JOURNAL OF DAIRY SCIENCE

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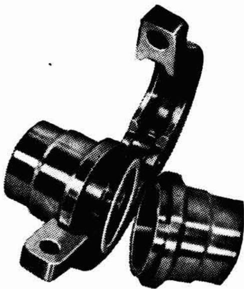

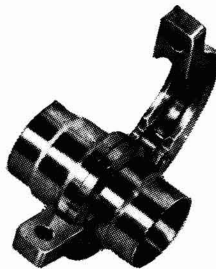

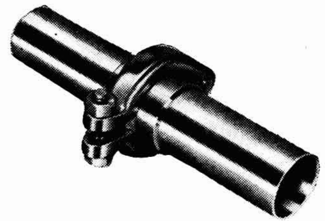

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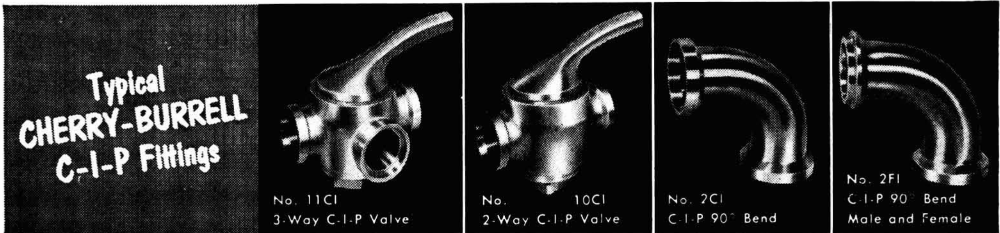

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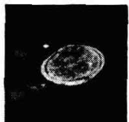

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ASSAY OF COWS' URINE FOR PREGNANEDIOL [1, 2]

D. L. HILL,[3] W. E. PETERSEN, AND S. H. COHEN

Division of Dairy Husbandry

University of Minnesota, St. Paul

The presence of pregnanediol in bovine urine has been reported *(3, 5, 7)*. Venning and Browne *(12)* isolated a water-soluble pregnanediol complex from the urine of women and were able to correlate its presence with an active corpus luteum. That progesterone is a precursor of pregnanediol was demonstrated experimentally by Muller *(6)*, who recovered 11.5 mg. pregnanediol from the urine of a hysterectomized woman who had received 30.0 mg. progesterone. Heard *et al. (2)* were able to recover pregnanediol in the urine of rabbits as a result of administering progesterone to them before and after hysterectomy.

Marker *(3)* obtained 25.0 mg. pregnanediol per gallon of pregnant cows' urine by hydrolyzing the butanol extract by steam distillation in the presence of strong alkali. Other reports, from Marker *(4)* and Marker *et al. (5)*, state that bull urine contained 100.0 mg. pregnanediol per gallon and that steers' urine contained none. Strickler *et al. (9)* used the method of Venning and were not able to find sodium pregnanediol glucuronidate in urine from bulls or steers. O'Moore *(7)* reported a concentration of pregnanediol in bovine urine ranging from 3.2 to 7.2 mg. per liter, whereas Stevenson *(8)* found that pregnanediol is either absent from the urine of pregnant cows or is excreted in quantities too small to be detected by the usual procedures.

It was the purpose of this study to ascertain the amounts of pregnanediol excreted in the urine of cattle and whether or not its presence could be used as an index of the activity of the corpus luteum.

MATERIALS AND METHODS

Urine samples were collected from cows in the third, fourth, fifth, sixth, seventh, and eighth month of gestation and examined for pregnanediol according to the procedure published by Astwood and Jones *(1)*. They found that this method gave good results on human urine when the concentration of pregnanediol

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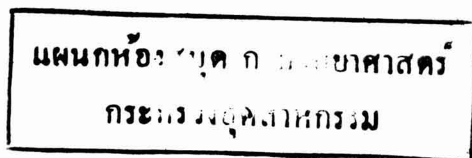
[2] Data presented in this paper are from a thesis submitted by the senior author to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree.

[3] Present address: Department of Dairy Husbandry, Purdue University, Lafayette, Indiana.



was more than 3.0 mg. per liter. An assay for sodium pregnanediol glucuronidate was made according to Venning *(11)*. This method is considered unreliable when there is less than 5.0 mg. per sample, but it can be used as a qualitative test for presence or absence. Since the chemical properties of bovine urine differ from human urine, recovery trials with pregnanediol and sodium pregnanediol glucuronidate were conducted to test the validity of using these methods. These procedures were also tested with human pregnancy urine. Further, the adaption of the Astwood and Jones procedure for colorimetric determination of pregnanediol by Talbot *et al.* *(10)* was used, and absorption spectra were made with a Beckman Model B spectrophotometer.

RESULTS

Using the absence of a characteristic whitish crystalline material on which to determine a melting point as negative for pregnanediol, the cow urines examined according to the Astwood and Jones procedure in this study were all negative. When the method was used on a 1.0-l. sample of human pregnancy urine, 18.3 mg. material with a melting point of 224-229° C. was obtained.

The efficiency of the method for recovering pregnanediol added to cow urine was tested by adding 15.0 mg. pregnanediol at different stages of the procedure

TABLE 1

The recovery of pregnanediol added to cow urine

Vol. of sample	Pregnanediol added	Pregnanediol recovered	Melting point[a]	Remarks
(ml.)	*(mg.)*	*(mg.)*	*(° C)*	
500 (water)	15.0	12.9	232-237	Without HCl hydrolysis
500 (urine)	15.0	10.2	232-235	Without HCl hydrolysis
500 (urine)	15.0	9.6	225-234	Pregnanediol added prior to HCl hydrolysis
500 (urine)	15.0	10.7	215-220	Pregnanediol added prior to HCl hydrolysis
500 (urine)	15.0	10.1	227-232	Pregnanediol added prior to HCl hydrolysis
500 (urine)	15.0	13.2	219-225	Pregnanediol after HCl hydrolysis
500 (urine)	Control	0.8		Waxy residue only

[a] Pure pregnanediol melts at about 235° C.

TABLE 2

The excretion of pregnanediol in pregnant cow urines

Vol. of sample	Stage of gestation	Pregnanediol recovered	Remarks
(ml.)	*(mo.)*	*(mg.)*	
3,000	4.0	None	Waxy residue only
250	5.0	None	Waxy residue only
1,000	6.0	None	Waxy residue only
2,500	7.0	None	Waxy residue only
850	8.5	None	Waxy residue only
250	8.5	None	Waxy residue only
250	ovariectomized	None	Waxy residue only

to aliquots of a 3,000-ml. sample. The results of the recovery trial are given in Table 1, and the results obtained on cow urine are given in Table 2.

Cows' pregnancy urines also gave negative results for excretion of pregnanediol as the water-soluble complex, sodium pregnanediol glucuronidate. The residues obtained were only waxy or oily appearing substances. The method recovered 33.9 mg. from 1.0 l. of human pregnancy urine. The results of recovery trials on cow urine to which sodium pregnanediol glucuronidate was added are given in Table 3, and a summary of the tests on pregnant cow urines is given in Table 4.

TABLE 3

The recovery of sodium pregnanediol glucuronidate added to cow urine

Vol. of sample	Sodium pregnanediol glucuronidate added	Wt. of residue recovered	Melting point [a]	Remarks
(ml.)	*(mg.)*	*(mg.)*	*(° C.)*	
2,500	46.6	36.9	242	
2,500	Control	9.2		Waxy residue only
1,000	20.0	16.8		
1,000	Control	4.8		Waxy residue only

[a] Pure sodium pregnanediol glucuronidate melts at 268-71° C.

TABLE 4

The excretion of sodium pregnanediol glucuronidate in pregnant cow urines

Vol. of sample	Stage of gestation	Sodium pregnanediol glucuronidate recovered	Remarks
(ml.)	*(mo.)*	*(mg.)*	
3,000	4.5	None	Waxy residue only
2,600	5.0	None	Waxy residue only
4,000	5.5	None	Waxy residue only
4,000	6.0	None	Waxy residue only
2,000	7.0	None	Waxy residue only

When the method of Talbot *et al.* *(10)* for the colorimetric determination of pregnanediol was used on cow urines, results indicating a concentration of 5.2 mg. pregnanediol per liter of urine were obtained regardless of whether the urine was collected from a pregnant cow or an ovariectomized subject. However, absorption spectra made on the residues extracted from these bovine urines do not agree with the absorption spectrum of purified pregnanediol. The absorption spectra obtained are shown in Figure 1.

DISCUSSION

The application to cow urine of methods suitable for determining the presence of pregnanediol in human urine did not reveal pregnanediol to be present in cows' pregnancy urines. It is desirable, however, to be mindful that the chemical composition and characteristics of cow urine and human urine differ greatly. Therefore, the methods developed for use on human urine may not be entirely effective when used on cow urine. It was observed that the initial extraction removed rather large amounts of material from the cow urine, but the purification

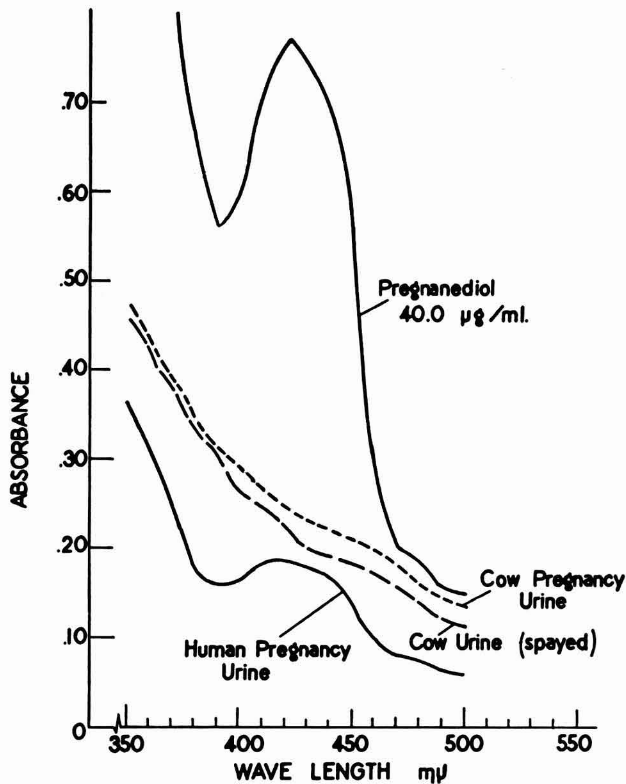


FIG. 1. Absorption spectra of residues extracted from various urines compared to pregnanediol.

procedure did not produce the characteristic whitish colored crystals. Since recovery rates were 59.4-62.2% when known quantities were added to cow urine at a concentration of 20-30 mg. per liter and compare rather favorably with 61.5-80.0% recovery rates which Astwood and Jones *(1)* report for their method, it does not seem likely that the negative results were obtained solely because any pregnanediol present was being adsorbed to the waxy material and lost in the purification procedure. No difficulty was encountered in applying the methods to human pregnancy urine, and the opinion is held that the methods should have detected pregnanediol in cow urine if it were present in weighable quantities.

The colorimetric determination for pregnanediol on cow urine gave results that would indicate the presence of small amounts of pregnanediol in bovine urine. The concentration of 5.2 mg. per liter is in good agreement with the concentration between 3.2 and 7.2 mg. per liter reported by O' Moore *(7)*. However, inspection of Figure 1 reveals that the color developed apparently was not not due to pregnanediol but more likely was the result produced by a mixture of steroidal compounds. It is also of interest that the concentration of these

compounds in the urine of a spayed cow did not differ greatly from the concentration found in the urine of a pregnant cow.

The results obtained in this study tend to concur with the findings of Stevenson *(8)*, who concluded that pregnanediol is either absent from pregnant cow urine or present in quantities too small to be measured by these procedures. Hence, it is apparent that the conversion of progesterone to pregnanediol and excretion via the urine is not the important pathway of progesterone metabolism by the bovine.

SUMMARY AND CONCLUSIONS

Cows' pregnancy urines were assayed for pregnanediol, using the gravimetric methods for recovering this substance from human urine. No pregnanediol was recovered. If present, the quantities were too small to be measured by these procedures.

REFERENCES

(1) Astwood, E. B., and Jones, G. E. A Simple Method for the Quantitative Determination of Pregnanediol in Human Urine. *J. Biol. Chem.*, **137**: 397. 1941.

(2) Heard, R. D. H., Bauld, W. S., and Hoffman, M. M. Steroids. V. Estradiol and Progesterone Metabolism. *J. Biol. Chem.*, **141**: 709. 1941.

(3) Marker, R. E. The Steroid Content of Cows' Pregnancy Urine. *J. Am. Chem. Soc.*, **60**: 2442. 1938.

(4) Marker, R. E. The Steroidal Content of Steers' Urine. *J. Am. Chem. Soc.*, **61**: 1287. 1939.

(5) Marker, R. E., Wittle, E. L., and Lawson, E. J. The Isolation of Pregnanediol from Bulls' Urine. *J. Am. Chem. Soc.*, **60**: 2937. 1938.

(6) Muller, H. A. The Excretion of Pregnanediol as an Indication of Corpus Luteum Function. *Klin. Wochschr.*, **19**: 390. 1940. (From *Chem. Abstr.*, **36**: 3828. 1942.)

(7) O'Moore, L. B. Sex Hormones in the Urine of Pregnant Cattle. *J. Comp. Pathol. Therap.*, **57**: 36. 1947.

(8) Stevensen, W. G. Pregnanediol Content of Urine Excreted by Cows and Mares during Gestation. *Am. J. Comp. Med. Vet. Sci.*, **11**: 25. 1947.

(9) Strickler, H. S., Walton, M. E., and Wilson, D. A. Failure to Find Sodium Pregnanediol Glucuronidate in Bulls' Urine. *Proc. Soc. Exptl. Biol. Med.*, **48**: 37. 1941.

(10) Talbot, N. B., Berman, R. A., MacLachlan, E. A., and Wolfe, J. K. The Colorimetric Determination of Neutral Steroids (Hormones) in a 24-Hour Sample of Human Urine. (Pregnanediol, Total alpha and beta Alcoholic and Non-alcoholic 17-Ketosteroids). *J. Clin. Endocrinol*, **1**: 668. 1941.

(11) Venning, E. H. Gravimetric Method for the Determination of Sodium Pregnanediol Glucuronidate (an Excretion Product of Progesterone). *J. Biol. Chem.*, **119**: 473. 1937.

(12) Venning, E. H., and Browne, J. S. L. Isolation of a Water-Soluble Pregnanediol Complex from Human Pregnancy Urine. *Proc. Soc. Exptl. Biol. Med.*, **34**: 792. 1938.

THE INFLUENCE OF DIETARY CALCIUM AND PHOSPHORUS ON THE INCIDENCE OF MILK FEVER IN DAIRY CATTLE

J. M. BODA AND H. H. COLE
Division of Animal Husbandry
University of California, Davis

This paper is concerned with the influence of various amounts and ratios of dietary calcium and phosphorus on the postpartum blood calcium and phosphorus levels of cows and with the development of a method for preventing a metabolic disease of dairy cattle, parturient paresis or milk fever. This method is based on three general assumptions: first, that milk fever results from a hypocalcemia induced by the rapid loss of calcium from the blood to the milk at the onset of lactation; second, that this hypocalcemia will not occur if the parathyroid glands secrete a sufficient amount of parathyroid hormone to mobilize calcium from the skeletal reserves, and, third, that a low-calcium high-phosphorus diet fed for some time before parturition will tend to lower blood calcium, thus causing a compensatory hypertrophy of the parathyroids such that at parturition sufficient hormone will be secreted to maintain a normal serum calcium level, irrespective of the calcium drain through lactation.

That milk fever is due to parathyroid insufficiency was first proposed, without experimental evidence, by Dryerre and Greig *(8)*. They reasoned that the disease probably resulted from the rapid loss of calcium from the blood stream to the mammary glands and postulated that the predisposing cause of milk fever might be parathyroid dysfunction on the basis of the following facts: The disease is intimately associated with the onset of lactation; it occurs most frequently in high producing cows; colostrum contains large amounts of calcium relative to the total circulating blood calcium; and tetany is often one of the early symptoms. As yet it has not been shown definitely that the parathyroids are involved in milk fever, but the majority of evidence indicates that this is true. It has been shown repeatedly that hypocalcemia is definitely associated with milk fever. Little and Wright *(27)*, the first to report low serum calcium levels in cows with milk fever, observed that the severity of the symptoms was roughly proportional to the fall in serum calcium. This finding has been confirmed by a number of investigators *(9, 11, 16, 36)*. Greig *(17)* demonstrated that the serum calcium levels of cows treated for milk fever by the method of udder inflation rise rapidly to normal and continue to above normal levels after recovery and that milk fever can be successfully treated by the intravenous injection of calcium gluconate. Seekles *(34)* injected sodium oxalate into cattle, obtaining hypocalcemia and symptoms similar to those of milk fever. Recent experiments by Niedermeier *et al. (32)* have demonstrated the importance of lactation in the drop of serum

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calcium at parturition. They found that the consistent decline of serum calcium which occurs in cows immediately after calving can be partially prevented by mastectomy.

In addition to the severe drop of serum calcium in milk fever, a marked decrease of serum inorganic phosphorus *(12, 20, 43)* and an increase in serum magnesium levels occur *(14, 20)*. The hypermagnesemia, in particular the lowered calcium-magnesium ratio, has been proposed as the cause of the coma which invariably accompanies acute attacks of milk fever *(20)*.

The essential role of the parathyroid gland in regulating the metabolism of calcium and phosphorus has been recognized since the original investigation of Collip *(6)*. Parathyroid hormone tends to maintain blood calcium at normal levels either by the direct dissolution of bone *(24, 39)* or indirectly by controlling the renal excretion of phosphate *(1)*. The secretion of parathyroid hormone in turn is determined by the levels of circulating blood calcium *(33)*. When the blood calcium falls, the parathyroids are stimulated; when it rises, they are depressed. Thus factors which tend to increase blood calcium levels will indirectly inhibit the parathyroids, whereas those which tend to lower blood calcium stimulate the parathyroids.

The relative amounts of dietary calcium and phosphorus influence markedly the size and, presumably, the activity of the parathyroids. Marine *(29)* demonstrated that a low calcium diet would cause parathyroid hypertrophy and hyperplasia in the fowl. Luce *(28)* confirmed this finding in rats. Since this early work, a large number of investigators have studied the influence of various dietary calcium and phosphorus levels on the activity of the parathyroids. Baumann and Sprinson *(2)* produced hypertrophy of the parathyroids in rabbits by feeding a low-calcium high-phosphorus diet. They reported an enlargement of the parathyroids of two to three times normal and an apparent increase in the circulating parathyroid hormone levels. Ham *et al.* *(18)* observed increased parathyroid size in rats fed a low-calcium high-phosphorus diet but not in rats fed a high-calcium low-phosphorus diet. De Robertis *(7)* submitted evidence that the parathyroids of rats fed a low-calcium diet exhibited increased cytological activity. Sinclair *(35)* found that a dietary calcium-phosphorus ratio of 1 : 5 increased gland size and that both hypertrophy and hyperplasia were involved. Stoerk and Carnes *(38)* found a direct proportionality between the logarithm of dietary calcium-phosphorus ratio and the serum calcium concentration over a wide range of dietary calcium and phosphorus and a close inverse relation between the logarithm of the dietary calcium-phosphorus ratio and the volume of the parathyroid glands of rats. Liegeois and Derivaux *(25)* demonstrated hyperparathyroidism in swine fed dietary calcium-phosphorus ratios of 1 : 5 to 1 : 7.3. Although changes in calcium requirements apparently do affect parathyroid function in ruminants, studies on the influence of dietary calcium and phosphorus levels on parathyroid size and activity in these species have not been reported. Campbell and Turner *(4)* have shown that the parathyroid glands of lactating goats are about 15% heavier than those of nonlactating controls.

EXPERIMENTAL METHODS

The animals used in this study were from a relatively high producing herd of purebred Jersey cattle in which there is a rather high incidence of milk fever. Only cows which previously had calved at least three times were employed. The cows were randomly divided into four groups over a period of about 2 years and were fed diets containing various amounts of calcium and phosphorus for varying periods before parturition. Immediately after calving, they were fed the normal herd ration used during lactation. The cows were individually fed in stanchions during the experimental period. Since roughages are high in calcium in comparison with the cereal grains, it was necessary to restrict the amount of such feeds the cows received in order to limit the total calcium intake. This, in turn, limited the amount of concentrate which could be fed, for if the roughage: concentrate ratio became too small, the animals bloated and would not eat.

The ration each group received was as follows: Group I, numbering 14 cows, received alfalfa hay ad libitum during the entire dry period of 2 to 3 months. The calcium: phosphorus ratio of alfalfa hay was approximately 6:1 (Table 1). Group II, 16 cows, received each day a low-calcium high-phosphorus diet consisting of 5 lb. of oat hay and 5 lb. of ground barley, to which was added monosodium phosphate at the rate of 1.3 lb. per 100 lb. barley. This ration was fed once a day in the morning. The daily intake of calcium and phosphorus amounted to 0.0135 and 0.0447 lb., respectively, the calcium: phosphorus ratio being 1:3.3. This ration was fed for periods ranging from 9 to 73 days before parturition (Table 2). Group III, 20 cows, received what is usually considered a balanced

TABLE 1

Cows fed alfalfa hay during the dry period (Ca:P ratio, approximately 6:1)

Cow No.	Age of cow *(years)*	Calving date	Symptoms of milk fever	Preceding lactation record *(lb. butterfat)*	Subsequent lactation record *(lb. butterfat)*	Serum Ca and P *(mg/100 ml)* 1st day postpartum Ca	P
288	11	7-18-51	—	452	540[a]	8.43	2.70
298	11	10-20-51	—	412	237		
350	10	9-24-51	—	365	......		
379	10	11-28-51	+[b]	604	773		
386	10	10-17-51	—	449	472		
397	9	10-18-51	—	492	435[a]		
413	9	8-24-51	—	559	295		
418	9	9- 1-51	+	350	......		
429	9	7-25-51	—	351	368		
519	8	6-13-51	—	355	392[a]	11.37	4.54
556	8	7- 4-51	+	616	......	5.59	......
598	8	7- 9-51	+	524	498[a]	4.35	0.90
607	8	8-25-51	+	312	336		
704	6	10-26-51	—	468	493		
			Mean	451	441		
			s	±45	±145		

[a] Corrected to 305-day record.
[b] + = Symptoms of milk fever.

