspberries, loganberries, boysenberries, youngberries, dewberries, gooseberries and currents. Strawberries may be handled in the same way, but better results are obtained if at least one pound of sugar is used for six pounds of fruit. Slicing the berries ⅜ of an inch in thickness further improves the product. Peaches and apricots require either sucrose sirup, invert sirup, or "high-conversion" corn sirup to prevent browning. These sirups may also be used for other fruits. W.C.C.

376. Tests for Quality in Egg Pulp. II. Further Experiments on the Resazurin Rednetase Test. W. J. SCOTT AND J. M. GILLESPIE, Div. Food Preservation and Transport, Australia. Jour. Council Sci. Indus. Res. Australia, *17*, No. 4: 299–304. Nov., 1944.

Further tests on eggs from widely scattered areas showed the method satisfactory. The best temperature for the test was 30° C. and 25° C. was best for plate counts used in checking the results. W.C.F.

377. Further Bacteriological Studies Relating to Egg Drying. C. K. JOHNS AND H. L. BERARD, Science Service, Dept. of Agr., Ottawa, Canada. Sci. Agr. *25*, No. 9: 551. 1945.

Bacteriological examinations of successive carlots of Grade A egg powder during 1944 indicated that the numbers of micro-organisms as revealed by plate count were quite satisfactory but that from May to December many samples displayed high direct microscopic counts. While a few of these were the result of inadequate cooling of melange due to refrigeration failures, the majority were due to the inclusion of a small percentage of eggs,

which, while apparently normal in appearance and odor, yet contained enormous numbers of bacteria. Fluorescence under ultraviolet light was of limited value in the detection of such eggs. Holding the shell eggs at temperatures around 60° F. appeared to be responsible for this deterioration. *Achromobacter, Flavobacterium* and *Pseudomonas* types grow at these temperatures while organisms of the latter genus were found in inoculated eggs held at 58° F. for one week and 40° F. for seven weeks. O.R.I.

MILK

378. Cost of Producing Milk in Northern Illinois. R. H. WILCOX AND C. S. RHODE. Ill. Agr. Expt. Sta. Bul. 511. 24 pages, June, 1945.

Milk production per cow averaged 8,328 pounds yearly in the northern Illinois dairy herds included in this study—a high milk yield compared with that of all cows in the area. Feed expenses constituted 53.9 per cent and man labor 18.5 per cent of the net cost of producing milk. Thirteen other items made up the other 27.6 per cent of the cost. The higher producing cows required less feed and labor per unit of milk produced than the lower producing cows. The greatest feed variation was in hay and pasture; there was less difference in the amounts of protein feed and silage, and very little difference in the amounts of grain used. Fifteen per cent of the costs entering into the production of milk other than the costs of feed and labor, were found to fluctuate with feed costs and 45 per cent with farm wages.

The above facts concerning feed and labor costs supplied the basis for working out formulas and graphs by which the cost of producing milk in this area with cows of different levels of production can be computed closely enough for most practical purposes. The only variables in the formulas are the local price of shelled corn and the monthly cash wages of a hired man. 12 tables, 4 figures. J.G.A.

379. Approved Milking Parlors for the State of Washington. H. A. BENDIXEN AND L. J. SMITH. Wash. Agr. Expt. Sta. Bul. 461. 40 pages, illus. May, 1945.

Contains detailed instructions, plans, and bill of materials for the erection of various types of milking parlors. 19 figures, 7 illustrations. J.G.A.

PHYSIOLOGY

380. Growth of Mammary Glands of Hypophysectomized Rats Following Estrogen and Lactogen Administration. RALPH P. REECE AND JAMES H. LEATHEM, Rutgers University. Proc. Soc. Expt. Biol. and Med., *59*, No. 2: 122. June, 1945.

Forty-eight castrated and hypophysectomized rats were used in the

study. Estrogen and/or lactogen were injected subcutaneously once daily for 10 days and the animals were autopsied 24 hours after the last injection. Mammary glands were studied as whole mounts. Estrogen did not stimulate mammary duct growth. The injection of the lactogenic hormone caused a thickening of the duct system and in combination with estrogen it induced mammary duct growth similar to that seen in intact rats following estrogen injections. R.P.R.

381. Effects of Thiouracil on the Mammary Gland. J. F. SMITHCORS, Cornell University. Proc. Soc. Expt. Biol. and Med., *59,* No. 2: 197. June, 1945.

Eighty-five male and female rats of the Long-Evans strain with body weights ranging from 37 to 238 gm. were used. The administration of thiouracil either in an aqueous suspension in daily doses of 10 mg. per 100 gm. of body weight or in the drinking water as a 0.1 of one per cent solution for 18 to 35 days failed to induce changes in the mammary glands similar to that seen following thyroidectomy. Mammary glands of rats treated with thiouracil and with estrogen for the last 10 days of the experimental period possessed well-developed lobules and alveoli whereas the control glands showed end bud growth and duct extension. The administration of thyroxin along with thiouracil and estrogen produced glands similar to those of rats receiving estrogen alone. R.P.R.

382. Augmentation of Sheep Pituitary Gonadotrophin by Insoluble Metallic Hydroxides. W. H. MCSHAN AND ROLAND K. MEYER, Univ. of Wisc. Proc. Soc. Expt. Biol. and Med., *59,* No. 2: 239. June, 1945.

A study was made of the effect of aluminum, magnesium, iron and zinc hydroxides on increasing the effectiveness of relatively crude and purified sheep pituitary extracts, a follicle-stimulating preparation, and an extract of rat pituitary glands. The gonadotrophic preparations were tested alone in the same quantities as was used with the hydroxides in 21-day-old female rats. Aluminum, iron, and zinc hydroxides augmented the action of sheep pituitary gonadotrophin while magnesium hydroxide was less effective. Aluminum hydroxide did not augment the action of an extract of rat pituitary glands. The effect of these hydroxides was explained on the basis of adsorption of the hormone by the hydroxide with slow release of the active substance from the site of injection. R.P.R.

MISCELLANEOUS

383. Some Studies on the Properties of Rubber Used in Dairy Equipment. Part. II. W. A. WHITTLESTON, Anim. Res. Sta., Dept. of

Agr., Wallaceville, New Zeal. New Zeal. Jour. Sci. and Technol. *26A*, No. 6: 382–398. April, 1945.

The suitability of different rubber stocks and types of rubberware for use on milking machinery was investigated. W.C.F.

384. Medical Examination of Employees in Hotels, Restaurants, and Other Food Places. ARTHUR WILSON, M. H. O., Saskatoon, Sask. Canad. Public Health Jour., *36*, No. 3: 111. 1945.

Results experienced in the city of Saskatoon with a strict control plan for food handlers are given. Apparently dairy employees were not included, however. O.R.I.

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