TABLE 2
Cows fed a low-calcium high-phosphorus ration before parturition
(Ca:P ratio, 1:3.3)

Cow No.	Age of cow *(years)*	Calving date	Time fed experimental ration *(days)*	Symptoms of milk fever	Preceding lactation record *(lb. butterfat)*	Subsequent lactation record *(lb. butterfat)*	Serum calcium and phosphorus *(mg/100 ml)*			
							1st day postpartum		4th day postpartum	
							Ca	P	Ca	P
213	13	5-29-51	28	—	364	280[a]	9.82	4.64	10.32	5.85
233	13	11- 1-51	78	—	275	454	8.77	......	11.02	......
362	10	5-24-51	23	—	448	410	9.16	4.86	7.84	3.12
365	10	12-25-51	43	—	493	609	9.10	6.88	9.20	5.45
372	10	7 -4-51	47	—	412	416	7.81	2.52	9.78	5.62
382	12	2-17-53	22	—	468	......	9.70	5.13	9.48	4.73
416	9	12-19-51	37	—	321	335	12.13	......	10.37	4.81
491	10	3-19-53	19	—	474	......	......	......	......	......
593	8	10-29-51	73	—	290[b]	399	9.98	5.04	10.46	3.82
604	9	1- 3-52	52	—	422	464	......	......	......	......
643	7	7-20-51	33	—	541	500	9.35	4.59	11.67	......
650	7	9-30-51	9	—	454	465	9.60	5.58	10.28	5.00
660	7	7- 9-51	9	—	440	420	9.48	3.69	10.36	3.42
678	7	6-17-51	47	—	638	415	7.86	7.06	......	......
751	6	7- 8-51	68	—	439	388	11.11	5.71	10.96	3.82
758	7	2-22-53	27	—	385	......	9.53	4.17	10.55	3.42
				Means	438 ± 78	427 ± 79	9.53 ±1.12	4.99 ±1.26	10.18 ± 1.01	4.51 ±0.98

[a] Corrected to 305-day record.
[b] Mastitis.

ration as regards calcium and phosphorus. The daily ration consisted of 4 lb. of oat hay and 10 lb. of ground barley. To the latter, spent bone black was added at the rate of 2 lb. per 100 lb. barley. Half of this ration was fed in the morning, the other half in the late afternoon. The daily calcium and phosphorus intakes were 0.0744 and 0.0746 lb., respectively; the calcium:phosphorus ratio was 1:1. The cows received this experimental diet for periods of 5 to 45 days before parturition (Table 3). Group IV, 19 cows, received a high-calcium low-phosphorus diet consisting of 4 lb. of oat hay, 10 lb. of ground barley, and 6.25 lb. calcium carbonate per 100 lb. barley. The calcium carbonate was added in order to bring the calcium-phosphorus ratio of this ration to approximately the same value as that of alfalfa hay. The daily calcium intake was 0.264 lb.; the phosphorus intake, 0.0446 lb.; the calcium-phosphorus ratio of the diet was 5.9:1. Half of the ration was fed in the morning, the other half in the afternoon. The animals in this group received this ration 20 to 40 days prepartum (Table 4). All of the animals used in the experiment were fed alfalfa hay ad libitum and were pastured on permanent pasture during the interval between the start of the dry period and the time they were placed on the experimental rations.

In order to obtain information regarding the changes in blood calcium and phosphorus following parturition in the cows on the various diets and to diagnose definitely milk fever when outward symptoms occurred, blood samples were collected from most of the animals from the jugular vein by venous puncture on the first and fourth days after parturition. The blood was allowed to clot and

TABLE 3
Cows fed a high-calcium high-phosphorus ration before parturition (Ca:P ratio, 1:1)

Cow No.	Age of cow (*years*)	Calving date	Time fed experimental ration (*days*)	Symptoms of milk fever	Preceding lactation record (*lb. butterfat*)	Subsequent lactation record (*lb. butterfat*)	Serum calcium and phosphorus (*mg/100 ml*) 1st day postpartum Ca	1st day postpartum P	4th day postpartum Ca	4th day postpartum P
213	14	7-15-52	5	—	200	244[a]	9.98	4.59	8.38	2.88
230	14	3- 2-52	29	—	377	248[a]	......	......	......	......
282	12	8- 6-52	12	—	380	368[a]	12.12	......	9.82	4.53
302	12	4-30-52	12	—	428	305[a]	9.63	......	8.31	......
355	11	6-21-52	45	—	290	287[a]	8.80	......	9.70	......
357	11	8- 2-52	25	—	454	295[a]	8.38	7.20	9.45	3.87
362	11	6- 3-52	28	—	410	340[a]	7.10	4.74	11.10	3.97
380	11	4-20-52	30	—	412	372	9.15	5.26	11.43	4.41
413	10	8-23-52	28	—	295[b]	383[a]	9.36	5.17	7.95	2.52
512	9	5-14-52	9	—	430	320	9.60	3.78	9.80	4.93
519	9	8-13-52	17	—	505	493[a]	8.23	2.70	9.59	5.89
660	8	8-10-52	23	+[c]	419	353[a]	6.05	1.03	......	......
678	8	7-14-52	5	+	415	362	......	......	......	......
679	8	3-19-52	33	—	495	550	7.92	3.78	10.95	6.03
725	7	7- 4-52	22	—	541	585[a]	6.20	1.76	10.21	6.56
751	7	8-13-52	8	+	388	400[a]	6.38	0.63	......	......
756	6	8-28-52	35	—	402	276[a]	6.45	2.27	9.00	......
850	5	6-23-52	24	—	429	362[a]	7.80	......	10.50	......
7700	6	6-27-52	27	—	425	441[a]	......	......	8.90	......
FOV	6	8-28-52	33	—	568	382[a]	9.36	5.08	9.75	3.60
				Mean	423	368	8.68	4.43	9.68	4.47
				s	± 68	± 88	±1.28	±1.43	±0.99	±1.29

[a] Corrected to 305-day record.
[b] Mastitis.
[c] + = Symptoms of milk fever.

undergo syneresis. The serum was then decanted and shipped by parcel post. Sometimes several days elapsed between collection and the time the analyses were made. This led in some cases to considerable hemolysis, and in such instances serum inorganic phosphorus was not determined. Analysis of serum calcium was made by the method of Clark and Collip *(5)* and analysis for serum inorganic phosphorus by the method of Fiske and Subbarow *(13)*.

Three-hundred-five-day production records on twice daily milking were obtained for the experimental cows under the program of the Dairy Herd Improvement Association for the years immediately preceding and following the experimental period in order to determine whether or not the experimental ration had any influence on lactation. If lactation had not been completed at the time the data were collected, the production records were estimated to 305 days, using the correction factors given by Turner *(40)*.

RESULTS

Table 1 presents the results obtained from the cows receiving alfalfa hay during the entire dry period. Five of the 14 animals exhibited clinical symptoms of milk fever shortly after parturition. Only a few blood samples were collected

TABLE 4

Cows fed a high-calcium low-phosphorus ration before parturition (Ca:P ratio, 5.9:1)

Cow No.	Age of cow *(years)*	Calving date	Time fed experimental ration *(days)*	Symptoms of milk fever	Preceding lactation record *(lb. butterfat)*	Subsequent lactation record *(lb. butterfat)*	Serum calcium and phosphorus *(mg/100 ml)*			
							1st day postpartum		4th day postpartum	
							Ca	P	Ca	P
55	11	12-11-52	20	—	504	......	9.60	......	......	......
233	14	1- 3-53	40	—	454	......	8.50	5.16	9.07	4.39
237	13	12-22-52	32	—	443	......	9.80	3.19	9.13	6.76
243	13	1-14-53	32	—	421	......	8.50	......	......	......
303	12	9-18-52	22	—	429	462[a]	......	......	......	......
352	11	12-15-52	27	+[c]	422	......	7.30	2.70	......	......
359	11	9-13-52	38	—	306	150[b]	9.87	......	......	......
386	11	1-22-53	39	—	472	......	8.82	2.97	10.30	5.18
397	10	11- 1-52	31	+	413	425[a]	6.40	3.29	......	......
407	10	11-28-52	29	+	644	475[a]	4.90	1.30	......	......
593	9	10-29-52	31	—	399	450[a]	8.55	5.63	9.87	6.13
604	9	1-18-53	36	+	464	......	6.20	1.96	......	......
607	9	10-21-52	22	—	336	536[a]	......	......	9.10	5.30
696	7	10-29-52	22	—	336	406[a]	8.45	3.91	11.17	5.20
704	7	11-12-52	33	—	492	485[a]	8.30	4.13	10.50	4.75
708	7	1-18-53	36	+	515	......	9.32	......	......	......
726	7	12-26-52	32	—	589	......	10.20	5.17	9.55	4.93
784	6	10-24-52	30	—	431	492[a]	7.90	3.82	10.17	4.30
786	6	12- 7-52	23	—	455	......	8.15	4.37	......	......
				Mean	448	476	8.89	4.26	9.87	5.22
				s	± 88	± 38	±1.03	±0.91	±0.68	±0.78

[a] Corrected to 305-day record.
[b] Mastitis.
[c] + = Symptoms of milk fever.

from the animals of this group, not enough to show any trends in serum calcium and phosphorus. There was no difference in the average amount of milk produced for the years immediately preceding and following the experimental period.

The data obtained from the cows receiving the low-calcium high-phosphorus diet before parturition appear in Table 2. None of the 16 animals in this group developed milk fever. There was a slight fall in serum calcium and a rise in serum inorganic phosphorus at parturition; the values returned to normal by the fourth day postpartum. These changes in calcium and phosphorus are in line with those reported in the literature for normal cows, that is, cows which do not develop milk fever *(3)*. Comparison of production records made before and after the experimental period show that the prepartum ration, although deficient in protein and total digestible nutrients compared with the Morrison Feeding Standards *(31)*, had no appreciable influence on the subsequent lactation. In calculating the means and standard deviation of the means, the production records of the cows with severe mastitis were excluded in this table, as in following ones, because of the marked influence of this disease on lactation.

Table 3 presents data obtained from the cows fed the high-calcium high-phosphorus ration before parturition. Three of the 20 cattle in this group developed clinical symptoms of milk fever (Cows 660, 678, and 751). However,

two of these animals (Cows 660 and 751) received the experimental ration for only a short time, and since they were fed alfalfa hay for the majority of the dry period, they probably should be considered comparable with the animals in Table 1. Analysis of the serum collected from these two cows on the day of parturition showed that a rather severe hypocalcemia had occurred, confirming the original diagnosis of milk fever. In addition to the three cows which exhibited clinical manifestations of milk fever, three others of this group (Cows 362, 725, and 756) were hypocalcemic on the day of parturition, but outward symptoms of milk fever were not evident. Possibly the lowered blood calcium was only transitory, not of sufficient duration to cause the typical syndrome associated with milk fever. Determination of the means and standard deviations of the serum calcium and phosphorus levels of this group for the first and fourth days postpartum, excluding the cows which exhibited outward symptoms of milk fever, led to calcium values which are approximately 1 mg. % and 0.5 mg. % lower than similar values for the cows in Table 2. However, the variation between individuals is too great for the differences to be statistically significant. There is no appreciable difference in the average serum phosphorus levels between the first and fourth days postpartum. There is also no difference in this respect between the cows in this group and those which received the low-calcium high-phosphorus diet before parturition. Comparison of the average lactation records preceding and following the experimental period indicates a decrease in production, although the difference between means is not significant. It is doubtful that this drop in production was due to the experimental ration, since the majority of the cows in this group were on experiment less than 1 month. A more plausible explanation is the fact that most of the production records for the year following the experimental period were not complete when the data were collected. These incomplete records were corrected to 305 days, using correction factors *(40)*, whose derivation appears to be based on animals with less lactation persistence than those used in this experiment.

Table 4 presents data obtained from the cows fed a relatively high-calcium low-phosphorus ration. Five of the 19 cows in this group developed milk fever. Analysis of the blood collected on the day of parturition from four of these five cows (Cows 352, 397, 407, and 604) demonstrated that they were hypocalcemic. The serum calcium level of the fifth cow was normal on the day of parturition (Cow 708). This apparent discrepancy can be explanied by the fact that this particular animal did not develop clinical symptoms of milk fever until several days postpartum, some time after the blood sample was collected. Determination of the means of the calcium levels of serum collected on the first and fourth days of parturition led to values similar to those obtained from the cows of Group III. Here again, however, the difference between the means is not statistically significant. There was no difference between the production records for the years preceding and those following the experimental period. Records made during the latter period were all incomplete and, except for lactations covering less than 3 months, were converted to a 305-day basis, using the correction factors already referred to.

Several of the cows fed the low-calcium high-phosphorus diet before parturition (Table 2) also received either the high-calcium high-phosphorus ration or the high-calcium low-phosphorus ration during the subsequent dry period. The data obtained from these eight cows for the two successive years are compiled in Table 5. Four of the eight cows which did not develop milk fever when fed a low-calcium diet were affected with the disease when they received a high-calcium diet before parturition. Three of the cows (Cows 213, 678, and 751) were on experiment only a short time the second year. Since they received alfalfa hay with a calcium-phosphorus ratio approximating 6:1 for most of the dry period, they probably can be considered comparable with those receiving the diet containing 5.9:1 calcium-phosphorus ratio. The mean serum calcium levels were approximately 1 mg. and 0.5 mg. % lower on the first and fourth days postpartum, respectively, when the cows received a high-calcium, as compared with a low-calcium, ration. From this it appears that a low-calcium diet tends to prevent a fall in serum calcium at parturition and beginning lactation.

A summary of the results obtained for the cows fed the various rations is presented in Table 6. The data indicate that the low-calcium high-phosphorus diet has effectively prevented milk fever and that the incidence of this disease

TABLE 5

Cows fed a low-calcium ration during one dry period and a high-calcium ration the subsequent dry period

Cow No.	Calving date	Time fed experimental ration (*days*)	Experimental ration Ca:P ratio	Symptoms of milk fever	Preceding lactation record (*lb. butterfat*)	Subsequent lactation record (*lb. butterfat*)	Serum calcium and phosphorus (*mg/100 ml*) 1st day postpartum Ca	1st day postpartum P	4th day postpartum Ca	4th day postpartum P
233	11- 1-51	78	1:3.3	—	275	454	8.77	......	11.02	......
604	1- 3-52	9	1:3.3	—	422	464	......	......	......	......
593	10-29-51	73	1:3.3	—	290[b]	399	9.98	5.04	10.46	3.82
213	5-29-51	28	1:3.3	—	364	280[a]	9.82	4.64	10.32	5.85
362	5-24-51	23	1:3.3	—	448	410	9.16	4.86	7.84	3.12
660	7- 9-51	9	1:3.3	—	440	419	9.48	3.69	10.36	3.42
678	6-17-51	47	1:3.3	—	638	415	7.86	7.06	......	......
751	7- 8-51	68	1:3.3	—	439	388	11.11	5.71	10.16	3.82
				Mean	432	404	9.45	5.17	10.16	4.01
				s	±102	± 53	±0.94	±1.04	± 1.17	±0.96
233	1- 3-53	40	5.9:1	—	454	......	8.50	5.16	9.07	6.76
604	1-18-53	36	5.9:1	+[c]	464	......	6.20	1.96	......	......
593	10-29-52	31	5.9:1	—	399	450[a]	8.55	5.63	9.87	6.13
213	7-15-52	5	1:1	—	280[a]	244[a]	10.14	4.59	8.38	2.88
362	6- 3-52	28	1:1	—	410	340[a]	7.10	4.74	11.10	3.97
660	8-10-52	23	1:1	+	419	353[a]	6.05	1.03	......	......
678	7-14-52	5	1:1	+	415	362	......	......	......	......
751	8-13-52	8	1:1	+	388	400[a]	6.38	0.63	......	......
				Mean	404	358	8.57	5.03	9.60	4.94
				s	± 53	± 63	±1.07	±0.68	±1.11	±1.57

[a] Corrected to 305-day lactation.
[b] Mastitis.
[c] + = Symptoms of milk fever.

TABLE 6
Summary of results

Group No.	Exptl. ration Ca:P ratio	Incidence of milk fever (%)	Serum calcium and phosphorus (mg/100 ml) 1st day postpartum Ca	1st day postpartum P	4th day postpartum Ca	4th day postpartum P
I	6:1	36				
II	1:3.3	0	9.53 ± 1.12	4.99 ± 1.26	10.18 ± 1.01	4.51 ± 0.98
III	1:1	15	8.68 ± 1.28	4.43 ± 1.43	9.68 ± 0.99	4.47 ± 1.29
IV	5.9:1	26	8.89 ± 1.03	4.26 ± 0.91	9.87 ± 0.68	5.22 ± 0.78

increases as the calcium-phosphorus ratio of diet increases. Statistical evaluation of the data demonstrates the following: There was no difference in the incidence of milk fever between Group I, fed alfalfa hay with a 6:1 calcium-phosphorus ratio, and Group IV, fed a ration with a ratio of 5.9:1. The difference and standard error of the difference between the two groups in this respect were 0.10 ± 0.16. This would indicate that the calcium-phosphorus ratio of the diet, and not the differences between alfalfa hay and a ration of oat hay and barley, is the important factor in milk fever prevention. Since the incidence of milk fever and the dietary calcium-phosphorus ratios of these two diets were essentially the same, the two groups were combined for statistical comparison with the animals receiving the low-calcium high-phosphorus ration (Group II). The proportion of animals which developed symptoms of milk fever in the combined total is 10/33, or 30%. The 90% confidence interval for this estimated proportion is 0.18-0.43. Using this lower limit, that is 0.18, it follows that the probability of 16 cows not developing milk fever (as occurred in Group II) is 0.04. Thus, only four times in 100 will this occur by chance alone. and under the conditions of this experiment it appears that feeding a low-calcium high-phosphorus diet before parturition is an effective means of preventing milk fever.

DISCUSSION

The results of this experiment indicate that a low-calcium high-phosphorus ration fed for approximately 1 month before parturition will prevent milk fever in dairy cattle. Although no direct evidence is available, the suggestion is made that prevention results from a compensatory hypertrophy of the parathyroid glands due to the low-calcium diet, such that at parturition and the initiation of lactation the increased calcium drain is compensated for by the increased mobilization of calcium from the skeletal reserves. That this may be the case is indicated from results obtained by Campbell and Turner *(4)*, who demonstrated parathyroid hypertrophy in rabbits fed a ration consisting of blue grass hay and corn, but not in rabbits fed alfalfa hay, corn, and oats. These rations are somewhat comparable with those fed to the cows in Groups II and I of this experiment.

Several other procedures have been suggested in the literature for the prevention of milk fever. Gould *(15)* and Mattick and Little *(30)* claim to have reduced the incidence of milk fever by feeding high-calcium diets. However, the results of these investigations are inconclusive, and the value of high-calcium

diets in milk fever prevention has not been demonstrated. Since high-calcium diets should depress rather than stimulate the parathyroids, such a procedure is in direct opposition to the theory that the disease is a condition resulting from parathyroid dysfunction.

Turner *(41)* suggested that prepartum milking might reduce the incidence of milk fever by gradually introducing lactation, thus giving the calcium mobilizing factors a chance to become functional and meet the demands of lactation. Smith and Blosser *(37)* and later Eaton *et al.* *(10)* investigated the influence of prepartum milking on the incidence of milk fever and the changes in serum calcium and phosphorus accompanying parturition. Smith and Blosser *(37)* found no reduction in the incidence of milk fever between the cows milked prepartum and the controls. This fact appears to be in conflict with the supposition that a mild calcium stress, as imposed by low-calcium diets or prepartum milking, might stimulate the parathyroids and prevent milk fever. However, the cows milked before parturition which developed milk fever produced an average of only 8.4 lb. of milk per day on the day preceding parturition. This represents a calcium loss through lactation of approximately 4.5 g. per day. Although no information concerning the daily calcium intake is presented, in all probability it exceeded the calcium loss to the milk by a considerable amount. In this case then, prepartum milking probably did not actually present a stimulation to calcium mobilization. It would be interesting to see if prepartum milking superimposed on a low-calcium dietary regime would prevent milk fever.

Little and Mattick *(26)* suggested that vitamin D might be beneficial in the prevention of milk fever. Campbell and Turner *(4)* made a rather thorough investigation of the relation of vitamin D to calcium metabolism and parathyroid function. They demonstrated with rats that a low-calcium low-vitamin D diet caused parathyroid enlargement; the addition of vitamin D (D_2 or D_3) partially prevented increase in gland size. When rats were fed a high-calcium low-vitamin D diet, parathyroid enlargement occurred, and the addition of vitamin D to the ration returned the parathyroids to normal. They also demonstrated that excessive doses of A.T. 10 (Hytakeral) led to parathyroid depression in rats. In experiments with lactating goats, they found that feeding a total of 32 million I.U. of vitamin D in the form of A.T. 10 over a period of 4 days led to increased blood calcium and phosphorus. On the basis of these results, they suggested the following: Vitamin D is a factor which enables calcium metabolism to proceed normally with a minimum of parathyroid activity. When vitamin D is unavailable, the calcium of the blood can remain normal only by increased parathyroid function. When vitamin D is supplied, calcium absorption and utilization is so improved that parathyroid activity can be minimal. They suggested the use of vitamin D to buffer the parathyroid gland from the sudden demand for greatly increased activity following the initiation of lactation as a measure for preventing milk fever and cautioned against the use of excessive amounts of A.T. 10, which might depress parathyroid activity.

Hibbs and coworkers *(19, 20, 21, 22, 23)* have investigated the use of vitamin D in the prevention of milk fever over a period of several years. They found that

feeding one million I.U. of vitamin D (irradiated ergosterol) daily to cows for 4 weeks prepartum and 1 week postpartum did not reduce the incidence of milk fever and had no influence on serum calcium, phosphorus, or magnesium levels. Because they suspected, in view of the findings of Campbell and Turner *(4)*, that the use of vitamin D at these relatively low levels for prolonged periods might be inhibiting the parathyroids, they shortened the prepartum feeding period to 5-7 days and fed 5, 10, and 30 million I.U. of vitamin D daily.- They reported that at the higher levels of vitamin D the incidence of milk fever was significantly reduced and that the usual drop of serum calcium at parturition was prevented.

Recently, Ward, Blosser, and Adams *(42)* have reported that a severe negative calcium balance occurs before parturition in cows which subsequently develop milk fever. They suggested that milk fever may be preceded either by a period of lowered calcium absorption or by a period of excessive calcium excretion through the intestines and that this may explain the value of vitamin D feeding in milk fever prevention.

SUMMARY

Sixty-nine aged Jersey cows were divided into four groups and fed diets containing various calcium-phosphorus ratios for varying periods of time before parturition. The incidence of milk fever was determined.

The results indicate that a low-calcium high-phosphorus diet (calcium-phosphorus ratio of 1:3.3) fed during the last month of the dry period effectively prevents milk fever. There appeared to be a direct correlation between the calcium-phosphorus ratio of the prepartum diet and the incidence of milk fever. Thus, 30% of the animals receiving a dietary calcium-phosphorus ratio of 6:1, 15% of the cows fed a dietary calcium-phosphorus ratio of 1:1, and none of those fed a 1:3.3 dietary calcium-phosphorus ratio exhibited clinical symptoms of milk fever.

The postpartal serum calcium levels of the cows receiving the low-calcium high-phosphorus diet tended to be higher than those receiving the high-calcium diets, although the results are not statistically significant.

The various rations fed appeared to have no appreciable influence on the subsequent lactation.

The suggestion is made that the low-calcium high-phosphorus diet exerts its milk fever preventive action by inducing a compensatory hypertrophy of the parathyroid glands, thus tending to avert the drop of serum calcium accompanying parturition and early lactation.

REFERENCES

(1) ALBRIGHT, F., AND REIFENSTEIN, E. C. *The Parathyroid Glands and Metabolic Bone Disease.* Williams and Wilkins, Baltimore, Md. 1948.

(2) BAUMANN, E. J., AND SPRINSON, D. B. Hyperparathyroidism Produced by Diet. *Am. J. Physiol.*, **125**: 741. 1950.

(3) BLOSSER, T. H., AND SMITH, V. R. Parturient Paresis. V. Blood Serum Levels of Citric Acid and Calcium in Normal Parturient Cows and Cows with Parturient Paresis. *J. Dairy Sci.*, **33**: 81. 1950.

(4) CAMPBELL, I. L., AND TURNER, C. W. The Relationship of the Endocrine System to the Regulation of Calcium Metabolism. Mo. Agr. Expt. Sta., *Bull.* 352. 1950.

(5) CLARK, E. P., AND COLLIP, J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium with Suggested Modifications. *J. Biol. Chem.*, **63**: 461. 1925.

(6) COLLIP, J. B. The Extraction of a Parathyroid Hormone Which Will Prevent or Control Parathyroid Tetany and Which Regulates the Level of Blood Calcium. *J. Biol. Chem.*, **63**: 395. 1925.

(7) DE ROBERTIS, E. The Cytology of the Parathyroid and Thyroid Glands of Rats with Experimental Rickets. *Anat. Record*, **79**: 417. 1941.

(8) DRYERRE, H., AND GREIG, J. R. Milk Fever: Its Possible Association with Derangements in the Internal Secretions. *Vet. Record*, **5**: 225. 1925.

(9) DRYERRE, H., AND GREIG, J. R. Further Studies on the Etiology of Milk Fever. *Vet. Record*, **8**: 721. 1928.

(10) EATON, H. D., JOHNSON, R. E., HELMBOLDT, C. F., SPIELMAN, A. A., MATTERSON, L. D., JUNGHERR, E. L., KRAMER, J. H., AND SLATE, R. J. Prepartum Milking. I. The Effect of Prepartum Milking on Some Blood Constituents of the Cow. *J. Dairy Sci.*, **32**: 870. 1949.

(11) FISH, P. A. Recent Progress in Our Knowledge of Milk Fever. *J. Am. Vet. Med. Assoc.*, **75**: 695. 1929.

(12) FISH, P. A. The Physiology of Milk Fever. III. The Blood Phosphates and Calcium. *Cornell Vet.*, **19**: 147. 1929.

(13) FISKE, C. H., AND SUBBAROW, Y. The Colorimetric Determination of Phosphorus. *J. Biol. Chem.*, **66**: 375. 1925.

(14) GODDEN, W., AND DUCKWORTH, J. Variations in Serum Magnesium and the Partition of Serum Calcium in Normal Parturitions and Milk Fever. *Biochem. J.*, **29**: 445. 1935.

(15) GOULD, G. N., JR. The Provision of Minerals for Milk Cows by Feeding and Fertilizing. *Vet. Record*, **13**: 7. 1933.

(16) GREIG, J. R. Studies in the Prevention of Milk Fever. *Vet. Record*, **10**: 301. 1930.

(17) GREIG, J. R. Calcium Gluconate as a Specific in Milk Fever. *Vet. Record*, **10**: 115. 1930.

(18) HAM, A. W., LITTNER, N., DRAKE, T. G. H., ROBERTSON, E. C., AND TISDALL, F. F. Physiological Hypertrophy of the Parathyroids, Its Cause and Its Relation to Rickets. *Am. J. Pathol.*, **16**: 277. 1940.

(19) HIBBS, J. W., KRAUSS, W. E., MONROE, C. F., AND SUTTON, T. S. Studies on Milk Fever in Dairy Cows. I. The Possible Role of Vitamin D in Milk Fever. *J. Dairy Sci.*, **29**: 617. 1946.

(20) HIBBS, J. W., KRAUSS, W. E., POUNDEN, W. D., MONROE, C. F., AND SUTTON, T. S. Studies on Milk Fever in Dairy Cows. II. The Effect of Vitamin D on Some of the Blood Changes in Normal and Milk Fever Cows at Parturition. *J. Dairy Sci.*, **29**: 767. 1946.

(21) HIBBS, J. W., POUNDEN, W. D., AND KRAUSS, W. E. Further Studies on the Effect of Vitamin D and of Parathyroid Extract, "Paroidin," on the Blood Changes of Normal and Milk Fever Cows at Parturition. *J. Dairy Sci.*, **30**: 564. 1947.

(22) HIBBS, J. W., AND POUNDEN, W. D. Recent Developments in the Use of Vitamin D in the Prevention of Milk Fever in Dairy Cows. *J. Dairy Sci.*, **34**: 498. 1951.

(23) HIBBS, J. W., POUNDEN, W. D., AND KRAUSS, W. E. Studies on Milk Fever in Dairy Cows. III. Further Studies on the Effect of Vitamin D on Some of the Blood Changes at Parturition and the Composition of Colostrum in Normal and Milk Fever Cows. *J. Dairy Sci.*, **34**: 855. 1951.

(24) JAFFE, H. L. Hyperparathyroidism (Recklinghausen's Disease of Bone). *Arch. Pathol.*, **16**: 63. 1933.

(25) LIEGEOIS, R., AND DERIVAUX, J. Èquilibre phospho-calcique alimentaire et èquilibre phospho-calcique sanquin chez le porc. *Compt. rend. soc. biol.*, **143**: 126. 1949.

(26) LITTLE, W. L., AND MATTICK, E. C. V. The Calcium Content of Cow's Blood. I. The Effect of Feeding Cod Liver Oil. *Vet. Record*, **13**: 238. 1933.

(27) LITTLE, W. L., AND WRIGHT, N. C. The Aetiology of Milk Fever in Cattle. *Brit. J. Exptl. Pathol.*, **6**: 129. 1925.

(28) LUCE, E. M. The Size of the Parathyroid of Rats and the Effect of a Diet Deficient in Calcium. *J. Pathol. Bacteriol.*, **26**: 200. 1923.

(29) MARINE, D. Parathyroid Hypertrophy and Hyperplasia in Fowls. *Proc. Soc. Exptl. Biol. Med.*, **11**: 117. 1914.

(30) MATTICK, E. C. V., AND LITTLE, W. L. The Calcium Content of Cow's Blood. II. (a) Calcium in the Blood of Parturient Heifers. (b) Effect of the Injection of Parathyroid Extract, Irradiation, etc. (c) Some Case Reports of Milk Fever. *Vet. Record*, **13**: 1091. 1933.

(31) MORRISON, F. B. *Feeds and Feeding.* 21st ed. Morrison Publ. Co., Ithaca, N. Y. 1950.

(32) NIEDERMEIER, R. P., SMITH, V. R., AND WHITEHAIR, C. K. Parturient Paresis. III. A Study of Various Blood Constituents at Parturition in Mastectomized Cows. *J. Dairy Sci.*, **32**: 927. 1949.

(33) PATT, H. M., AND LUCKHARDT, A. B. Relationship of a Low Blood Calcium Level to Parathyroid Secretion. *Endocrinol.*, **31**: 384. 1942.

(34) SEEKLES, L., SJOLLEMA, B., AND VAN DER KAAY, F. C. Injection of Sodium Oxalate Decreasing the Calcium Content of the Blood Serum in Relation to Symptoms of Decreased Blood Calcium Contents. *Tijdschr. Diergeneesk.*, **58**: 750. 1931. (*Biol. Abstr.*, **6**: 4234. 1932.)

(35) SINCLAIR, J. G. Size of the Parathyroid Glands of Albino Rats as Affected by Pregnancy and Controlled Diets. *Anat. Record*, **80**: 479. 1941.

(36) SJOLLEMA, B. Significance of the Electrolyte Balance in the Organism. The Biochemistry of Milk Fever of Cows. *Biochem. Z.*, **200**: 300. 1928. (*Biol. Abstr.*, **5**: 4684. 1931.)

(37) SMITH, V. R., AND BLOSSER, T. H. Parturient Paresis. I. The Incidence of Parturient Paresis and Changes in the Total Serum Calcium at Parturition in Prepartum Milked Cows. *J. Dairy Sci.*, **30**: 861. 1947.

(38) STOERK, H. C., AND CARNES, W. H. The Relation of the Dietary Ca:P Ratio to Serum Ca and to Parathyroid Volume. *J. Nutrition*, **29**: 43. 1945.

(39) THOMPSON, D. L., AND COLLIP, J. B. The Parathyroid Glands. *Physiol. Rev.*, **12**: 309. 1932.

(40) TURNER, C. W. The Mode of Inheritance of Yearly Butterfat Production. An Analysis of the Progeny Performance of Jersey Sires and Dams. Mo. Agr. Expt. Sta., *Research Bull.* 112. 1927.

(41) TURNER, C. W. The Development of the Mammary Gland as Indicated by the Initiation and Increase in the Yield of Secretion. Mo. Agr. Expt. Sta., *Research Bull.* 156. 1931.

(42) WARD, G. M., BLOSSER, T. H., AND ADAMS, M. F. The Relation of Prepartal and Postpartal Mineral Balances to the Occurrence of Parturient Paresis in Dairy Cows. *J. Dairy Sci.*, **35**: 587. 1952.

(43) WILSON, L. T., AND HART, E. B. The Chemistry of the Blood of Dairy Cows Before and After Parturition and its Relation to Milk Fever. *J. Dairy Sci.*, **15**: 116. 1932.

USE OF SPECIAL PROCESSED SOYBEAN FLOUR AND WHEY SOLUBLES IN MILK REPLACEMENT FORMULAS FOR DAIRY CALVES [1]

J. F. STEIN, C. B. KNODT,[2] AND E. B. ROSS[3]

Department of Dairy Husbandry
Pennsylvania Agricultural Experiment Station
State College

Many calves have been raised successfully on milk replacement formulas *(1, 2, 3, 4, 8)*. In recent years the supply of some of the ingredients used in these formulas has fluctuated greatly. The development of formulas based on more economical and generally available ingredients appears desirable. Shoptaw *(6)* reported that the use of soybean flour as a substitute for cow's milk for dairy calves resulted in poor growth, scouring, and poor physical appearance.

The further development of soybean flour for the feeding of human infants, as well as its availability and comparatively low cost, indicated that use of this material in milk replacement formulas for dairy calves should be studied. The results of feeding trials relative to the value of a soybean flour (solvent extracted) in calf milk replacement formulas are presented in this report.

EXPERIMENTAL PROCEDURES

The procedure followed was identical for the three trials presented. Thirty-six male Holstein calves were obtained for each trial from Pennsylvania state institutional herds. The calves were assigned individual solid-walled pens on a randomized basis to avoid positional effects. Steam heat, thermostatically controlled, was used to keep the temperature at a minimum of 65° F.

The test animals were divided into six groups of six calves each, which were similar in body weight at 4 days of age. They were placed on trial on the 6th day and remained on trial through the 49th day of age. The milk replacement formulas fed in the three trials are presented in Table 1. The formulas were all ground after mixing, using a hammer mill equipped with a 1/32-in. screen. All formulas were suspended in water at 100° F. in open pails and fed according to the following schedule twice daily: birth-5th day, dam's milk or 4 lb. milk; 6th-10th day, 0.3 lb. replacement, 2 lb. water, 2 lb. milk; 11th-18th day, 0.5 lb. replacement, 5 lb. water; 19th-28th day, 0.6 lb. replacement, 5 lb. water; 29th-36th day, 0.7 lb. replacement, 6 lb. water; 37th-49th day, 0.8 lb. replacement, 7 lb. water.

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[2] Present address: General Mills, Inc., Detroit, Mich.

[3] Present address: Clark's Summit, Pa.

TABLE 1

Milk replacement formulas[a] fed in Trials I, II, and III

Ingredients	Trial I						Trial II						Trial III					
	Groups						Groups						Groups					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Nonfat dry milk solids	49.0	49.0	49.0	38.25	27.75	....	49.0	28.0	27.75	14.75	....	....	49.0	38.45	38.45	38.50	28.45	38.45
Dried whey	10.0	10.0	10.0	....	....	....	10.0	10.0	10.0	....	....	....	10.0	....	....	....	....	10.0
Blood meal	10.0	10.0	....	....	....	....	10.0	....	....	....	....	....	10.0	....	....	....	....	....
Dist. dried corn sol.	15.0	....	....	....	....	....	15.0	....	....	....	....	....	15.0	....	....	....	....	....
Oat flour	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Dextrose	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	17.0	9.0	7.0	7.0	7.0	7.0	7.0	7.0
Dried brewers' yeast	....	5.0	5.0	5.0	5.0	5.0	....	5.0	5.0	5.0	5.0	5.0	....	5.0	5.0	5.0	5.0	5.0
Whey solubles	....	....	....	10.0	10.0	10.0	....	....	....	....	....	....	....	10.0	5.0	5.0	5.0	....
Quadrex[b]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	2.0	2.0	2.0	2.5	2.5	3.0	2.0	2.5	2.5	2.5	3.0	3.0	2.0	2.5	2.5	2.5	2.5	2.5
dl-methionine	....	....	....	0.25	0.25	0.25	....	....	0.25	0.25	0.25	0.25	....	0.05	0.05	....	0.05	0.05
Soybean flour	....	10.0	20.0	30.0	40.0	66.0	....	40.0	40.0	62.0	66.25	66.25	....	30.0	35.0	35.0	45.0	30.0
Mico-phos	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aurofac D[c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Salt	....	....	....	....	....	1.0	....	....	....	1.0	1.0	1.0	....	....	....	....	....	....
Limestone	....	....	....	....	0.5	0.75	....	0.5	0.5	0.5	0.5	0.5	....	....	....	....	....	....
Lactose	....	....	....	....	....	....	....	....	....	....	....	8.0	....	....	....	....	....	....

[a] Expressed in pounds.
[b] 4,000 U.S.P. units vitamin A and 500 U.S.P. units vitamin D_2 per gram.
[c] 5 g. aureomycin per pound.

All calves were fed a fair quality timothy-clover hay ad libitum, with a calf starter fed ad libitum to a maximum of 5 lb. daily. The starter was composed of 416 lb. yellow corn meal, 300 lb. wheat bran, 400 lb. crimped whole oats, 100 lb. linseed oil meal, 300 lb. soybean oil meal (44% protein–solvent process), 150 lb. dehydrated alfalfa meal, 100 lb. cane molasses, 100 lb. nonfat dry milk solids, 100 lb. distillers' dried corn solubles, 10 lb. dicalcium phosphate, 10 lb. ground limestone, 10 lb. iodized salt, and 4 lb. vitamin A and D_2 meal (1,814,544 and 226,800 U.S.P. units per pound, respectively).

All calves were weighed on the same day of the week, at the same time of day, and by the same person. Hay and starter consumption, the condition of the feces, vigor, and general appearance of each calf were recorded daily.

EXPERIMENTAL RESULTS

Trial I. A summary of the data relative to growth rates obtained in Trial I is presented in Table 2. These data were analyzed statistically according to the methods of Snedecor *(7)*. A significant difference in mean daily body weight gains was found between groups. All groups were significantly better than Group VI, in which complete substitution was made for nonfat dry milk solids, dried whey, blood meal, and distillers' dried corn solubles present in the control formula by the addition of whey solubles, dried brewers' yeast, DL-methionine, and soybean flour.

Removal of distillers' dried corn solubles from the control formula, when

TABLE 2
Growth rates of calves in Trials I, II, and III (expressed as gains in body weight and height of withers)

	Mean daily body weight gain		Mean daily gain in withers height	
	0-28 days	0-49 days	0-28 days	0-49 days
	(lb.)	*(lb.)*	*(in.)*	*(in.)*
Groups		Trial I		
I	1.33	1.43	0.05	0.06
II	1.13	1.52	0.06	0.06
III	1.09	1.28	0.04	0.06
IV	1.44	1.54	0.04	0.06
V	1.31	1.43	0.04	0.05
VI	0.63	0.76	0.04	0.05
		Trial II		
I	1.45	1.42	0.07	0.06
II	1.04	1.27	0.06	0.06
III	0.81	0.99	0.06	0.05
IV	0.76	0.79	0.04	0.04
V	0.52	0.64	0.05	0.04
VI	0.60	0.75	0.04	0.04
		Trial III		
I	0.99	1.24	0.04	0.06
II	1.05	1.30	0.04	0.05
III	1.28	1.29	0.07	0.06
IV	1.07	1.23	0.06	0.06
V	0.91	1.08	0.06	0.06
VI	0.80	1.19	0.04	0.05

accompanied by additions of soybean flour and dried brewers' yeast (Group II), gave body weight gains comparable to those of Group IV, in which 21.9% of the nonfat dry milk solids and all of the dried whey, blood meal, and distillers' dried corn solubles in the control formula were replaced by soybean flour, dried brewers' yeast, DL-methionine, and whey solubles (Table 1). Both Groups II and IV gained more rapidly in body weight than Group III ($P < 0.05$), in which blood meal and distillers' dried corn solubles were removed and replaced by additions of dried brewers' yeast and soybean flour. Animals in Group V received a formula in which the dried whey, blood meal, distillers' dried corn solubles, and 43.3% of the nonfat dry milk solids were replaced by dried brewers' yeast, whey solubles, DL-methionine and soybean flour. No statistically significant differences were found between the control (Group I) and Groups II, IV, and V in body weight gains. Groups I, II, III, IV, and V gained in body weight at the rate of 14.0, 22.0, 2.9, 23.6, and 14.0%, respectively, above the Ragsdale *(5)* standards, with Group VI below these standards (39.6%).

Analysis of the body weight gains by the individual test animals indicates a more consistent rate of gain for the animals in Groups II and IV than for the other groups studied. The rate of growth as measured by height of withers indicated no statistically significant differences in this respect between the groups studied.

Trial II. Statistical analyses of the data of Trial II indicated no significant difference in body weight gains between the controls (Group I) and Group II when blood meal, distillers' dried corn solubles, and 42.8% of the nonfat dry milk solids were replaced by additions of dried brewers' yeast and soybean flour. Both Groups I and II gained more rapidly in body weight than the other groups studied ($P < 0.01$). The replacement fed Group III differed from that of Group II only in that 0.25% DL-methionine was added at the expense of nonfat dry milk solids, with Group II showing a greater mean gain in body weight as compared to the DL-methionine supplemented group. The nonfat dry milk solids, dried whey, blood meal and distillers' dried corn solubles were replaced by dried brewers' yeast, DL-methionine, and additional dextrose in the formula fed to Group V animals, whereas those in Group VI received additional sugar in the form of lactose. There was no significant difference between these latter groups in body weight gains.

Trial III. On the basis of the results of Trials I and II, a factor or factors present in whey solubles appeared to be of value in milk replacement formulas containing soybean flour for dairy calves. Trial III was designed to study the value of whey solubles and DL-methionine levels with formulas as presented in Table 1.

Statistical analyses of growth data presented in Table 2 revealed no significant difference in gains in body weight between groups of calves fed the various formulas in Trial III. Groups II and III, which received 10% and 5% whey solubles in their milk replacement formulas, respectively, had mean gains in body weight greater than that of the Group I calves, as well as the calves in Group VI receiving 10% dried whey. The lower level of nonfat dry milk solids

fed to Group V may have been responsible, in part, for the decreased growth rate of this group. Individual calves in Groups II and III were more consistent in rate of body weight gains than calves in Group VI. In comparing formulas with and without DL-methionine, no significant difference was found between Groups III and IV during the 48 days trial. One calf in Group IV averaged only 0.86 lb. daily gain in body weight and is responsible for the slightly less mean group gain than that found in Group III. No difference existed in rate of growth as

TABLE 3

Consumption of starter, hay and efficiency of gains in Trials I, II, and III

	Total starter consumption		Total hay consumption		Starter per lb. body weight gain
	0-28 days	0-49 days	0-28 days	0-49 days	0-49 days
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(lb.)*
Groups			Trial I		
I	105	502	35	193	1.19
II	129	538	34	183	1.20
III	120	484	23	152	1.28
IV	136	581	40	183	1.28
V	146	546	26	116	1.30
VI	100	417	21	114	1.88
			Trial II		
I	132	429	79	243	1.03
II	153	455	60	181	1.23
III	132	444	36	131	1.52
IV	125	425	75	161	1.83
V	95	372	42	133	1.98
VI	107	333	79	198	1.49
			Trial III		
I	126	456	19	85	1.25
II	127	480	38	109	1.25
III	156	554	32	94	1.46
IV	133	490	39	154	1.35
V	123	460	35	112	1.45
VI	95	409	30	99	1.17

measured by height of withers, nor was there any significant difference found in starter and hay consumption, as presented in Table 3. A poorer quality mixed hay was fed in this trial and probably is responsible for a lower intake than in Trials I and II.

All calves readily consumed their replacements. The replacements with the higher levels of soybean flour gave more desirable-appearing preparations in respect to color and degree of suspension of material. Scouring was extremely low in all groups in this trial, with no relation of incidence to any particular group. The animals in Groups II, III, and IV maintained a smoother finish throughout the trial than those in the other groups studied. On the basis of the results obtained, it appears that whey solubles may be of benefit in the nutrition of the young dairy calf under the conditions of this trial. The data

also suggest that the addition of 0.05 and 0.25% DL-methionine had little or no significance in stimulating growth rate when used in the replacements studied.

DISCUSSION

The objective of the experimental work presented in this report was to develop economical formulas from readily available ingredients which would produce acceptable growth in dairy calves. A special processed soybean flour and whey solubles were used to replace blood meal, distillers' dried corn solubles, dried whey and part of the nonfat dry milk solids used in milk replacement formulas developed previously *(1)*. In Trial I of these studies, it was found that dried whey, blood meal, distillers' dried corn solubles, and a maximum reduction of 43% of the nonfat dry milk solids could be accomplished by additions of dried brewers' yeast, DL-methionine, and whey solubles with soybean flour. A higher level (66%) of soybean flour at the expense of all nonfat dry milk solids was found unsatisfactory in terms of growth and appearance of calves.

In supplementing formulas high in soybean flour with additions of 10% dextrose, or 8% lactose and 2% dextrose, no improvement was found in growth rates of the calves. Because of cost and possible nutritional requirements, the value of DL-methionine in replacement formulas was studied in Trials II and III. Supplementation with these amino acids at the 0.05 and 0.25% level gave no improvement in growth rates.

Results obtained in Trials I and II indicate that whey solubles may be of particular value in the nutrition of the young dairy calf. Calves receiving whey solubles appeared to gain more rapidly during the first 4 to 5 weeks period and had a more uniform growth response within groups. A level of 5% whey solubles increased appetite, as calves receiving this ingredient consumed more starter than their corresponding controls.

SUMMARY

Soybean flour when fed with dried brewers' yeast and whey solubles can replace dried whey, distillers' dried corn solubles, blood meal, and up to a maximum of 43% of the nonfat dry milk solids in the milk replacement formulas fed to calves. Increases in soybean flour levels at the expense of nonfat dry milk solids of over a 43% substitution depressed appetite and retarded growth and resulted in poor physical appearance of the calves. The addition of dextrose or lactose to high level soybean flour replacement formulas did not improve growth rates of calves fed such formulas. Whey solubles stimulated appetite and produced more uniformity of gains than did dried whey. At levels of 0.25 and 0.05% DL-methionine failed to stimulate growth of calves fed formulas studied in these trials.

ACKNOWLEDGMENTS

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REFERENCES

(1) BLOOM, S., AND KNODT, C. B. The Value of Aureomycin and Vitamin B_{12} in Milk Replacement Formulas for Dairy Calves. *J. Dairy Sci.*, **35**: 910. 1952.

(2) HATHAWAY, J. L., TRIMBERGER, G. W., AND DAVIS, H. P. The Use of Dried Whey and Blood Meal in the Raising of Calves on Limited Amounts of Milk. Neb. Agr. Expt. Sta., *Bull.* 132. 1943.

(3) HAYWARD, H. Rearing of Calves on Milk Substitutes. Pa. Agr. Expt. Sta., *Bull.* 60. 1902.

(4) MAYNARD, L. A., AND NORRIS, L. D. A System of Rearing Calves with Limited Use of Milk. *J. Dairy Sci.*, **6**: 483. 1923.

(5) RAGSDALE, A. C. Growth Standards for Dairy Cattle. Mo. Agr. Expt. Sta., *Bull.* 336. 1934.

(6) SHOPTAW, LA VAN. Soybean Flour as a Substitute for Cow's Milk in Feeding Dairy Calves. *J. Dairy Sci.*, **19**: 95. 1936.

(7) SNEDECOR, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.

(8) WALLACE, H. D., LOOSLI, J. K., AND TURK, K. L. Substitutes for Fluid Milk in Feeding Dairy Calves. *J. Dairy Sci.*, **34**: 256. 1951.

THE UNSATURATED FATTY ACIDS OF MILK FAT[1]
I. METHYL ESTER FRACTIONATION AND ISOLATION OF MONOETHENOID CONSTITUENTS

L. M. SMITH AND E. L. JACK

Department of Dairy Industry, University of California, Davis

Knowledge of the component unsaturated fatty acids of milk fat is relatively meager. With the exception of oleic acid, which is a major constituent, the monoethenoid and polyethenoid[2] fatty acids occur in comparatively small amounts in milk fat *(10, 14)*. Nevertheless, because of their reactivity, they may be of considerable importance in the synthesis, nutritional value, physical and chemical properties, and deterioration of this complex fat. The present study was initiated to obtain further information concerning the isolation, identification, and configuration of the unsaturated fatty acids of milk fat. This paper describes experiments on the fractionation of methyl ester mixtures and the isolation of monoethenoid fatty acids as their methyl esters.

Jack and Henderson *(10)* reviewed the literature on ester fractionation analysis of milk fat. Analytical data for methyl ester fractions *(6, 7, 9, 10)* confirm the presence of unsaturated fatty acids of lower molecular weight than oleic. Bosworth and Brown *(2)* isolated decenoic acid and methyl tetradecenoate from milk fat by bromination and subsequent fractional distillation of fractions containing saturated and unsaturated esters of the same molecular weight. Brown and Orians *(4)* resolved the C_{12}, C_{14}, and C_{16} methyl ester fractions of human milk fat by low temperature crystallization, but the application of this technique to similar methyl ester fractions prepared from milk fat of the cow has not been reported.

The conventional method of preparing milk fat methyl esters for distillation requires the use of strong alkali, strong acid, and steam distillation *(8, 10)*. Subsequent fractional distillation of the esters exposes the higher molecular weight fractions in the still pot to heating for several hours. Such treatments may change the polyunsaturated constituents originally present in the milk fat *(15)*. Markley *(12)* has discussed the advantages of alkali-catalyzed methanolysis of fats over the conventional method of saponification and reesterification for the preparation of methyl esters because of the milder reaction conditions involved.

Fractionation of mixtures of fatty acids or their derivatives by the formation of crystalline complexes with urea has been studied by Schlenk and Holman

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[1] The data in this paper are taken from a thesis presented by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California.

[2] For the purposes of brevity, polyethenoid is used to include all fatty acids having more than one double bond.

(17) and others *(16, 20)*. The method separates straight-chain from branched or cyclic compounds, and the more saturated from the more unsaturated fraction in mixtures of polar compounds. Since the technique does not involve reaction with double bonds, there should be no isomeric changes in the unsaturated constituents.

The low temperature fractional crystallization technique of Brown *(3)* has been widely employed to separate mixtures of fatty acids or esters into saturated and unsaturated fractions. The process is comparatively simple and rapid and should not result in changes of chemical structure.

APPARATUS AND METHODS

The laboratory fractionation column assembly[3] described by Todd *(21)* was employed for the fractional distillation of methyl esters. The interchangeable 12-mm. and 25-mm. columns were packed with 0.125-in. single-turn glass helices. Figure 1 shows the modified reflux regulator and sample receiver which was designed to minimize admixture of successive fractions and to facilitate sample removal. A Glas-Col mantle controlled by a Powerstat was used to heat the still pot and the charge was stirred by means of a Mag-Mix magnetic stirrer.

Skelly Solve A (pentane) was purified by percolation through a column of silica gel *(22)*.

The methyl esters prepared by methanolysis were obtained by the method of Kurz *(11)* except that pentane was substituted for ethyl ether as solvent.

Methyl esters were crystallized from solvents at —21° C. and filtered in a —21° C.-refrigerated cabinet. For temperatures below —21° C. a constant temperature bath similar in principle to that described by Foreman and Brown *(5)* was employed. The heat transfer medium was ethanol cooled by solid carbon dioxide. Figure 2 shows the apparatus used in the working compartment of the bath for filtration of crystallized esters.

Solvents were removed by distillation under reduced pressure followed by holding for 24 hours in a vacuum desiccator at room temperature.

Iodine values (Hanus) and saponification equivalents were determined by standard procedures *(1)*. Refractive indices of methyl esters were observed at 25 ± 0.05° C. with a Bausch and Lomb "Abbe-56" refractometer.

Whenever possible, the samples were protected from light and oxygen during the experiments and were stored at —21° C. under nitrogen.

EXPERIMENTAL AND DISCUSSION

A. METHYL ESTER FRACTIONATION

Fractional distillation. A milk fat produced in May, 1951, by a cow in the herd of the University of California at Davis was analyzed by the ester-fractionation procedure *(10)*. Compositions of the different fractions obtained from the esterified "solid" and "liquid" acids were calculated from the analytical

[3] Precise Fractionation Assembly, manufactured by the Todd Scientific Co., Springfield, Pa.

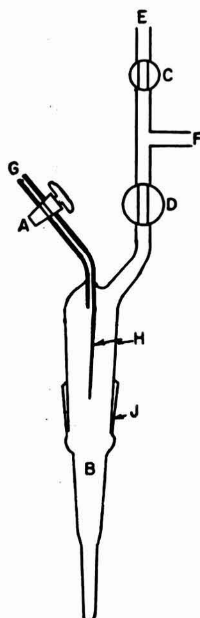


FIG. 1. Modified reflux regulator and sample receiver for Todd fractionation assembly. A—reflux regulator, B—graduated sample receiver, C—2-way stopcock, D—3-way, oblique bore stopcock open at top, E—to McLeod Gauge and top of distillation column, F—to vacuum, G—to distillation head, H—"finger" to direct distillate into center of sample receiver, J—24/40 S/T joint.

data *(8, 10)*. The concentrations (weight per cent) of the unsaturated fatty acids were as follows: decenoic, 0.19; dodecenoic, 0.27; tetradecenoic, 1.52; hexadecenoic, 3.12; octadecenoic, 33.14; linoleic plus other unsaturated acids, 4.03. These data are in agreement with results published by others (*8*, p. 115).

Although fractional distillation of the methyl esters of "liquid" acids provided a means of obtaining fractions from C_{10} to C_{18}, it did not permit the separation of pure monoethenoid fractions in the presence of the saturated esters.

Extractive crystallization with urea. Progressively increasing amounts of urea-saturated methanol were added to six lots of methyl esters prepared from milk fat. The mixtures were dissolved by warming and then were held at 22° C. for 24 hours. The complexes were separated by filtration, washed twice with pentane, and decomposed by warming to 50° C. with 0.5 *N* hydrochloric acid. The freed esters were taken up in pentane and the aqueous phase removed by means of a separatory funnel. Pentane extracts were dried with anhydrous sodium sulfate and the solvent removed. The filtrates also were treated with 0.5 *N* hydrochloric acid and the esters recovered by a similar procedure. All complex fractions were colorless at 25° C. whereas the filtrates ranged in color from light yellow to orange red.

Table 1 shows that increasing amounts of both saturated and unsaturated

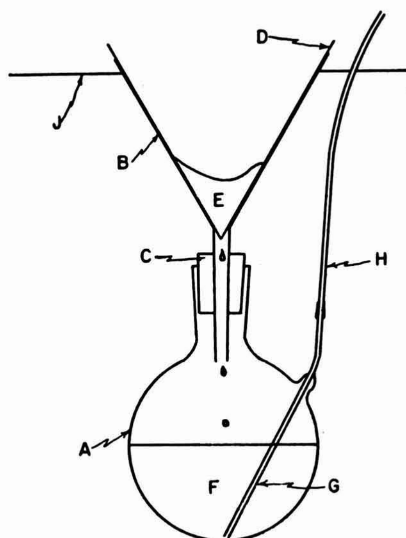


FIG. 2. Apparatus for filtering methyl ester fractions in constant temperature refrigerated bath. A—1-liter flask, B—6″ funnel, C—rubber stopper, D—filter paper, E—precipitate, F—filtrate, G—glass tube, H—tygon tube to collection flask, J—alcohol level in working tank.

methyl esters were isolated in the urea complexes as the proportion of urea to original esters increased. The comparatively high iodine numbers of the complex fractions indicated that separation of saturated from unsaturated esters was incomplete under the above conditions. Large quantities of urea were required to precipitate the bulk of the saturated methyl esters. For these reasons, the urea adduct technique was not applied to the mixed methyl esters of milk fat for the separation of saturated from unsaturated components.

In a second experiment, pentane was used as solvent for "liquid" esters and the reaction time was 45 minutes instead of 24 hours. The "liquid" esters were those remaining in the filtrate after separation of a saturated fraction crystallized from pentane at —21° C.

Table 2 shows that the resultant filtrate esters progressively increased in

TABLE 1

Extractive crystallization of methyl esters with urea

Esters	MeOH	Urea	Filtrate fraction	Complex fraction	
(g.)	*(ml.)*	*(g.)*	*(g.)*	*(g.)*	*(I.V.)*[a]
5.0	25	4	3.7	0.9	7.24
5.0	50	8	3.0	1.6	7.44
5.0	75	12	2.7	2.1	9.94
5.0	100	16	2.3	2.7	11.36
5.0	150	24	1.6	3.2	17.24
5.0	200	48	1.3	3.3	22.46

[a] *I.V.* as used in this paper means Iodine Value (Hanus).

TABLE 2

Influence of urea concentration on fractionation of "liquid" methyl esters[a]

Esters	Pentane	Urea	MeOH	Esters recovered in filtrate	
(g.)	*(ml.)*	*(g.)*	*(ml.)*	*(g.)*	*(I.V.)*
5.75	50	3.0	16.0	4.59	49.6
5.75	50	6.0	16.0	3.83	55.8
5.75	50	12.0	16.0	2.11	67.5
5.75	50	18.0	16.0	0.55	85.7

[a] —21° C. methyl ester filtrate. Iodine Value 45.8.

iodine value. These results may be explained by the methyl oleate and saturated esters forming urea adducts more readily than the polyethenoid and lower molecular weight monoethenoid esters. The technique can be considered as a possible method for the concentration of polyethenoid esters from mixtures with methyl oleate *(16)*.

Low temperature crystallization of saturated esters. Three lots of methyl esters prepared from milk fat by methanolysis were subjected to low temperature crystallization in absolute ether, absolute methanol, and pentane respectively. Iodine values were determined on each of the three resultant precipitates. Table 3 shows that pentane gave the most complete separation of saturated esters. Furthermore, this solvent was easiest to remove from the fractions. Possibly because of hydrogen bonding, an extremely high proportion of methanol to methyl esters was necessary to obtain a precipitate which could be filtered.

On the basis of preliminary results, crystallization from pentane was used in subsequent experiments for removing the bulk of the stearate and palmitate from the mixed methyl esters of milk fat before distillation, and for separating saturated components from distilled fractions containing monoethenoid esters.

B. ISOLATION OF MONOETHENOID METHYL ESTER FRACTIONS

Preparation of the fat. The milk fat was obtained in March, 1952, from an Ayrshire herd pastured on legumes and annuals near Davis, California. The milk was separated at 32° C., and the cream was cooled and subsequently churned at 16° C. in a laboratory crock churn. Following two washes with distilled water, the butter granules were melted at 50° C. and the foam removed from the surface of the liquid. The fat was filtered at 45-50° C. in small lots to obtain a clear, dry product.

TABLE 3

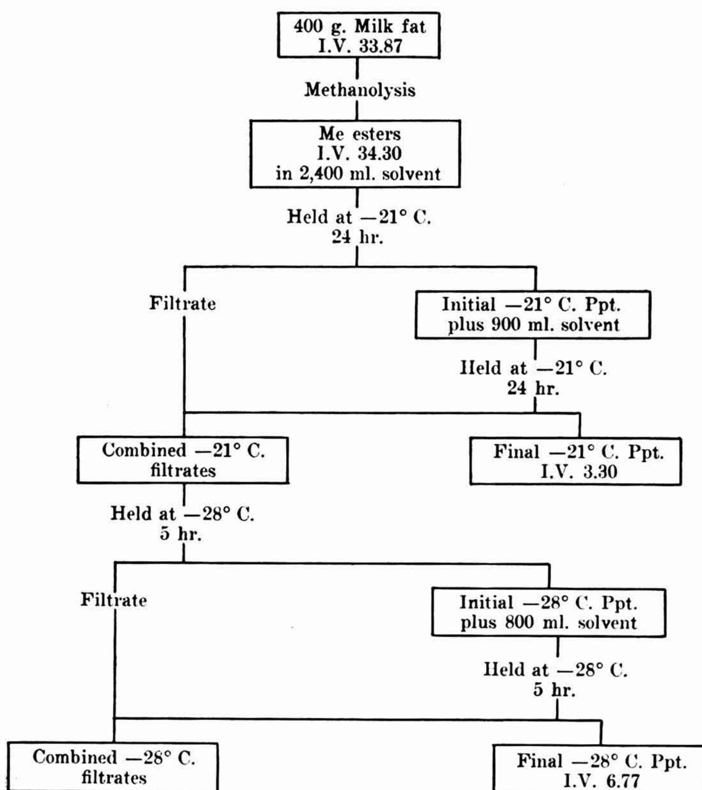
Influence of different solvents on precipitation of saturated methyl ester fractions by crystallization at —21° C. for 24 hours

Fat	Crystallization solvent	Ppt.	Filtrate	Total Me esters	Ppt.
(g.)	*(ml.)*	*(g.)*	*(g.)*	*(g.)*	*(I.V.)*
50	Ether —180	6.9	39.6	46.5	14.28
5	Methanol—300	2.4	2.3	4.7	15.31
50	Pentane —180	11.3	37.3	48.6	6.67

Preparation of methyl esters. To 200 g. milk fat in 800 ml. pentane were added 700 ml. neutralized absolute methanol and 11 ml. 1.0 *N* potassium hydroxide in methanol. The mixture was gently swirled and allowed to stand at 22° C. for 48 hours. The solution was then divided into two equal volumes, each of which was washed once with 250 ml. 0.06 *N* hydrochloric acid and three times with 0.02 *N* hydrochloric acid. Then the two lots of esters were each increased to a volume of 1,200 ml. with pentane, dried for 4 hours with anhydrous sodium sulfate, and filtered. Methyl esters were prepared as above from three 400-g. portions of milk fat designated A, B, and C.

Low temperature crystallization of saturated esters. Each of the three lots of methyl esters was fractionated by crystallization from pentane as shown for Lot A in Scheme 1. On the basis of iodine values, the —21° C. and —28° C. precipitates consisted mainly of saturated methyl esters, whereas the unsaturated esters remained in the —28° C. filtrate.

Distillation of —28° C. filtrates. The —28° C. filtrates were divided into fractions by distillation at pressures below 0.1 mm. mercury without the use of



SCHEME 1

Fractionation of Lot A mixed methyl esters by low temperature crystallization from pentane

a fractionating column. The very low pressure drop in the apparatus facilitated rapid distillation at comparatively low still pot temperatures. Although this operation did not accomplish efficient fractionation, it avoided excessive heating of the polyethenoid esters in the still pot. Distillation of each filtrate was terminated when the refractive index of the distillate exceeded 1.4460 so that nearly all the C_{18} and higher molecular weight esters remained in the still pot *(13)*. Temperatures of the still pot and vapor did not exceed 124° and 122° C., respectively, in these distillations.

The C_{18-20} methyl esters were combined and investigated as reported elsewhere *(18)*.

Fractional distillation of C_{4-16} esters. The C_{4-16} methyl esters of Lots A, B, and C were fractionally distilled in an electrically heated, packed column. On completing each distillation, the refractive index of each "cut" was determined. Table 4 presents typical column-operating data. The presence of unsaturated esters of similar carbon chain lengths but with slightly lower boiling points than the homologous saturated series made clean-cut separation difficult.

Distillation cuts with refractive indices within —0.0006 and +0.0010 of reported values for methyl caprate, laurate, myristate, palmitate, and oleate *(13)* were combined. Holdup and pot residue fractions were not included. Refractive index, saponification equivalent, and iodine value were determined for each of the combined samples.

TABLE 4

Fractionation of Lot B-2 C_{4-16} methyl esters

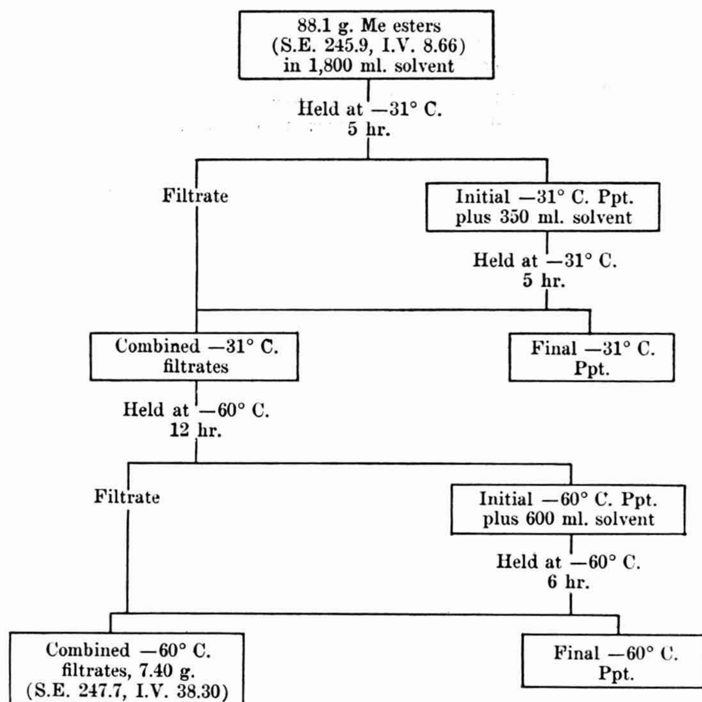
Fraction	Vapor temperature	Volume [a]	n_D^{25}
	(°C.)	*(ml.)*	
1	29 – 30	0.48	1.4144
2	42 – 49	1.14	1.4232
3	45.5– 46.5	5.20	1.4240
4	47 – 51.5	1.11	1.4262
5	50 – 51.5	2.50	1.4290
6	51.5– 52.5	2.35	1.4294
7	52.5– 55	2.00	1.4299
8	57 – 68.5	1.08	1.4346
9	68.5– 73	1.40	1.4350
10	74 – 75.5	3.20	1.4348
11	75 – 77	6.60	1.4347
12	77 – 76	5.70	1.4343
13	76 – 72	4.00	1.4341
14	72 – 80	1.80	1.4346
15	79 – 85.5	2.80	1.4362
16	84.5– 91.5	4.20	1.4390
17	92 – 91	3.00	1.4400
18	85.4	6.00	1.4392
19	84.5– 81.5	2.10	1.4385
20	82 – 90	3.90	1.4383
21	90 – 99	3.10	1.4398
22	100 –103	3.12	1.4476
23	105 –105.5	3.10	1.4510
24	105 –107	4.60	1.4510
25	(Holdup)	10.50	1.4483
26	(Residue)	6.15 g.	1.4521

[a] Initial charge 81.6 g.

The C_{18} sample appeared to be reasonably pure methyl oleate, but the C_{16} to C_{10} fractions contained both saturated and unsaturated components and were further purified as described below. The iodine number of the combined cuts of molecular weight less than methyl caprate was 0.00 ($n_D^{25} = 1.4144$). This agrees with the observation of others *(9)* that the fatty acids of milk fat with shorter carbon chains than ten do not contain double bonds.

Further purification of monoethenoid fractions. The C_{16}, C_{14}, C_{12}, and C_{10} methyl ester fractions were subjected to low temperature crystallization from pentane in an attempt to remove the saturated component from each sample. Scheme 2 shows the fractionation of the C_{14} esters and illustrates the procedures used.

Table 5 compares analytical data for the monoethenoid methyl ester fractions with the calculated theoretical values. Saponification equivalents show that the samples had predominantly C_{18}, C_{16}, C_{14}, C_{12}, and C_{10} carbon chains respectively. Saturated and unsaturated esters of the same chain length were separated with decreasing efficiency as the chain length decreased. The purity of the samples with respect to monoethenoid component could have been increased by further low temperature crystallizations from polar solvents, especially if the esters were converted to their respective fatty acids. For the purposes of this investigation,



SCHEME 2

Fractionation of C_{14} methyl esters by low temperature crystallization from pentane

TABLE 5

Analytical data for C_{18} to C_{10} monoethenoid methyl ester fractions

Carbon chain length	n_D^{25}	Saponification equivalent		Iodine value		Per cent Purity [a]
		Theoretical	Found	Theoretical	Found	
C_{18}	1.4506	296.5	294.7	85.62	87.10	101.7
C_{16}	1.4446	268.4	274.3	94.57	56.80	60.3
C_{14}	1.4389	240.4	247.7	105.60	38.30	36.3
C_{12}	1.4315	212.3	216.5	119.55	15.68	13.1
C_{10}	1.4297	184.3	188.1	137.75	17.58	12.8

[a] Calculated as monoethenoid from Iodine Value.

however, it was deemed preferable to avoid subjecting the samples to further manipulation.

Studies on the configuration of the above fractions, as determined by infrared spectrophotometry, are reported in a subsequent paper *(19)*.

SUMMARY

The concentrations of the unsaturated fatty acids of a typical California milk fat were calculated from methyl ester distillation data obtained by conventional methods.

Methyl esters of milk fat were prepared conveniently by a methanolysis procedure employing mild reaction conditions and pentane as solvent. This solvent was more satisfactory than ethyl ether or methanol in low temperature fractionation of methyl ester mixtures. Low temperature crystallization from pentane was more convenient and efficient than extractive crystallization with urea for the separation of saturated and unsaturated methyl esters.

The methyl esters of the monoethenoid fatty acids of milk fat from decenoic to octadecenoic were isolated by vacuum distillation and partially purified by low temperature crystallization techniques.

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REFERENCES

(1) Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis.* Washington, D. C. 1945.

(2) BOSWORTH, A. W., AND BROWN, J. B. Isolation and Identification of Some Hitherto Unreported Fatty Acids in Butter Fat. *J. Biol. Chem.*, **103**: 115. 1933.

(3) BROWN, J. B. Low-Temperature Crystallization of the Fatty Acids and Glycerides. *Chem. Revs.*, **29**: 333. 1941.

(4) BROWN, J. B., AND ORIANS, BETTY M. The Fatty Acids of Human Milk Fat. *Arch. Biochem.*, **9**: 201. 1946.

(5) FOREMAN, H. D., AND BROWN, J. B. Solubilities of the Fatty Acids in Organic Solvents at Low Temperatures. *Oil and Soap*, **21**: 183. 1944.

(6) HANSEN, R. P. The Accuracy of Ester Fractionation Analysis of Butterfat. *J. Am. Oil Chemists' Soc.*, **28**: 375. 1951.
(7) HANSEN, R. P., AND SHORLAND, F. B. Seasonal Variations in Fatty Acid Composition of New Zealand Butterfat. *Biochem. J.*, **52**: 207. 1952.
(8) HILDITCH, T. P. *The Chemical Constitution of Natural Fats*, 2nd ed. 1947. John Wiley and Sons, Inc., New York.
(9) HILDITCH, T. P., AND LONGENECKER, H. E. Further Determination and Characterization of the Component Acids of Butter Fat. *J. Biol. Chem.*, **122**: 497. 1938.
(10) JACK, E. L., AND HENDERSON, J. L. The Fatty Acid Composition of Glyceride Fractions Separated from Milk Fat. *J. Dairy Sci.*, **28**: 65. 1945.
(11) KURZ, H. Zur katalytischen Umesterung fetter Oele durch alkoholische Kalilauge. *Fette u. Seifen*, **44**: 144. 1937.
(12) MARKLEY, K. S. *Fatty Acids*, p. 299. 1947. Interscience Publishers, Inc., New York.
(13) MATTIL, K. F., AND LONGENECKER, H. E. The Use of Refractive Index Measurements in Fatty Acid Ester Analyses. *Oil and Soap*, **21**: 16. 1944.
(14) McDOWELL, A. K. R. The Properties of New Zealand Butters and Butterfats. III. Seasonal Variations in the Nature of the Unsaturated Acids of Butterfat as Estimated by Spectrophotometric Methods. *J. Dairy Research*, **20**: 101. 1953.
(15) MITCHELL, J. H., JR., KRAYBILL, H. R., AND ZSCHEILE, F. P. Quantitative Spectral Analysis of Fats. *Ind. Eng. Chem.*, Anal. ed., **15**: 1. 1943.
(16) NEWEY, H. A., SHOKAL, E. C., MUELLER, A. C., BRADLEY, T. F., AND FETTERLY, L. C. Drying Oils and Resins. Segregation of Fatty Acids and Their Derivatives by Extractive Crystallization with Urea. *Ind. Eng. Chem.*, **42**: 2538. 1950.
(17) SCHLENK, H., AND HOLMAN, R. T. Separation and Stabilization of Fatty Acids by Urea Complexes. *J. Am. Chem. Soc.*, **72**: 5001. 1950.
(18) SMITH, L. M., AND JACK, E. L. The Unsaturated Fatty Acids of Milk Fat. II. Conjugated and Nonconjugated Constituents. *J. Dairy Sci.*, **37**: 390. 1954.
(19) SMITH, L. M., FREEMAN, N. K., AND JACK, E. L. The Unsaturated Fatty Acids of Milk Fat. III. Geometrical Isomerism. *J. Dairy Sci.*, **37**: 399. 1954.
(20) SWERN, D., AND PARKER, W. E. Application of Urea Complexes in the Purification of Fatty Acids, Esters, and Alcohols. I. Oleic Acid from Inedible Animal Fats. *J. Am. Oil Chemists' Soc.*, **29**: 431. 1952.
(21) TODD, F. Efficient and Versatile Laboratory Fractionation Assembly. *Ind. Eng. Chem.*, Anal. ed., **17**: 175. 1945.
(22) TUNNICLIFF, D. D., BRATTAIN, R. R., AND ZUMWALT, L. R. Benzene, Toluene, Ethylbenzene, *o*-Xylene, *m*-Xylene, and *p*-Xylene. Determination by Ultraviolet Spectrophotometry. *Anal. Chem.*, **21**: 890. 1949.

THE UNSATURATED FATTY ACIDS OF MILK FAT[1]
II. CONJUGATED AND NONCONJUGATED CONSTITUENTS

L. M. SMITH AND E. L. JACK
Department of Dairy Industry, University of California, Davis

The occurrence in milk fat of certain polyethenoid fatty acids and their configuration are still controversial. McDowell *(10)* recently compared the concentrations of conjugated and nonconjugated constituents in milk fat as reported in the literature. Although Schaffer and Holm *(12)* found practically no conjugated dienoic and trienoic systems, other investigators *(10, 11)* reported their presence. The amounts of conjugated and nonconjugated dienoic acids and of nonconjugated trienoic acid varied with type of feed. In contrast to the report of White and Brown *(19)*, Hilditch *(7)* and Shorland *(14)* concluded that the nonconjugated dienoic acid was not linoleic but was probably an isomer. Shorland also presented evidence that the nonconjugated octadecatrienoic acid of New Zealand milk fat was linolenic acid. Although McDowell found no trace of conjugated tetraenoic acid, Shorland and Johannesson *(15)* and Morris *et al. (11)* detected its presence. Bosworth and Sisson *(2)* isolated arachidonic acid from milk fat by chemical means. Mattsson *(9)* found "arachidonic acid" to be extremely sporadic, whereas McDowell reported small but fairly consistent quantities of nonconjugated tetraenoic acids. Shorland and Johannesson showed the presence of nonconjugated tetraenoic and pentaenoic systems in concentrates of C_{20} unsaturated acids of milk fat.

Limitations of the methods used for the analysis of polyunsaturated fatty acids may partly explain the disagreement in the literature. Bailey *(1)* discussed the difficulties involved in the identification of octadecadienoic and octadecatrienoic acids by the formation and separation of their insoluble bromine addition compounds. Spectrophotometric techniques for determination of nonconjugated acids are highly empirical *(3)* and do not provide information regarding chain length, positions of double bonds, and geometrical isomerism of polyethenoid constituents.

The present paper gives the results of a study of polyethenoid fatty acids of California milk fats. Since these components occurred in minor proportions, they were concentrated by combinations of low temperature crystallization, preferential urea complex formation, and vacuum distillation procedures before an attempt was made to investigate their chain lengths and configurations.

APPARATUS AND METHODS

Milk fats were prepared as described previously *(16)*.

Ultraviolet absorption measurements were made with a Beckman quartz spectrophotometer, model DU, equipped with hydrogen lamp. The fused silica

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[1] The data in this paper are taken from a thesis presented by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California.

absorption cells were 48 mm. high and had 10-mm. light paths and ground glass stoppers.

The fats were isomerized in a solution of 11% potassium hydroxide in glycerol *(4)*.

Skelly Solve A (pentane) and 2-2-4-trimethylpentane (isooctane) were purified by percolation through a column of silica gel *(18)*. Isooctane was used as solvent for conjugated constituents, and methanol for determinations of nonconjugated acids in isomerized products. Whenever possible, the dilutions were adjusted so that the observed absorbances were between 0.1 and 0.8.

Absorptivity a (extinction coefficient k) is defined as

$$a = \frac{A}{bc} = (-\log_{10} T)/bc$$

where A is the absorbance and T the transmittance of a solution relative to that of the solvent in an equal cell, b is the inside length in centimeters of the cell used, and c is the concentration of the solution in grams per liter *(8)*.

The spectrophotometric procedure of Brice *et al.* *(4)*, including background corrections, was followed for the determination of polyethenoid constituents reported in section B. Their restandardized method *(3)* was employed in section C.

Apparatus and materials used in low temperature crystallization, urea complex, and vacuum fractional distillation techniques have been described previously *(16)*.

EXPERIMENTAL AND DISCUSSION

A. ABSORPTIVITY OF MILK FAT BEFORE AND AFTER ALKALI ISOMERIZATION

Figure 1 shows typical ultraviolet absorption curves for milk fats before and after alkali isomerization. The absorption peak at 233 mμ in the nonalkali isomerized spectra is attributable to dienoic conjugation. Minor peaks at 300 and 315 mμ are evident in the absorption curves of the isomerized fats. Maxima at 233, 268, 300, and 315 mμ in the case of the isomerized fats denote the presence of nonconjugated dienoic, trienoic, and tetraenoic constituents. Spectral positions of the nonconjugated absorption maxima are in agreement with data characteristic of alkali isomerized methyl esters of linoleic, linolenic, and arachidonic acids *(5)*. It has not been definitely shown, however, that the observed maxima for milk fat can be attributed solely to the presence of these acids and not partly to their isomers *(14)*. The results are in agreement with those of Mattsson over the spectral range 210-280 mμ which he reported.

B. VARIABILITY OF POLYETHENOID CONSTITUENTS

Determinations of polyethenoid constituents in representative California milk fats produced at different seasons are summarized in Table 1. The data are too limited to justify conclusions regarding seasonal variability.

Conjugated acids. The content of conjugated dienoic acids varied less than in Swedish and New Zealand milk fats as reported by Mattsson and McDowell, respectively, but the average value agreed with the data of Morris *et al.*

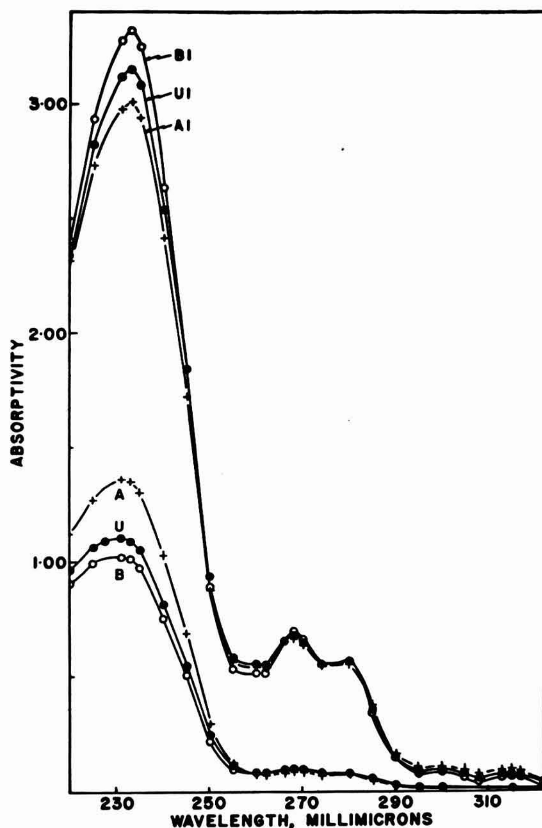

FIG. 1. Ultraviolet absorption spectra of three milk fats. A, B, and U—before alkali isomerization; AI, BI, and UI—after alkali isomerization.

and McDowell. The small values for conjugated trienoic constituents were relatively constant and were of the same order as those reported by Morris *et al.* and McDowell. The trace amounts of conjugated tetraenoic systems were in agreement with the results of Morris *et al.* and Shorland and Johannesson.

Nonconjugated acids. The amounts of nonconjugated dienoic acids fluctuated less than the values found by Mattsson. They were somewhat higher than McDowell's data but consistently lower than those of Schaffer and Holm. Concentrations of nonconjugated trienoic acids varied less than results reported by Mattsson, but were in line with those of Schaffer and Holm, and McDowell. Results for nonconjugated tetraenoic acids agree with the values of McDowell but disagree with the minute quantities found by Mattsson.

Possibly the observed differences in concentrations of both conjugated and nonconjugated constituents may be largely attributed to variations in pasture conditions as suggested by others *(9, 10, 14)*.

TABLE 1

Variability of polyethenoid constituents of milk fat [a]

Sample description		Per cent					
		Conjugated			Nonconjugated		
		Diene	Triene	Tetraene	Diene	Triene	Tetraene
June 1951.	U. C. creamery	0.74	0.02	0.004			
July 1951.	U. C. creamery	0.88	0.02	0.004			
July 1951.	U. C. creamery	0.95	0.02	0.003	1.59	0.75	0.30
Nov. 1951.	U. C. creamery	0.94	0.03	0.003	1.16	0.91	0.44
Mar. 1952.	Herd B	0.79	0.02	0.002	1.78	0.97	0.36
Aug. 1952.	U. C. herd	0.86	0.02	0.002	1.54	0.78	0.28
Aug. 1952.	Herd A	1.08	0.02	0.002	1.17	0.73	0.29
	Av.	0.89	0.02	0.003	1.45	0.83	0.35

[a] All spectrophotometric determinations in this paper are reported as percentage of acid in sample.

C. CONCENTRATION OF POLYETHENOID CONSTITUENTS

Lot C milk fat, its mixed methyl esters, and the $C_{4\text{-}16}$ and $C_{18\text{-}20}$ methyl ester fractions prepared as described previously *(16)*, were examined in the 215-322 mμ spectral range. Figures 2 and 3 show absorptivity curves of the fractions before and after alkali isomerization.

The absorption maxima of the mixed methyl esters were slightly higher than those of the original milk fat because glycerol and part of the lower esters were removed during methyl ester preparation. The polyethenoid constituents were concentrated mainly in the $C_{18\text{-}20}$ fraction.

Extractive crystallization with urea. Approximately 65 g. of the combined $C_{18\text{-}20}$ methyl esters in 1,000 ml. pentane were added to 384 g. methanol containing 156 g. urea. The slurry was stirred and held 12 hours at room temperature. After the liquid phase had been decanted, the crystalline complexes were washed three times with 250 ml. pentane and transferred to a Buchner funnel with 100 ml. pentane. The urea adducts were dried with gentle suction and decomposed by 500 ml. 0.1 *N* hydrochloric acid. The freed esters were washed twice with 250 ml. water, dried with anhydrous sodium sulfate, and recovered from the solvent. The filtrate was divided into two parts and each washed twice with 250 ml. 0.1 *N* hydrochloric acid and once with the same amount of water. Solvent was removed from the recombined, dried esters. Thirty-six g. methyl esters were recovered from the complex fraction and 28 g. from the filtrate.

Crystallization from pentane at —60° C. A second 65 g. of the combined $C_{18\text{-}20}$ methyl esters, dissolved in 1,000 ml. pentane, were held at −60° C. for five hours. The resulting precipitate was recrystallized from 500 ml. pentane under the same conditions. Twelve g. methyl esters were recovered from the precipitate and 52 g. from the filtrate.

Table 2 compares concentrations of polyethenoid constituents in the fractions obtained by the two fractionation techniques. More of the polyunsaturated components were found in the esters recovered from the urea complex fraction than in the −60° C. precipitate fraction. Under the experimental conditions, low temperature crystallization was more efficient and convenient than extractive

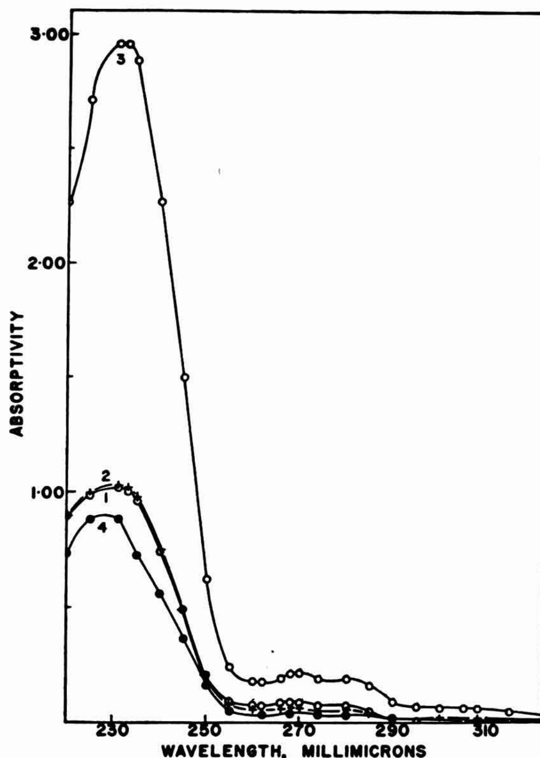

FIG. 2. Ultraviolet absorption spectra of Lot C milk fat and methyl ester fractions before isomerization. 1—milk fat, 2—mixed methyl esters, 3—C_{18-20} methyl ester fraction, 4—C_{4-16} methyl ester fraction.

crystallization with urea for the concentration of polyethenoid methyl esters. Relatively more of the conjugated than the nonconjugated components formed urea complexes. This is in agreement with the observation of Schlenk and Holman *(13)* that conjugated isomers give higher yields of urea adducts than do nonconjugated compounds.

TABLE 2

Concentrations of polyethenoid constituents in fractions prepared from C_{18-20} methyl esters by low temperature crystallization with pentane and by extractive crystallization with urea

Sample description	Per cent					
	Conjugated			Nonconjugated		
	Diene	Triene	Tetraene	Diene	Triene	Tetraene
C_{18-20} −60° C. Filtrate	3.16	0.04	0.000	5.89	3.41	2.24
C_{18-20} −60° C. Ppt.	0.50	0.01	0.001	0.68	0.66	0.19
Ratio	6.3:1	4:1	....	8.7:1	5.2:1	11.8:1
C_{18-20} Urea Filtrate Fraction	3.87	0.04	0.000	9.20	4.50	4.06
C_{18-20} Urea Complex Fraction	1.55	0.04	0.002	1.46	0.97	0.18
Ratio	2.5:1	1:1	....	6.3:1	4.6:1	22.5:1

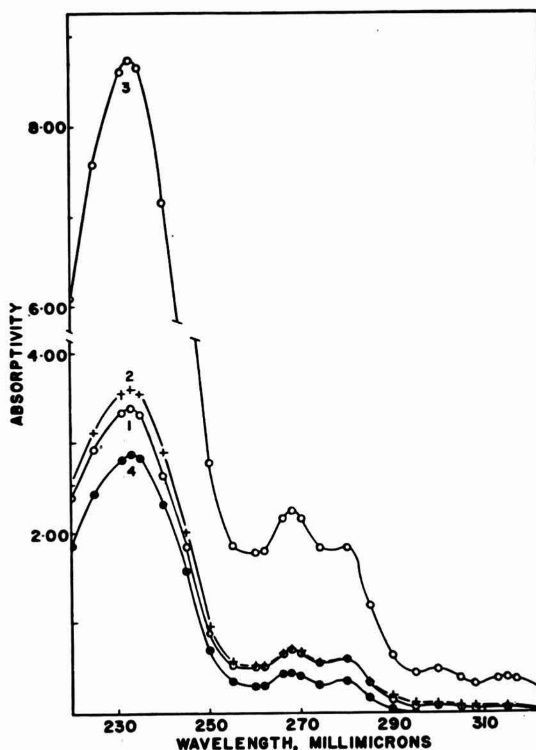

FIG. 3. Ultraviolet absorption spectra of Lot C milk fat and methyl ester fractions after alkali isomerization. 1—milk fat, 2—mixed methyl esters, 3—C_{18-20} methyl ester fraction, 4—C_{4-16} methyl ester fraction.

The absorptivities at 348 mμ of the —60° C. filtrate and noncomplex-forming filtrate fractions after alkali isomerization were 0.26 and 0.37, respectively. These data provide evidence for the presence of a nonconjugated pentaene constituent in milk fat, and are in agreement with the report of Shorland and Johannesson. However, absorption in the 375 mμ region, attributed to the presence of hexaenoic constituents, was very slight with no absorption maximum.

Fractional distillation of C_{18-20} —60° C. filtrate methyl esters. The C_{18-20} —60° C. filtrate methyl ester fraction was distilled in vacuum, and a series of cuts approximately 2-5 ml. each was obtained. Saponification equivalent and polyethenoid constituents were determined for the original charge, holdup, pot residue, and most of the cuts. Table 3 summarizes the data.

The results illustrate the difficulty of separating by distillation complex mixtures of the methyl esters of monoethenoid and polyethenoid fatty acids having 18 or more carbon atoms in their chains. However, appreciable fractionation was achieved. The conjugated dienoic and trienoic constituents were more concentrated in the later fractions. Nonconjugated dienoic and trienoic components distilled over more uniformly, although the former had some tendency to concen-

TABLE 3

Fractionation of C_{18-20} —60° C. filtrate methyl esters

Fraction	Volume	Vapor Temperature	Saponification equivalent	Per cent					
				Conjugated			Nonconjugated		
				Diene	Triene	Tetraene	Diene	Triene	Tetraene
	(ml.)	*(°C.)*							
Charge	53		308.8	3.16	0.042	0.000	5.89	3.41	2.24
1	1.4	90 – 98		0.53	0.071	0.004	1.22	0.45	0.00
2	5.2	98 –110	292.1	0.36	0.013	0.001	8.13	3.49	0.01
3	2.9	110							
4	2.9	110	298.0	0.12	0.009	0.000	9.32	4.15	0.06
5	3.5	110 –111.5							
6	3.4	111.5–112	297.8	0.13	0.007	0.000	7.74	4.78	0.02
7	3.5	113							
8	3.4	111	299.0	0.22	0.009	0.000	6.63	4.34	0.03
9	3.0	110							
10	3.0	110 –114	297.4	0.38	0.004	0.000	5.49	4.26	0.00
11	2.0	114	297.9	0.60	0.004	0.000	4.97	4.06	0.00
12[a]	10.0		298.0	6.96	0.019	0.000	3.48	4.47	1.00
13[b]	1.0			15.14	0.079	0.001	1.04	2.13	4.00
Residue	6.3		352.5	10.90	0.299	0.002	4.16	0.00	12.80

[a] Holdup from top part of column.
[b] Holdup from bottom part of column.

trate in the earlier cuts. The data confirm the results of Mattsson, who reported that the methyl ester of a conjugated C_{18} acid became concentrated in later distillation fractions, whereas the nonconjugated dienoic ester distilled more uniformly.

The isomerized samples were also examined in the 348 mμ and 375 mμ regions, where absorption characteristic of pentaenoic and hexaenoic conjugation is found *(15)*. Minor absorptivity peaks were observed at 348 mμ in fractions 12 and 13, and the pot residue (with absorptivities of 0.16, 0.26, and 0.96, respectively). The pot residue also had a very slight peak (absorptivity 0.13) at 375 mμ. The results provide further evidence for the presence of nonconjugated pentaenoic acids in milk fat, although conditions for isomerization of their methyl esters were not optimum *(6)*.

Saponification equivalents for fractions 2 to 12 were relatively constant, although these fractions contained varying concentrations of conjugated and nonconjugated dienoic components. The data indicate that the conjugated and nonconjugated dienoic acids of milk fat are predominantly 18 carbons in chain length. Although the pot residue was clear and liquid at room temperature, less confidence can be placed in the saponification equivalent and spectrophotometric data for this fraction because of the heat treatment to which it was subjected. However, the results suggest that the nonconjugated tetraenoic and pentaenoic components of milk fat have carbon chain lengths of 20 or longer.

In the present paper, the constituents of milk fat which exhibit ultraviolet absorption maxima characteristic of dienoic and trienoic conjugated systems have been assumed tentatively to be conjugated dienoic and trienoic acids. However, more highly unsaturated acids with two or three of their double bonds in conjugation could show the same maxima at 233 mμ and 268 mμ.

Infrared spectrophotometric studies of three methyl ester fractions containing polyethenoid constituents are reported elsewhere *(17)*.

SUMMARY

Ultraviolet absorption curves for milk fats before and after alkali isomerization showed the presence of conjugated and nonconjugated polyethenoid constituents. The amounts of these polyunsaturated fatty acids in representative California milk fats were estimated by spectrophotometry and compared with other published data.

Methyl esters of the polyethenoid fatty acids were concentrated by combinations of vacuum distillation, preferential urea complex formation, and low temperature crystallization procedures. The latter technique was superior to the urea complex method for the concentration of polyethenoid esters by removal of saturated and monoethenoid components. Although individual conjugated and nonconjugated polyunsaturated constituents were not isolated in a pure state from a mixture of C_{18-20} methyl esters by fractional distillation in vacuum, appreciable fractionation was achieved. The results showed that the milk fat contained small amounts of conjugated and nonconjugated dienoic, trienoic, and tetraenoic fatty acids with carbon chain lengths of 18 or longer. Spectrophotometric evidence also indicated the presence of a nonconjugated pentaenoic constituent.

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REFERENCES

(1) BAILEY, A. E. *Industrial Oil and Fat Products*, 2nd ed., p. 15. 1951. Interscience Publishers, Inc., New York.

(2) BOSWORTH, A. W., AND SISSON, E. W. Arachidonic Acid in Butter Fat. *J. Biol. Chem.*, **107**: 489. 1934.

(3) BRICE, B. A., SWAIN, M. L., HERB, S. F., NICHOLS, P. L., JR., AND RIEMENSCHNEIDER, R. W. Standardization of Spectrophotometric Methods for Determination of Polyunsaturated Fatty Acids Using Pure Natural Acids. *J. Am. Oil Chemists' Soc.*, **29**: 279. 1952.

(4) BRICE, B. A., SWAIN, MARGARET L., SCHAEFFER, B. B., AND AULT, W. C. Spectrophotometric Determination of Small Proportions of Polyunsaturated Constituents in Fatty Materials. *Oil and Soap*, **22**: 219. 1945.

(5) HERB, S. F., AND RIEMENSCHNEIDER, R. W. Influence of Alkali Concentration and Other Factors on the Conjugation of Natural Polyunsaturated Acids as Determined by Ultraviolet Absorption Measurements. *J. Am. Oil Chemists' Soc.*, **29**: 456. 1952.

(6) HERB, S. F., WITNAUER, L. P., AND RIEMENSCHNEIDER, R. W. Isolation of Eicosapentaenoic and Docosapentaenoic Acids from Natural Sources as Their Methyl Esters by Adsorption and Distillation Techniques. *J. Am. Oil Chemists' Soc.*, **28**: 505. 1951.

(7) HILDITCH, T. P. *The Chemical Constitution of Natural Fats*, 2nd ed., p. 116. John Wiley and Sons, Inc., New York.

(8) HUGHES, H. K., *et al.* Suggested Nomenclature in Applied Spectroscopy. *Anal. Chem.*, **24**: 1349. 1952.

(9) MATTSSON, S. Polyunsaturated Fatty Acids in Butter and Their Influence on the Oxidation of Butter. *Proc. XIIth Intern. Dairy Congr.*, **2**: 308. 1949.

(10) McDOWELL, A. K. R. The Properties of New Zealand Butters and Butterfats. III. Seasonal Variations in the Nature of the Unsaturated Acids of Butterfat as Estimated by Spectrophotometric Methods. *J. Dairy Research*, **20**: 101. 1953.

(11) MORRIS, R. J., MACPHEE, R. D., AND RANDALL, E. L. Ultraviolet Spectrophotometry in Detection of Food Product Substitutes. New Applications. *Anal. Chem.*, **24**: 1396. 1952.

(12) SCHAFFER, P. S., AND HOLM, G. E. The Determination of Linoleic Acid in Milk Fat. *J. Dairy Sci.*, **33**: 865. 1950.

(13) SCHLENK, H., AND HOLMAN, R. T. Separation and Stabilization of Fatty Acids by Urea Complexes. *J. Am. Chem. Soc.*, **72**: 5001. 1950.

(14) SHORLAND, F. B. C_{18} Unsaturated Acids of Butterfat. *Nature*, **166**: 745. 1950.

(15) SHORLAND, F. B., AND JOHANNESSON, D. L. C_{20-22} Unsaturated Acids of Butterfat. *Nature*, **168**: 75. 1951.

(16) SMITH, L. M., AND JACK, E. L. The Unsaturated Fatty Acids of Milk Fat. I. Methyl Ester Fractionation and Isolation of Monoethenoid Constituents. *J. Dairy Sci.*, **37**: 380. 1954.

(17) SMITH, L. M., FREEMAN, N. K., AND JACK, E. L. The Unsaturated Fatty Acids of Milk Fat. III. Geometrical Isomerism. *J. Dairy Sci.*, **37**: 399. 1954.

(18) TUNNICLIFF, D. D., BRATTAIN, R. R., AND ZUMWALT, L. R. Benzene, Toluene, Ethylbenzene, *o*-Xylene, *m*-Xylene, and *p*-Xylene. Determination by Ultraviolet Spectrophotometry. *Anal. Chem.*, **21**: 890. 1949.

(19) WHITE, MARY F., AND BROWN, J. B. A Study of the Tetrabromide Method of Estimating Linoleic Acid in Fatty Acid Mixtures. *J. Am. Oil Chemists' Soc.*, **26**: 385. 1949.

THE UNSATURATED FATTY ACIDS OF MILK FAT[1]
III. GEOMETRICAL ISOMERISM

L. M. SMITH, N. K. FREEMAN, AND E. L. JACK
Department of Dairy Industry, University of California, Davis, and Division of Medical Physics, Donner Laboratory, University of California, Berkeley

The monoethenoid fatty acids of animal fats are largely, but not exclusively, of cis configuration. Bertram *(2)* first reported the isolation of vaccenic acid from beef tallow, sheep fat, and milk fat and concluded that it was a $\triangle^{11,12}$-octadecenoic acid of trans configuration. Other investigators *(3, 6)* have confirmed the presence of vaccenic acid in milk fat, and Rao and Daubert *(13)* have presented additional evidence confirming the trans configuration of this acid. Swern *et al.* *(21)* isolated elaidic acid (trans-$\triangle^{9,10}$-octadecenoic acid) from oleo oil prepared from beef fat. Although Hilditch and Longenecker *(8)* reported that milk fat contained C_{16}, C_{14}, C_{12}, and C_{10} monoethenoid fatty acids with double bonds in the $\triangle^{9,10}$-position, the geometrical configuration of these acids has not been determined.

Very little is known concerning isomerism of the C_{18} and longer chain polyethenoid fatty acids of milk fat. Spectrophotometric evidence *(19)* indicates the presence of both conjugated and nonconjugated polyunsaturated acids, but the positions of double bonds or spatial arrangements at these bonds have not been established. Hilditch *(7)* and others *(14, 22)* have suggested the presence of different geometric isomers of linoleic acid (cis-$\triangle^{9,10}$-cis-$\triangle^{12,13}$-octadecadienoic acid) in milk fat.

By comparison of the infrared absorption spectrogram of an unknown compound with those of known compounds, it is often possible to identify the unknown or to obtain significant information about its chemical structure *(1)*. O'Connor *et al.* *(12)* reviewed infrared spectrophotometry in relation to fatty acid chemistry. Shreve *et al.* *(16)* determined the infrared absorption spectra for a number of pure long-chain saturated and monounsaturated fatty acids, methyl esters, triglycerides, and alcohols. Within each class, trans compounds were distinguished readily from cis and/or saturated compounds. Terminally unsaturated structures were distinguished readily from the internally unsaturated and/or the saturated types.. Shreve *et al.* *(15)* developed an infrared spectrophotometric method, based on differences in absorption at 10.36μ, for the determination of trans-octadecenoic acids, esters, and alcohols in mixtures. Jackson *et al.* *(10)* found that methyl octadecadienoic esters with isolated trans, conjugated trans-trans, or conjugated cis-trans double bonds were characterized by different bands in the spectral region from 9.8 to 11.0μ.

The occurrence of cis-trans isomerization during hydrogenation *(5)* or autoxi-

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[1] The data in this paper are taken from a thesis presented by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California.

dation of methyl oleate *(20)* has been established. Feuge *et al.* *(5)* found that the formation of trans forms did not occur in the absence of hydrogenation, even where the nickel catalyst was added to methyl oleate and the mixture heated to 200° C. for four hours and agitated by bubbling purified nitrogen through it. Swern *et al.* *(20)* concluded that hydroperoxides were the major early products of autoxidation of methyl oleate and that the trans configuration predominated in the hydroperoxides.

In the present investigation, the infrared absorption spectra of methyl ester fractions were studied in an attempt to resolve the structure of certain unsaturated fatty acids occurring in milk fat.

EXPERIMENTAL

APPARATUS AND METHODS

Preparation and subsequent examination of methyl ester fractions containing monoethenoid and polyethenoid constituents have been reported *(18, 19)*. The fractions were not exposed to reagents which catalyze the conversion of cis to trans isomers *(11)*, but they were unavoidably subjected to heating during distillation.

The infrared absorption curves in the spectral range between 2μ and 16μ were recorded on a Baird Associates Model B double-beam spectrophotometer equipped with sodium chloride prism. The determinations were made on methyl ester fractions dissolved in carbon disulfide in the concentration of 30 g. per liter using absorption cells 0.9 mm. in thickness.

RESULTS AND DISCUSSION

A. MONOETHENOID METHYL ESTER FRACTIONS

Infrared spectral absorption curves of the C_{18}, C_{16}, C_{14}, C_{12}, and C_{10} monoethenoid methyl ester fractions are shown in Figure 1. The spectrograms were compared with the spectra of purified methyl esters as determined by Shreve *et al.* *(16)*. Absorption bands related to known specific structural features of the molecules were readily identified. In general, the locations of the bands in the spectrograms were independent of chain length. However, the relative intensity of the unassigned absorption peak near 9μ appeared to decrease progressively with increasing chain length, while absorption at 13.8μ increased. The bands discussed below are usually attributed to the specific features mentioned *(16)*.

Spectral similarities.

1. The strong band near 3.4μ is attributed to C-H stretching vibrations.

2. The strong band near 5.75μ is attributed to C=O stretching vibrations.

3. The band near 7.3μ is attributed to symmetrical deformation vibrations of the methyl group.

4. The strong triplet absorption at about 8.0, 8.3, and 8.5μ is characteristic

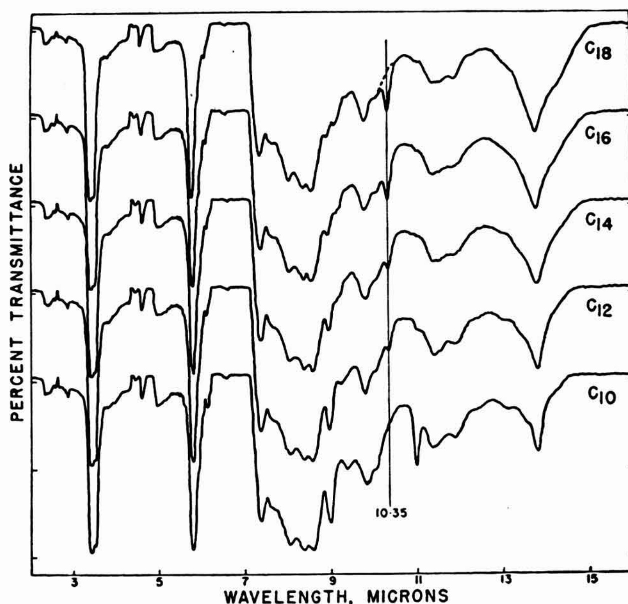
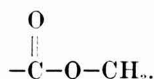


FIG. 1. Infrared absorption spectrograms of C_{18}, C_{16}, C_{14}, C_{12}, and C_{10} monoethenoid methyl ester fractions: 30 g./liter in carbon disulfide; cell thickness, 0.9 mm. (Spectral regions from 4.3 to 4.9μ and 6.2 to 7.1μ are obscured by solvent absorption bands.)

of the spectra of methyl esters of long chain acids. One or more of these bands is believed to be related to vibrations involving the C-O linkages in the ester group



5. The band near 13.8μ has been observed in spectra of a large number of hydrocarbons containing an unbranched chain of four or more carbon atoms. It is usually attributed to the methylene rocking vibration. In cis unconjugated compounds, out-of-plane bending vibrations of the hydrogens attached to the double bond carbons probably contribute to the total absorption observed in this region.

Spectral differences.

1. The spectrogram for the C_{10} monoethenoid methyl ester fraction shows a strong band near 11μ, which is absent in the other spectra. This band is one of a pair of bands which is known to be associated with bending motions of the hydrogens attached to a terminal double bond. The second band, which should be found at 10μ, is slightly weaker; in this instance it appears only as an increase in the intensity of absorption indicated by a small shoulder on the side of the 9.8μ band. At the present time, it is not possible to estimate quantitatively from the curve the amount of terminally unsaturated component in the sample.

The presence of these absorption peaks provides strong evidence that the unsaturated bond of decenoic acid of milk fat occurs between the ninth and tenth carbons. Obviously, geometrical isomerism does not occur in the case of a terminal double bond. There is no band at 10.35μ indicative of internal trans double bonds.

2. Spectrograms for the C_{18}, C_{16}, C_{14}, and C_{12} fractions exhibit an absorption band of varying intensity near 10.35μ. This band is attributed to out-of-plane vibrations of the two hydrogen atoms attached to an isolated trans double bond. Nothing can be inferred from the spectrograms regarding the location of this bond in the carbon chain.

The method of calculation for the estimation of trans components differed from that of Shreve *et al.* *(15)* because the esters were of different chain lengths and an adequate calibration was not feasible. The absorbance of the trans component only was calculated in each case from the peak transmittance with respect to an estimated background. As indicated by the dotted line in Figure 1, this background represents the assumed transmittance in the absence of any trans constituent. Calculation at 10.35μ on this background curve gives an absorptivity which is very close to the value given for pure methyl oleate by Shreve *et al.* *(15)*. The absorptivity a (extinction coefficient k) is given by the following equation *(9)*:

$$a = \frac{A}{bc} = \frac{-\log_{10} T}{bc} = \frac{0.119}{0.09 \times 30} = 0.044$$

where A is the absorbance and T the transmittance of a solution relative to that of the solvent in an equal cell, while b is the inside length in centimeters of the cell used, and c is the concentration of the solution in grams per liter of solution. Shreve *et al.* reported a value of 0.041. The calculated concentration of trans isomer corrected to molar basis in each monoethenoid fraction is presented in Table 1.

TABLE 1

Concentrations of transisomers in monoethenoid methyl ester fractions

	Per cent			
	Trans in fraction		Monoethenoid	*Trans* in
Fraction	Weight	Molar	in fraction, molar	monoethenoid, molar
C_{18}	14	14	~100	14
C_{16}	14	13	60	22
C_{14}	7	6	36	17
C_{12}	5	3.5	13	27

Although the results obtained from a calculation of this type are not very accurate, the data provide strong evidence for the presence of appreciable amounts of trans components in the C_{18}, C_{16}, C_{14}, and C_{12} monoethenoid fatty acids of milk fat.

B. METHYL ESTER FRACTIONS CONTAINING POLYETHENOID CONSTITUENTS

Figure 2 shows infrared absorption curves for C_{18-20} methyl esters of Lot C-1, and fractions 2 and 13 from the fractional distillation of C_{18-20} −60° C. filtrate

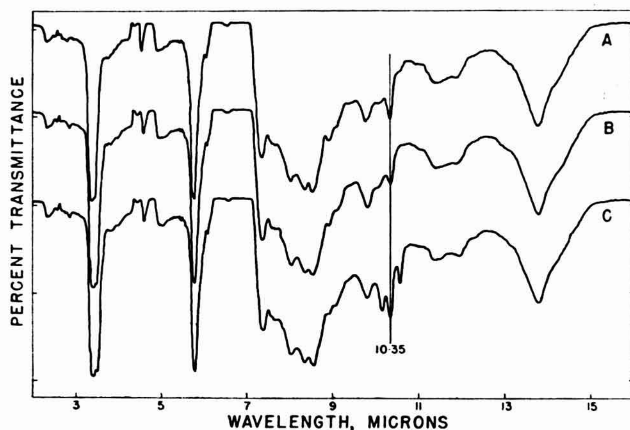


FIG. 2. Infrared absorption spectrograms of methyl ester fractions containing polyethenoid constituents: 30 g./liter in carbon disulfide; cell thickness, 0.9 mm. A — lot C-1 C_{18-20} fraction; B, C — fractions 2 and 13, respectively, from distillation of C_{18-20} −60° C. filtrate esters. (Spectral regions from 4.3 to 4.9μ and 6.2 to 7.1μ are obscured by solvent absorption bands.)

esters. The spectrograms were compared with Figure 1 and the curves given by Shreve *et al.* (*16*) and Jackson *et al.*

Spectral similarities.

Absorption bands similar to those found in the monoethenoid methyl ester fractions are present in the spectrograms near 3.4, 5.75, 7.3, 8.0, 8.3, 8.5, and 13.8μ. They are attributed to the same functional groups as those cited for the monoethenoid spectrograms.

Spectral differences.

1. The intensity of absorption at 10.35μ, characteristic of isolated trans double bonds, varied in the spectra of the three fractions. Concentrations of trans isomers in the C_{18-20} methyl esters and in distillation fraction 2 were calculated to be approximately 15 and 9% respectively, when background reference points at 10.35μ in the spectrograms were estimated. The spectra do not reveal whether the trans component(s) are isomeric forms of methyl oleate or of one of the polyethenoid esters present.

2. The spectrogram for fraction 13 has a very strong band at 10.35μ. In addition, strong peaks appear at 10.15 and 10.55μ. The presence of these bands makes it difficult to estimate the background reference point, even if it is assumed they do not affect the 10.35μ peak. Therefore, the reliability of the

calculated value of 25% isolated trans component in fraction 13 is open to question.

The C_{18-20} methyl ester fraction and distillation fractions 2 and 13 contained 2.43, 0.36, and 15.14% conjugated dienoic systems respectively *(19)*. The percentage of components with isolated trans double bonds in the three samples was estimated to be 15, 9, and 25 respectively. Therefore, the absorptivity at 10.35μ was probably not related to the C_{18} conjugated dienoic acid. Possibly the differences in absorptivity at this wavelength were caused by the different distribution of nonconjugated constituents containing isolated trans double bonds.

A doublet at 10.18 and 10.54μ was attributed by Jackson *et al.* to cis-trans conjugated linoleate, whereas a single band at 10.12μ was related to trans-trans conjugated linoleate. On the basis of these results, the peaks at 10.15 and 10.55μ in the spectrogram of fraction 13 provide good evidence for the presence of cis-trans conjugated isomers. These cis-trans isomers were undoubtedly in the 15.14% conjugated dienoic constituent(s). By comparison with the curves given by Jackson *et al.* (Figure 2), the relative intensities of the two peaks suggest that the conjugated dienoic esters are largely cis-trans, but the data do not exclude the possibility that a minor amount of trans-trans isomers may be present. These data do not provide information regarding the order of the cis and trans double bonds in the carbon chain.

In the absence of spectral data for pure geometrical and positional isomers of methyl linolenate and arachidonate, interpretation of infrared spectra of methyl ester fractions containing these constituents must be considered tentative.

Absorption near 2.8μ, attributed to hydroperoxidic hydroxyl and other types of hydroxyl groups *(17)*, is slight in the spectrograms presented in Figures 1 and 2. Hydroperoxides have been shown to be the major early products of oxidation of methyl oleate *(4, 20)* and to have the trans configuration predominantly *(20)*. In the present study, the percentage transmittance at 2.8 to 2.9μ remained relatively constant for all samples, although they had been exposed to different temperatures during preparation and were found to contain varying percentages of trans components. These data may be interpreted as evidence that formation of trans isomers as a result of oxidation during preparation of the samples was negligible.

SUMMARY

Infrared absorption spectra were recorded for C_{18}, C_{16}, C_{14}, C_{12}, and C_{10} monoethenoid methyl ester fractions of milk fat and for three C_{18-20} fractions, each containing different distributions of polyethenoid constituents. The spectrograms were compared with the available spectral data for pure saturated and unsaturated esters of long chain fatty acids. The presence of only minor absorption at 2.8 to 2.9μ in all curves was interpreted as evidence that sample preparation occasioned no appreciable oxidation with concurrent hydroperoxidic hydroxyl formation and geometric isomerism.

The C_{18} to C_{12} monoethenoid fractions showed an absorption band near 10.35μ, which is attributed to the presence of trans isomers. Approximate con-

centrations of trans components ranged from 14 to 27% of the monoethenoid methyl esters. An absorption peak characteristic of a terminal double bond in the C_{10} fraction provided strong evidence that the unsaturated bond of decenoic acid occurs between the ninth and tenth carbons.

Absorption bands at 10.35μ were observed in the C_{18-20} fractions containing polyethenoid methyl esters, but it was not clear whether the trans component(s) were isomeric forms of one or more of the nonconjugated polyethenoid esters or of the methyl oleate present. In one of the C_{18-20} fractions, which contained approximately 15% conjugated dienoic constituent, the infrared spectrum showed the configuration of the conjugated double bonds to be principally cis-trans.

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REFERENCES

(1) Barnes, R., and Gore, R. C. Infrared Spectroscopy. *Anal. Chem.*, **21**: 7. 1949.
(2) Bertram, S. H. Die Vaccensäure (Eine neue Fettsäure aus Rinder-, Schafs- und Butterfett). *Biochem. Z.*, **197**: 433. 1928.
(3) Boer, J., Jansen, B. C. P., and Kentie, A. On the Growth-Promoting Factor for Rats Present in Summer Butter. *J. Nutrition*, **33**: 339. 1947.
(4) Farmer, E. H., and Sutton, D. A. The Course of Autoxidation Reactions in Polyisoprenes and Allied Compounds. IV. The Isolation and Constitution of Photochemically-Formed Methyl Oleate Peroxide. *J. Chem. Soc.*, p. 119. 1943.
(5) Feuge, R. O., Pepper, M. B., Jr., O'Connor, R. T., and Field, Elsie T. Modification of Vegetable Oils. XI. The Formation of Trans Isomers During the Hydrogenation of Methyl Oleate and Triolein. *J. Am. Oil Chemists' Soc.*, **28**: 420. 1951.
(6) Geyer, R. P., Nath, H., Barki, V. H., Elvehjem, C. A., and Hart, E. B. The Vaccenic Acid Content of Various Fats and Oils. *J. Biol. Chem.*, **169**: 227. 1947.
(7) Hilditch, T. P. *The Chemical Constitution of Natural Fats*, 2nd ed., p. 116. 1947. John Wiley and Sons, Inc., New York.
(8) Hilditch, T. P., and Longenecker, H. E. Further Determination and Characterization of the Component Acids of Butter Fat. *J. Biol. Chem.*, **122**: 497. 1938.
(9) Hughes, H. K., *et al.* Suggested Nomenclature in Applied Spectroscopy. *Anal. Chem.*, **24**: 1349. 1952.
(10) Jackson, J. E., Paschke, R. F., Tolberg, W., Boyd, H. M., and Wheeler, D. H. Isomers of Linoleic Acid. Infrared and Ultraviolet Properties of Methyl Esters. *J. Am. Oil Chemists' Soc.*, **29**: 229. 1952.
(11) Markley, K. S. *Fatty Acids*, p. 57. 1947. Interscience Publishers, Inc., New York.
(12) O'Connor, R. T., Field, Elsie T., and Singleton, W. S. The Infrared Spectra of Saturated Fatty Acids with Even Number of Carbon Atoms from Caproic, C_6 (Hexanoic), to Stearic, C_{18} (Octadecanoic), and of Their Methyl and Ethyl Esters. *J. Am. Oil Chemists' Soc.*, **28**: 154. 1951.
(13) Rao, P. C., and Daubert, B. F. Configuration of Vaccenic Acid. *J. Am. Chem. Soc.*, **70**: 1102. 1948.
(14) Shorland, F. B. C_{18} Unsaturated Acids of Butterfat. *Nature*, **166**: 745. 1950.
(15) Shreve, O. D., Heether, M. R., Knight, H. B., and Swern, D. Determination of *trans*-Octadecenoic Acids, Esters, and Alcohols in Mixtures. *Anal. Chem.*, **22**: 1261. 1950.

(16) SHREVE, O. D., HEETHER, M. R., KNIGHT, H. B., AND SWERN, D. Infrared Absorption Spectra. Some Long-Chain Fatty Acids, Esters, and Alcohols. *Anal. Chem.*, **22**: 1498. 1950.

(17) SHREVE, O. D., HEETHER, M. R., KNIGHT, H. B., AND SWERN, D. Infrared Absorption Spectra of Some Hydroperoxides, Peroxides, and Related Compounds. *Anal. Chem.*, **23**: 282. 1951.

(18) SMITH, L. M., AND JACK, E. L. The Unsaturated Fatty Acids of Milk Fat. I. Methyl Ester Fractionation and Isolation of Monoethenoid Constituents. *J. Dairy Sci.*, **37**: 380. 1954.

(19) SMITH, L. M., AND JACK, E. L. The Unsaturated Fatty Acids of Milk Fat. II. Conjugated and Nonconjugated Constituents. *J. Dairy Sci.*, **37**: 390. 1954.

(20) SWERN, D., COLEMAN, J. E., KNIGHT, H. B., RICCIUTI, C., WILLITS, C. O., AND EDDY, C. R. Reactions of Fatty Materials with Oxygen. XIV. Polarographic and Infrared Spectrophotometric Investigation of Peroxides from Autoxidized Methyl Oleate. *J. Am. Chem. Soc.*, **75**: 3135. 1953.

(21) SWERN, D., KNIGHT, H. B., AND EDDY, C. R. Trans-Octadecenoic Acid Content of Beef Fat. Isolation of Elaidic Acid from Oleo Oil. *J. Am. Oil Chemists' Soc.*, **29**: 44. 1952.

(22) WHITE, MARY F., AND BROWN, J. B. A Study of the Tetrabromide Method of Estimating Linoleic Acid in Fatty Acid Mixtures. *J. Am. Oil Chemists' Soc.*, **26**: 385. 1949.

THE ANTIBACTERIAL ACTION OF PENICILLIN, STREPTOMYCIN, AND SULFANILAMIDE AGAINST HEAVY SUSPENSIONS OF VIBRIO FETUS ADDED TO SEMEN EXTENDER[1]

A. EUGENIA ORTHEY AND H. L. GILMAN
Department of Pathology and Bacteriology
New York State Veterinary College, Ithaca, New York

Although the clinical complex now known as bovine vibriosis was reported by MacFadyean and Stockman *(10)* in 1913 and from 1917 to 1923 was the subject of intensive studies by Smith and coworkers *(21-26)*, the disease for many years was regarded as of minor importance in the general problem of reproductive diseases of cattle. The gradual elimination of brucellosis confirms our belief of long standing that factors other than *Brucella abortus* are often responsible for a considerable degree of interference with the normal reproductive processes. The work of Plastridge *(11, 12, 15, 16)* and European workers revived interest in the disease and stimulated the initiation of intense study in this and other countries.

Until recently, observable abortion was generally regarded as the only significant symptom. We now know that delayed conception and sterility are common manifestations of the infection. Some investigators, such as Stegenga and Terpstra *(27-29)* in Holland, report that *Vibrio fetus* is the primary etiological agent in outbreaks of "enzootic sterility." Clinical observations made in our laboratory confirm the fact that the disease is very widespread. Unpublished experimental data by Hughes and McEntee *(7)* indicate that infertility is the principal manifestation of the disease. Observable abortion may be infrequent or absent.

In 1943, Plastridge and Williams *(14)* suggested that the bull might be an important factor in the preservation and dissemination of the disease. In 1949, Herrick *(6)*, and in 1950, Webster and Thorp *(31)* called attention to and emphasized the venereal nature of the disease and the fact that it may be readily transmitted by coitus. With the establishment of the male as the principal disseminator of the disease and the lack of a simple and accurate means to detect infected animals, the question of how to prevent spread of the disease becomes a matter of great importance. This is true especially when the semen from known infected bulls or from potentially infected bulls is used to artificially inseminate cattle over a wide area. We must know whether noninfected cattle can become infected in this manner and, if so, what means can be taken to prevent the occurrence. Plastridge and his coworkers *(17)*, Terpstra and Eisma *(30)*, and Hughes and McEntee *(7)* have infected virgin heifers by artificial

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insemination using infected extended semen. It is apparent, therefore, that artificial insemination, particularly when the semen is deposited in the uterus, may be an important factor in the spread of the disease. Rasbech *(19)* admits that in Denmark the use of semen from infected bulls has resulted in widespread dissemination of the disease.

It is an established fact *(4)* that the addition of antibiotics to extended semen of some low fertility bulls results in a significant increase in the rate of conceptions. Probably this effect is largely due to the inhibitory or lethal effect on bacteria present. A matter of great importance is whether these or other antibiotics are able to kill *V. fetus* without harmful effects on the sperm cells *(5)* and thereby effectively render semen, when properly used for artificial insemination, incapable of disseminating the disease. Preliminary observations by Roberts *et al. (20)* and Easterbrooks *et al. (1, 2, 3)* indicate that the commonly used addition of 500 units penicillin and 500 γ of streptomycin per milliliter of semen extender will inactive *V. fetus*. Opinions differ as to which of the two antibiotics is more effective against the organism. Jennings *(8)* states that *V. fetus* is not sensitive to concentrations of penicillin up to 100 units per milliliter when grown on blood agar. Prier *(18)* reports that 0.6 to 39.0 units, depending on the strain of *V. fetus* used, will inhibit the growth in thiol broth. No definite time exposure of the organism to the antibiotic is stated for either of these experiments, nor are the organisms removed from contact with the antibiotics at the end of the exposure periods. This also holds true for the streptomycin trials of Prier, in which he finds that 0.3 to 78.0 γ of the antibiotic will inhibit the growth of *V. fetus*. If the organism is never completely removed from contact with the antibiotic, it is impossible to determine whether the action is bactericidal or merely bacteriostatic.

Plastridge and associates *(13)* state that 500 units of penicillin have no adverse effect on the organisms, but death occurs within 24 hours in the presence of 500 γ of streptomycin at 37° C. However, the addition of 500 γ of streptomycin per milliliter to diluted semen did not destroy *V. fetus* at 5° C. The authors also indicated that *V. fetus*, if present, in diluted bull semen treated with 500 γ per milliliter of streptomycin would be rendered incapable of producing infection, at the time of or following insemination, because of the stability and increased activity of the streptomycin at body temperature. The validity of this latter statement is open to question, since it is based on supposition rather than fact. Lawson and MacKinnon *(9)* in England used 1,000 units of penicillin and 1,000 γ of streptomycin per milliliter. They concluded that neither antibiotic, as used in their studies, was capable of inactivating *V. fetus* in semen. However, they added the antibiotics to the semen "shortly before insemination." It is highly probable that exposure of the organism to the antibiotic was of too short duration to permit a bactericidal action to occur.

The work here presented was initiated in an attempt to study the viability of *V. fetus* when added to the commonly used citrate buffered yolk semen extender containing the antibacterial agents, penicillin, streptomycin, and sulfanilamide, singly and in combination, in varying concentrations, for varying periods of time,

and under different temperature conditions. The streptomycin used was Dihydro-Streptomycin Merck (present as the sulfate). The penicillin was Penicillin G Sodium Merck.

EXPERIMENTAL

Semen extender. In all these experiments, the vehicle for the antibacterial agents to which the *V. fetus* organisms were to be exposed was citrate-buffered egg yolk. This consists of equal parts of sterile 2.9% aqueous sodium citrate dihydrate solution and sterile egg yolk.

Extender–antibacterial agent mixtures. Eighteen matched centrifuge tubes were stoppered with cotton and sterilized by autoclaving. To each sterile centrifuge tube was added 0.5 ml. of sterile 2.9% aqueous sodium citrate dihydrate containing double the amount of antibacterial agent per milliliter that was desired for the final dilution to be used in the particular phase of the experiment. To each tube, 0.5 ml. of sterile egg yolk was added aseptically. This gave the correct final concentration of antibacterial agent per milliliter of extender. By varying the amounts of the antibacterial agents per milliliter of citrate solution, different concentrations used in the experiment were obtained. These mixtures were stored overnight at 5° C., inoculated with the standardized quantity of *V. fetus* organisms the next morning, and handled thereafter as indicated in the description of the various experiments. The 18 tubes here described were cultured at the end of 18 different exposure periods. This experiment was replicated ten or fifteen times for each concentration of antibacterial agent, and for each temperature exposure.

Vibrio fetus inoculum. A strain of *V. fetus* which had been recovered from an aborted bovine fetus, and which grew well on Difco thiol medium, was used. The organism was inoculated into tubes of semi-solid thiol (0.4% agar) and incubated at 37° C. for 4 days in jars containing 15-20% carbon dioxide. Slanted Roux flasks containing solid thiol (1.9% agar) were then heavily inoculated, each one with the entire growth from one of the above prepared tubes. About 75 sterile glass beads of 6 mm. diameter were then added to the surface of the medium. The beads are useful for spreading the inoculum over the agar surface and later for loosening the growth from the medium while harvesting the bacteria. Enough carbon dioxide to displace 15% of the air in each flask was forced through the cotton stopper. The flask was then sealed with a small sheet of pliofilm. After 4 days of incubation at 37° C. the beads were rolled around the surface of the agar by rotating the flask. This made an even distribution of the inoculum over the surface. The following day the bacteria were harvested by adding 25 ml. of sterile physiological saline solution to the flask and loosening the growth by again rotating the beads over the agar surface. The bacterial suspension was then adjusted to a density of approximately 500 on the Klett-Summerson photoelectric colorimeter. This suspension was then added to the tubes of test extender in the amount of 0.2 ml. per tube.

Tests for viable cells. The extender-antibacterial agent mixtures were cultured for viable cells at intervals of 4 or 8 hours up to a maximum of 72 hours.

When cultures were made over the entire exposure time, they were made after 0, 1, 2, 4, 8, 16, 20, 24, 28, 32, 40, 44, 48, 52, 56, 64, 68, and 72 hours. In the instance of the combined antibacterial agents, cultures were made only at 24 hours and subsequently.

In any experiment in which control of time of exposure to an antibacterial agent is an integral part of the work, the bacteria must be completely removed from any contact with the active agent at the time of culturing. In the early part of these studies, penicillinase and cysteine were used satisfactorily to inactivate penicillin at the desired time. Para-amino-benzoic acid proved equally effective against sulfanilamide. Several agents were tested in an effort to effectively neutralize streptomycin. None proved to be satisfactory. The bacteria were then separated from the antibacterial agent by the use of high speed centrifugation. All the streptomycin experiments and a repetition of those using penicillin and sulfanilamide utilized this technique. Since the results of the two methods to remove the bacteria from the action of the antibacterial agents, penicillin and sulfanilamide, are similar, only the data in which centrifugation is used will be reported here.

When the antibacterial agent and vibrios had been in contact the desired period of time, the following methods were used to remove the bacteria and test their viability. The tube containing the mixture of bacteria and antibacterial agent was shaken well and the cotton plug was replaced with a sterile rubber stopper. The tube was then centrifuged in an angle centrifuge for 20 minutes at about 2,045 G's. The supernatant fluid was then suctioned off into a trap flask, using sterile Pasteur pipettes and a water suction pump. Two ml. of sterile aqueous physiological saline solution were added, and the material was resuspended and recentrifuged. The supernatant fluid was again removed and 0.5 ml. of sterile physiological saline solution added. The sediment was resuspended by shaking, and a 4-mm. loopful of this material was inoculated into each of four tubes of semi-solid thiol medium. Incubation at 37° C. in jars containing 15-20% carbon dioxide followed. The cultures were observed daily for a period of 14 days.

Controls. For each tube of antibacterial-vibrio mixture, three controls were set up simultaneously and handled identically. None of the controls were treated with antibacterial agents. The three controls consisted of egg yolk and citrate, citrate solution only, and physiological saline only. The suspensions of *V. fetus* were added to each control tube at the same time and in the same manner as in the "test" tubes and then stored and cultured identically.

RESULTS

Action of penicillin against Vibrio fetus (Table 1). As stated previously, each separate experiment was replicated ten times and some instances fifteen times. Penicillin was present in each tube in a concentration of 500 units per milliliter. Controls without the antibiotics were used. In the tubes kept at 5° C. for the entire period of observation, there was no lethal effect on the bacteria. In the experiments using incubation at 37° C. for 1 or 2 hours followed

TABLE 1
Effects of penicillin on Vibrio fetus

Hr. of exposure	Per cent of viable tubes containing 500 units penicillin[a] when exposed			
	5° C.	1 hr. at 37° C. 5° C. thereafter	2 hr. at 37° C. 5° C. thereafter	37° C.
0	100	60	30	40
4	100	70	80	80
8	100	90	80	80
16–72	100	100	100	100

[a] With no penicillin all tubes up to 72 hr. were viable.

by refrigeration and also in the experiment when the tubes were incubated for the entire period of observation, the organisms were nonviable in some of the tubes. These were invariably in those exposed to the antibiotic 8 hours or less. In practically all the tubes the organisms were still alive and the growth compared favorably with that observed in the controls. In the tubes exposed 16 hours or more the organism was still viable in all replicates.

Action of sulfanilamide against Vibrio fetus. The organism was viable in all tubes after exposure to 0.3% sulfanilamide at all exposure times up to and including 72 hours. This was true of all ten replicates. Temperature treatment consisted of storage at 5° C. for the entire period of 1 or 2 hours of incubation followed by refrigeration, or storage at 37° C. for the entire observation period. In no instance under the conditions enumerated did the agent have any adverse effect on viability.

Action of streptomycin against Vibrio fetus (Table 2). In these experiments, streptomycin in a concentration of 500 γ per milliliter was used. All tests were conducted under the four temperature treatments as previously enumerated, and at least ten replicates of each were done. When refrigerated over the entire observation time, the organisms remained viable. The agent had no observable adverse effect. After 1 or 2 hours of initial incubation followed by refrigeration, some of the replicates for almost every exposure time were nonviable. However, the majority remained viable. When incubated for the entire observation period, some of the short exposure time cultures were nonviable. Practically all incubated replicates were alive after 16 hours.

When the cultures were kept at refrigeration or incubation temperatures for the entire period, the results were about the same for 1,000 γ per milliliter of streptomycin as for 500 γ. When a 1-hour initial incubation was used, the organisms were dead in some tubes from the exposure times up to 8 hours. All replicates exposed 8 to 24 hours contained viable organisms. Of those exposed up to 48 hours, a number of scattered tubes were "alive." At 48 hours and thereafter, the organisms were dead in most of the replicates. Two hours of incubation had less adverse effect on viability of the organisms than 1 hour incubation. All replicates were viable up to 32 hours, with the exception of a few tubes held for the shorter exposure times. Most organisms were alive up to 64 hours; a few were still alive at 72 hours.

TABLE 2

Effects of streptomycin on Vibrio fetus

Hr. of exposure	Per cent of viable tubes containing streptomycin[a] when exposed			
	5° C.	1 hr. at 37° C. 5° C. thereafter	2 hr. at 37° C. 5° C. thereafter	37° C.
		500 γ/ml. of streptomycin		
0	90	60	90	70
4	100	80	80	90
8	100	90	90	80
16	100	100	90	100
24	100	85	70	100
32	100	95	95	90
40	100	100	100	100
48	100	95	100	100
56	100	80	80	100
64	100	60	60	90
72	100	65	80	100
		1,000 γ/ml. of streptomycin		
0	100	70	80	80
4	100	90	80	80
8	100	100	100	90
16	90	100	100	100
24	100	100	100	100
32	100	70	80	100
40	100	60	90	100
48	90	30	80	100
56	100	30	60	100
64	100	40	60	90
72	100	0	30	100
		2,000 γ/ml. of streptomycin		
0	80	80	90	90
4	80	0	30	100
8	80	1	20	90
16	70	0	20	100
24	60	0	20	100
32	30	0	0	100
40	30	0	0	90
48	40	0	0	100
56	20	0	0	100
64	10	0	10	100
72	20	0	30	100

[a] With no streptomycin all tubes up to 72 hr. were viable.

When the antibiotic was used in a concentration of 2,000 γ per milliliter the anti-bacterial action was considerably more pronounced except in the tubes kept at incubator temperature for the entire exposure time. At incubation temperature the results were comparable to those observed when the lesser concentrations of antibiotic were used, but when exposed only at refrigerator temperature the effect of this greater concentration became noticeable. Most of the replicates were viable up to 24 hours. After that time all of the organisms in many of the tubes were killed by the streptomycin. However, some tubes at each time up to 72 hours showed viability.

Initial incubation of 1 hour gave the most uniformly satisfactory results. All cultures were killed except those given merely a token (never over 15 minutes)

exposure to the antibiotic and one weakly viable replicate at 8 hours. Under these temperature conditions, 2,000 γ per milliliter appears to yield effective bactericidal action. Exposure for an initial incubation period of 2 hours is less effective. Although most of the replicates were nonviable, some were viable up to 24 hours, and again at 64 and 72 hours; from 28 through 56 hours all were nonviable.

Action of combined antibacterial agents on Vibrio fetus (Table 3). Extender containing 500 units of penicillin, 0.3% of sulfanilamide, and 500 γ of streptomycin was also studied under the same conditions as in the previous experiments using the single antibacterial agents, except that the study of

TABLE 3
Effects of penicillin, streptomycin, sulfanilamide on Vibrio fetus

Hr. of exposure	Per cent of viable tubes containing 500 γ/ml. of streptomycin, 500 units/ml. of penicillin, 0.3% sulfanilamide[a] when exposed		
	5° C.	1 hr. at 37° C. 5° C. thereafter	2 hr. at 37° C. 5° C. thereafter
24	100	90	80
32	100	70	80
40	80	80	80
48	100	90	90
56	100	90	80
64	100	90	80
72	100	80	90

[a] With no antibacterial agents all tubes up to 72 hr. were viable.

exposure entirely at incubator temperature was omitted. The results of exposure of the organism to this mixture were approximately the same as those obtained when 500 γ per milliliter of streptomycin alone was used.

SUMMARY AND CONCLUSIONS

Of the three antibacterial agents, penicillin, sulfanilamide, and streptomycin, used under the conditions in these experiments, streptomycin appeared to be the only one to possess any observable bactericidal action against *Vibrio fetus*.

The streptomycin was most effective when the extender–antibiotic–bacterial mixture was incubated 1 hour and then refrigerated for the remainder of the observation period. The reason for the greater effectiveness of streptomycin against the organism when held at incubator temperature for 1 hour in contrast to prolonged incubation is probably that during the first hour the organism was in its lag phase and, therefore, more susceptible to unfavorable influences. After 1 hour incubation some bacteria tend to acquire a resistance to antibiotics. A combination of the three agents, penicillin, streptomycin, and sulfanilamide, was no more effective than streptomycin alone.

A concentration of 2,000 γ per milliliter of streptomycin appeared to be the minimum that would kill *Vibrio fetus* under the conditions here described. It is true that the number of organisms used was much greater than would

be encountered under natural conditions. Experiments are now under way to test the action of streptomycin and penicillin in varying concentrations against a graduated number of organisms.

REFERENCES

(1) EASTERBROOKS, H. L. Antibiotic Treatment of Bull Semen Used in Artificial Insemination. *Fertility and Sterility*, **2**: 430. 1951.

(2) EASTERBROOKS, H. L., HELLER, P., LIEBERMAN, M., PLASTRIDGE, W. N., AND JUNGHERR, E. L. Fertility and Bacterial Content of Diluted Bull Semen Treated with Various Concentrations of Dihydrostreptomycin Sulfate. *Am. J. Vet. Research*, **12**: 191. 1951.

(3) EASTERBROOKS, H. L., HELLER, P., PLASTRIDGE, W. N., AND JUNGHERR, E. L. Comparative Fertility of Diluted Bull Semen Treated with Calcium Chloride Complex Streptomycin or Dihydro Streptomycin Sulphate. *J. Dairy Sci.*, **33**: 851. 1950.

(4) FOOTE, R. H., AND BRATTON, R. W. The Fertility of Bovine Semen in Extenders Containing Sulfanilamide, Penicillin, Streptomycin and Polymyxin. *J. Dairy Sci.*, **33**: 544. 1950.

(5) HENNAUX, L., DIMITROPOULOS, E., AND CORDIEZ, E. L'Action de la Penicilline sur la Vitalité du Sperme de Taureau. Soc. de Biologie. *Comp. rend.*, **142**: 408. 1948.

(6) HERRICK, J. B. Laboratory and Field Technique to Determine Fertility in the Bull. *J. Am. Vet. Med. Assoc.*, **115**: 87. 1949.

(7) HUGHES, D. E., AND MCENTEE, K. Personal communication. Unpublished data. 1952.

(8) JENNINGS, I. W. The Action of Penicillin In Vitro on Various Bacteria of Animal Origin. *Vet. Record*, **59**: 369. 1947.

(9) LAWSON, J. R., AND MACKINNON, D. J. No. 5. Vibrio Foetus Infection in Cattle. *Vet. Record*, **64**: 763. 1952.

(10) MCFADYEAN, J., AND STOCKMAN, S. *Report of the Departmental Committee Appointed by the Board of Agriculture and Fisheries to Inquire into Epizootic Abortion.* London. 1913.

(11) PLASTRIDGE, W. N. Cultural and Serological Observations on Vibrio Fetus. *J. Bacteriol.*, **42**: 816. 1941.

(12) PLASTRIDGE, W. N. Vibriosis in Cattle. Univ. of Pa. Vet. Ext. *Quart.* **122**: 62. 1951.

(13) PLASTRIDGE, W. N., AND EASTERBROOKS, H. L. Effect of Antibiotics in Diluted Bull Semen on Vibrio Fetus. *Am. J. Vet. Research*, **13**: 145. 1952.

(14) PLASTRIDGE, W. N., AND WILLIAMS, L. F. Observations on Vibrio Fetus Infection in Cattle. *J. Am. Vet. Med. Assoc.*, **102**: 89. 1943.

(15) PLASTRIDGE, W. N., AND WILLIAMS, L. F. Vibrionic Abortion in Cattle. *Cornell Vet.*, **38**: 165. 1948.

(16) PLASTRIDGE, W. N., WILLIAMS, L. F., AND PETRIE, D. Vibrionic Abortion in Cattle. *Am. J. Vet. Research*, **8**: 178. 1947.

(17) PLASTRIDGE, W. N., WILLIAMS, L. F., CHERNAK, C., AND EASTERBROOKS, H. L. The Effect of Vibriosis on Breeding Efficiency in Cattle. *J. Am. Vet. Med. Assoc.*, **118**: 367. 1951.

(18) PRIER, J. E. The In Vitro Effect of Several Antibiotics Against Vibrio Fetus. *Vet. Med.*, **46**: 358. 1951.

(19) RASBECH, N. O. Study of the Spreading of V. Fetus by Bulls Used in A.I. (Abs.) *Vet. Record*, **63**: 657. 1951.

(20) ROBERTS, S. J., GILMAN, H. L., AND LARSEN, P. H. Vibrio Fetus Infection in Cattle. *Cornell Vet.*, **40**: 111. 1950.

(21) SMITH, T. Spirilla Associated with Disease of the Fetal Membranes in Cattle (Infectious Abortion). *J. Exptl. Med.*, **28**: 701. 1918.

(22) SMITH, T. The Etiological Relation of Spirilla (Vibrio Fetus) to Bovine Abortion. *J. Exptl. Med.*, **30**: 313. 1919.

(23) SMITH, T. Further Studies on the Etiological Significance of Vibrio Fetus. *J. Exptl. Med.*, **37**: 341. 1923.

(24) SMITH, T., AND ORCUTT, M. L. Vibrios from Calves and Their Serological Relation to Vibrio Fetus. *J. Exptl. Med.*, **45**: 391. 1927.

(25) SMITH, T., AND ORCUTT, M. L. Some Morphological and Biological Characters of the Spirilla (Vibrio Fetus, N.S.P.) Associated with Disease of the Fetal Membranes in Cattle. *J. Exptl. Med.*, **30**: 299. 1919.

(26) SMITH, T., LITTLE. R. B., AND TAYLOR, M. S. Further Studies on the Etiological Role of Vibrio Fetus. *J. Exptl. Med.*, **32**: 683. 1920.

(27) STEGENGA, TH. Vibrio Fetus En Enzootische Steriliteit. *Tijdschr. Diergeneesk.*, **75**: 753. 1950.

(28) STEGENGA, TH. Vibrio-Foetus et Sterilite Enzootique. These, Utrecht, 1950. (Abs.) *Ann. med. vet.*, **95**: 51. 1951.

(29) STEGENGA, TH., AND TERPSTRA, J. I. Over Vibrio Fetusinfecties Bij Het Rund en ''Enzootische'' Steriliteit. *Tijdschr. Diergeneesk.*, **74**: 293. 1949.

(30) TERPSTRA, J. I., AND EISMA, W. A. Vibrio Foetus Infection in Cattle and Enzootic Sterility. *Tijdschr. Diergeneesk.*, **76**: 433. 1951.

(31) WEBSTER, H. D., AND THORP, FRANK, JR. Vibrionic Abortion, a Venereal Disease of Cattle. *Mich. State Coll. Vet.*, **11**: 23. 1950.

THE ANTIBACTERIAL ACTION OF PENICILLIN AND STREPTOMYCIN AGAINST VIBRIO FETUS INCLUDING CONCENTRATIONS FOUND IN NATURALLY INFECTED SEMEN [1]

A. EUGENIA ORTHEY AND H. L. GILMAN
Department of Pathology and Bacteriology
New York State Veterinary College, Ithaca, New York

The clinical complex now known as bovine vibriosis was for many years regarded as a relatively unimportant disease of reproduction manifested solely by infrequent premature death and expulsion of the fetus. We now recognize the venereal nature of the disease and its role as a major factor in the production of delayed conception and infertility. In a previous paper *(6)* the authors discussed the nature of the disease in some detail and attempted to evaluate the contributions of other workers in this field of study.

Adler *et al.* *(1)* recently confirmed the observations of Plastridge and associates *(8)*, Terpstra and Eisma *(9)*, and Hughes and McEntee *(4)* that virgin heifers could be infected through the use of artificial insemination using semen from known infected bulls which was not treated with antibiotics.

Exact data on the effectiveness of antibiotics when added to infected semen to destroy *Vibrio fetus* were lacking. Plastridge and Easterbrooks *(7)* stated that 500 units of penicillin had no adverse effect on the organism and that death occurred within 24 hours in the presence of 500 γ of streptomycin at 37° C., but not at 5° C. The authors found it difficult to agree with their subsequent statement that *V. fetus*, when present in the diluted semen treated with streptomycin, would be "rendered incapable of producing infection, at the time of, or following insemination, owing to the increased activity of the streptomycin at body temperature." In order to be effective, all vibrios should be dead before the semen is used for insemination. Lawson and MacKinnon's work *(5)*, with the antibiotics added "shortly before insemination," employed an exposure time that was insufficient to permit accurate evaluation of the effectiveness of the antibiotics.

For data to be of practical value, the authors feel that the semen, whether infected naturally or artificially, should be handled according to the methods commonly used in artificial insemination organizations. In another article *(6)*, the authors reported that a concentration of 2,000 γ per milliliter of streptomycin appeared to be the minimum that would kill *V. fetus* using such methods. However, it must be emphasized that the concentrations of *V. fetus* organisms used were considerably greater than would be encountered in the semen of naturally infected bulls. In order to simulate normal conditions and numbers of organisms as closely as possible, the project here reported was initiated.

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EXPERIMENTAL

During the course of the experiments reported, ten strains of vibrio were studied. Six concentrations of each strain were prepared by serial dilution and exposed for three different lengths of time to four levels of penicillin alone, to four levels of streptomycin alone, and to 500 units of penicillin in conjunction with four levels of streptomycin. Because of the number of cultures involved, six concentrations of one strain of vibrio, for three lengths of exposure, and four levels of one antibiotic were studied simultaneously. Repeated experiments were conducted, to study the ten different strains, and the two antibiotics singly and in combination.

Semen extender. In all trials, the vehicle to which the antibiotics and vibrios were added was citrate-buffered egg yolk. This consists of equal parts of a sterile 2.9% sodium citrate dihydrate solution and sterile egg yolk.

Extender-antibacterial agent mixtures. These mixtures were prepared early each morning, warmed to 37° C., and inoculated immediately. The procedure for their preparation, as described in a previous article *(6)* by the authors, was employed except that the tubes in the present studies contained 2 ml. of extender-antibacterial agent mixture.

The four concentrations of a given antibiotic to be simultaneously prepared and studied consisted of one set each of 0, 500, 1,000, and 2,000 units or micrograms per milliliter. The set with *0* concentration of antibiotic served as controls for studying the effects of the three other concentrations, since they were conducted simultaneously. The streptomycin used was Dihydro-Streptomycin Merck (present as the sulfate). The penicillin was Penicillin G Sodium Merck.

Vibrio fetus inoculum. Ten strains of vibrio were studied. Six of these were freshly isolated from the semen of infected bulls and were known as VM 756, VM 770, VM 793, VM 805, VM 811, and VM 1154. Their growth and morphological characteristics were identical to the strains of *V. fetus* recovered from aborted fetuses. Since naturally infected bulls transmit vibriosis to females at the time of service, and since the disease complex may be induced in the cow inseminated with semen to which these bull strains have been added, whether freshly isolated from semen or reconstituted following lyophilization, it is probably valid to conclude that these were true strains of *V. fetus.* Four were fetal strains from the abomasum of an aborted bovine fetus which either were recently isolated or reconstituted from strains that had been lyophilized shortly after recovery. These were therefore definitely identifiable as *V. fetus.* These were known as strains 14,804, 15,124, CU 177, and 17,006. All the strains had been transferred on artificial media as few times as possible after the original isolation. The organism was cultured on flasks and harvested as described in the previous report *(6)* under *Inoculum.* An exception was that with slow growing strains 3 days were allowed after distributing the original inoculum over the surface, before harvesting. The raw bacterial suspension from the flask was then centrifuged at about 2,045 G's for 15 minutes, the supernatant fluid drawn off aseptically, and the bacterial cells resuspended in fresh sterile physiological saline solution. This produced a suspension too dense to give an

accurate reading on the Klett-Summerson photoelectric density meter, but this suspension was adjusted so that a 1 in 10 dilution gave a density of 125 on the meter with a green filter. The basic dense suspension will be referred to hereafter as suspension No. 1 and the 1 in 10 dilution which gave a reading of 125 on the meter will be referred to as suspension No. 2. Suspension No. 2, in turn, was diluted serially tenfold three more times and the total series numbered 1 through 5, respectively. From these five suspensions the six tubes of antibacterial agent-extender mixtures were inoculated. These tubes were numbered 2 through 7. To tube No. 7 was added 0.02 ml. of suspension No. 5; to tube No. 6, 0.02 ml. of suspension No. 4; to tube No. 5, 0.02 ml. of suspension No. 3; to tube No. 4, 0.02 ml. of suspension No. 2; to tube No. 3, 0.02 ml. of suspension No. 1; and to tube No. 2, 0.2 ml. of suspension No. 1. Each of these six inoculums was replicated 12 times to study simultaneously three exposure periods and four levels of antibiotic.

By the use of the above procedure the tube numbers corresponded to the suspension numbers and the tubes likewise contained serial dilutions of 1 ml. in 10 ml. Therefore, the bacterial counts and density readings made on the suspensions could be applied to their correspondingly numbered tubes. This was necessary since neither bacterial counts nor density readings could be made on the tube contents because of the presence of opaque egg yolk.

An estimate was made of the number of organisms per milliliter represented by the density readings. The Breed method was used except that methylene blue was replaced with Gram's crystal violet. Only suspension No. 4 in the series, with a density of 1.2, could be counted. To check the validity of this count, special suspensions with Klett-Summerson densities of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 were prepared and counted. From these counts the theoretical numbers of organisms in each of the other suspensions were calculated. These calculations are shown in Table 1.

TABLE 1
Estimated number of organisms and Klett-Summerson density readings of suspensions of Vibrio fetus employed

Tube No.	Suspension No.	Klett-Summerson density reading	No. of organisms per ml.
–	1	too dense to read	$200\text{-}225 \times 10^8$
2	2	125	$200\text{-}225 \times 10^7$
3	3	12.5	$200\text{-}225 \times 10^6$
4	4	1.2	$22\text{-}24 \times 10^6$
5	5	<0	$22\text{-}24 \times 10^5$
6	4 dil. 100 times	<0	$22\text{-}24 \times 10^4$
7	5 dil. 100 times	<0	$22\text{-}24 \times 10^3$

The authors have also attempted to count the number of *V. fetus* organisms present in naturally infected semen. This was done by making thin smears of semen and counting the organisms and sperm cells in a given area. Knowing the total number of sperm cells per milliliter of a given sample of semen, the number of vibrios per milliliter could be calculated. The highest count was

11,318,600 vibrios per milliliter. The count in many samples was around 4,000,000 organisms. No organisms could be observed in some samples that proved positive on culture. The highest count was about one-half that in the experimental tube No. 4, or about five times the number found in tube No. 5. The number of organisms observed and counted must be regarded as only approximating the actual number present in a given sample, but the observations did allow some conclusions to be drawn as to the efficacy of the antibiotics against the numbers of organisms found in naturally infected semen.

Temperature treatment of extender-vibrio mixture. The tubes were placed in a water bath at 37° C. and, as closely as possible, the cooling method for semen used in artificial insemination described by Foote and Bratton *(2)* was employed. After the temperature reached 5° C., the tubes continued to be stored at that temperature for the duration of the exposure time.

Tests for viable cells. The inoculated extender-antibiotic mixtures were cultured for viable cells at the end of 1 hour, 6 hours, and 24 hours. One group of six serially diluted tubes from each set of tubes used for each concentration of antibiotic was cultured at the end of each of these intervals according to the technique previously described *(6)*, except that after the material had been resuspended, recentrifuged, and the supernatant fluid drawn off a second time, no further physiological saline was added. The precipitate was divided into two parts by means of an inoculating loop, and each half was placed in a separate tube of semi-solid thiol medium. The inoculum was stirred into the medium to a depth of 2-5 mm. involving not over one quarter of the surface area, as described by Hughes and Gilman *(3)* for semen and mucus culture. Incubation was at 37° C. in jars containing 15-20% carbon dioxide. The inoculated tubes were observed daily for a period of 14 days.

Naturally infected extended semen samples. Semen from naturally infected bulls was used for this series of tests. Both raw semen and extended semen were cultured. The raw semen cultures served as controls to demonstrate the presence of the organism in the semen before being extended. Semen was extended with the standard citrate-buffered egg yolk extender containing 500 units of penicillin, 500 γ of streptomycin, and 0.3% sulfanilamide per milliliter. The semen was extended to the normal limits of no less than 10×10^6 sperm cells per milliliter and cooled to 5° C. by the same procedure as is commonly practiced for artificial insemination *(2)*. The samples were cultured at 1 and 6 hours by the same procedures described under *Tests for viable cells.*

RESULTS

The control tubes containing 0 units or micrograms of antibiotics were viable at all times in all tubes. The results are based on duplicate studies of each strain.

Action of penicillin against Vibrio fetus (Figure 1). At the end of 1 hour exposure to 500 units of penicillin, most strains were viable in the concentrations in tubes No. 1 to 5, inclusive. In the higher dilutions (tubes No. 6 and 7) the bactericidal action was complete. There was, however, considerable strain differ-

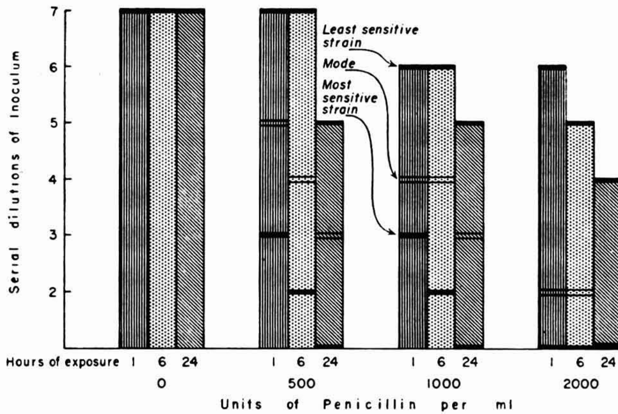

FIG. 1. Effects of penicillin on *V. fetus* in semen extender. The bars show the dilution tubes in which the organisms were viable. The highest point on any given bar represents the dilution tube in which the most resistant strain was viable. The lowest dark line on any given bar represents the most sensitive strain. The double lines express the mode.

ence. Two strains (VM 805 and CU 177) were apparently unaffected, whereas VM 1154, showing great penicillin sensitivity, was viable only in tubes No. 2 and 3. From this it can be seen that one hour exposure to penicillin is generally ineffective except in the highest dilutions of the organism.

After 6 hours of exposure to 500 units of penicillin, most strains were inhibited somewhat by penicillin but remained viable in the dilutions contained in tubes No. 2 through 4. One strain (CU 177) was again viable in all tubes, and VM 1154 was reduced to viability only in tube No. 2, containing the greatest number of organisms.

After 24 hours of exposure to 500 units of penicillin, all strains were affected more noticeably. The viability end point was generally reached in either tube No. 3 or tube No. 4. However, the difference in strain resistance caused a spread from VM 1154, nonviable in all tubes, to VM 805, viable in tube No. 5.

The organisms were more sensitive to 1,000 units of penicillin per milliliter. After 1 hour exposure, all strains showed some degree of sensitivity. Strains VM 805 and CU 177 were viable in the tubes up to and including No. 6. Again, the least resistant strain was VM 1154, but it was no less resistant to 1,000 than to 500 units per milliliter. All other strains were viable through tube No. 4.

After 6 hours of exposure to 1,000 units of penicillin per milliliter, the results were approximately the same as for 1 hour exposure at this concentration. The only exceptions to this were VM 770 and strain 17,006, which required the bacterial population density present in tube No. 3 in order to maintain viability.

At the end of 24 hours of exposure to 1,000 units of penicillin, only the two tubes (No. 2 and 3) containing the greatest density population of organisms were able to maintain viability for most of the strains. VM 1154 was nonviable in all tubes, VM 805 and CU 177 required the numbers of organisms in tube

No. 5 and tube No. 4, respectively, to maintain viability. VM 811 was viable only in tube No. 2.

Vibrio fetus showed even greater sensitivity to 2,000 units of penicillin per milliliter than to 1,000 units per milliliter, and 1 hour exposure was adequate to render most strains nonviable in all dilutions beyond the first two tubes (No. 2 and 3). Several (VM 793, VM 811, and 15,124) were so severely affected as to be nonviable in all concentrations. Again, VM 805 and CU 177 were the most resistant, remaining viable in dilutions as great as those found in tubes No. 6 and 5, respectively.

After 6 hours, only the heaviest concentration (tube No. 2) could maintain viability for many strains, and about half of the strains were nonviable. With longer exposures to greater quantities of penicillin the bactericidal action was more pronounced. Because of variation in strain sensitivity, two resistant organisms again were present. VM 805 required only a density population as present in tube No. 4, and CU 177 required only that in tube No. 5 to remain viable.

At 24 hours, the more drastic effect of 2,000 units of penicillin became very evident, and seven of the ten strains were nonviable. Only VM 805, CU 177, and 17,006 were viable in any of the tubes. The most resistant was VM 805, which was alive in tube No. 4. The other two were alive only in tube No. 2.

Action of streptomycin against Vibrio fetus (Figure 2). The same ten strains of *V. fetus* used in the penicillin trials were used in the streptomycin sensitivity tests. Streptomycin, since it is most active against Gram-negative

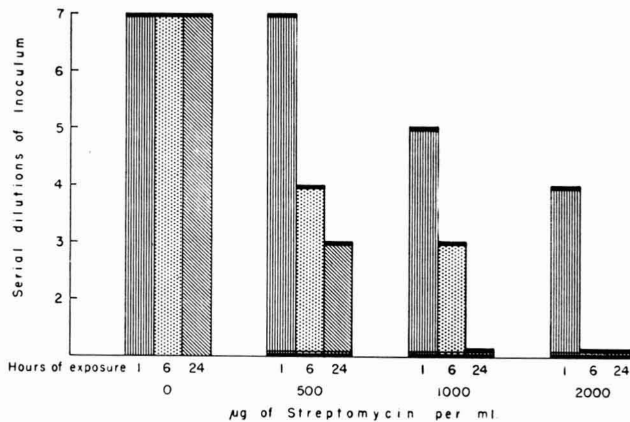

FIG. 2. Effects of streptomycin on *V. fetus* in semen extender. The bars show the dilution tubes in which the organisms were viable. The highest point on any given bar represents the dilution tube in which the most resistant strain was viable. The lowest dark line on any given bar represents the most sensitive strain. The double lines express the mode.

bacteria, was much more effective against vibrios than was penicillin. The effect was more rapid and rendered nonviable much greater numbers of organisms than did penicillin.

After 1 hour exposure to 500 γ of streptomycin, only one highly resistant strain (14,804) was viable in all tubes. All strains were more severely affected than they were when exposed to penicillin for 1 hour. The other strains viable at the end of this exposure period were VM 811 in all the dilutions up to and including tube No. 6; VM 756 up to and including tube No. 4; 15,124 in tubes No. 2 and 3; and VM 805 and VM 1154 in tube No. 2.

After 6 hours, all strains but two were nonviable even in the heaviest concentrations used in this experiment. The resistant strains, 14,804 and VM 756, were viable in dilutions as high as tubes No. 4 and 3, respectively.

After 24 hours, strains 14,804 and VM 756 were still highly resistant, being viable in tubes No. 2 and 3. All other strains were nonviable even in the heaviest concentrations used in the experiment.

At a concentration of 1,000 γ per milliliter, the ability of streptomycin to render nonviable larger quantities of organisms in shorter times was evident. Only three strains (14,804, VM 805, and VM 1154) were viable in any dilution after 1 hour, and after 6 hours only the highly resistant 14,804, alive in tubes No. 2 and 3, was viable. After 24 hours, all strains were nonviable in all tubes.

As reported in a previous communication *(6)*, 2,000 γ of streptomycin was highly bactericidal to the organism even when present in great numbers. The results were similar in the trials here reported. The only viable organisms were 14,804 and VM 1154, exposed 1 hour. They were viable up to and including tubes No. 4 and 2, respectively. Beyond exposure of 1 hour no recoveries of viable organisms were made on any strain exposed to this concentration of streptomycin.

Action of streptomycin plus penicillin against Vibrio fetus (Figure 3). Streptomycin in concentrations of 500, 1,000, and 2,000 γ per milliliter were used

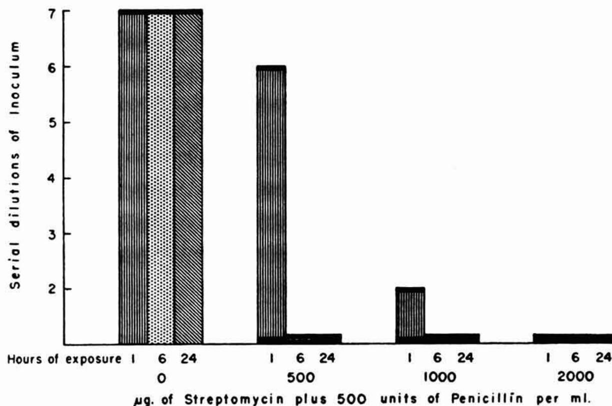

FIG. 3. Effects of streptomycin plus 500 units of penicillin on *V. fetus* in semen extender. The bars show the dilution tubes in which the organisms were viable. The highest point on any given bar represents the dilution tube in which the most resistant strain was viable. The lowest dark line on any given bar represents the most sensitive strain. The double lines express the mode.

in combination with 500 units of penicillin per milliliter to test the sensitivity of the same ten strains of *V. fetus*.

All strains were nonviable in all concentrations of the antibiotics in all tubes when the exposure time was longer than 1 hour. Only two strains were found viable in any dilution and these only at the 1 hour exposure period. At a concentration of 500 γ of streptomycin plus 500 units of penicillin, VM 805 was still viable in tube No. 2 and VM 811, surprisingly, in tubes up to and including tube No. 6. With 1,000 γ of streptomycin and 500 units of penicillin, VM 805 was again viable at 1 hour exposure in tube No. 2.

Action of penicillin, streptomycin, and sulfanilamide against Vibrio fetus organisms as found in extended, naturally infected semen. As previously stated, samples of semen from naturally infected bulls, and from which *V. fetus* had been cultured, were extended with the commonly used citrate-buffered egg yolk containing 500 γ of streptomycin, 500 units of penicillin, and 0.3% sulfanilamide per milliliter. Two hundred and one such known infected samples were cultured at the end of 1 hour and again at the end of 6 hours of exposure to the antibacterial agents in the extender. At no time was *V. fetus* recovered from any of these samples.

SUMMARY AND CONCLUSIONS

Both penicillin and streptomycin exhibit bactericidal action against *V. fetus* organisms under the conditions used in these experiments. Streptomycin was was by far the more effective of the two. A much greater density population of organisms per milliliter is required to maintain viability in the presence of streptomycin than with penicillin.

A combination of the two antibiotics, using 500 units of penicillin and 500 γ of streptomycin per milliliter in semen extender, killed all *V. fetus* organisms in concentrations up to 225×10^7 organisms per milliliter when exposed 6 hours under the temperature conditions used in these experiments. Since this was many more organisms than were found in naturally infected semen, this combination seems capable of rendering innocuous all *V. fetus* organisms that might be present in naturally infected semen.

At no time were the authors able to recover *V. fetus* from naturally infected semen extended with citrate-buffered egg yolk containing 500 units of penicillin, 500 γ of streptomycin, and 0.3% sulfanilamide per milliliter and handled by the common procedures employed by artificial breeding organizations.

ACKNOWLEDGMENT

The authors are indebted to David E. Hughes of this laboratory who isolated and supplied the original cultures of all bull strains used in this experiment.

REFERENCES

(1) ADLER, H. C., ALBERTSEN, B. E., RASBECH, N. O., AND SZABO, L. Diagnosticering of Vibrio fetus—Infektionen Hos Tyre Ved Smitteforsog. *Nord. Vet. Med.*, **4**: 462. 1952.

(2) FOOTE, R. H., AND BRATTON, R. W. The Fertility of Bovine Semen Cooled with and without the Addition of Citrate-Sulfanilamide-Yolk Extender. *J. Dairy Sci.*, **32**: 856. 1949.

(3) HUGHES, D. E., AND GILMAN, H. L. A Satisfactory Method for the Culture of Vibrio Fetus. *Cornell Vet.*, **43**: 463. 1953.

(4) HUGHES, D. E., AND MCENTEE, K. Unpublished data, 1952.

(5) LAWSON, J. R., AND MACKINNON, D. J. No. 5. Vibrio Fetus Infection in Cattle. *Vet. Record*, **59**: 369. 1952.

(6) ORTHEY, A. E., AND GILMAN, H. L. The Antibacterial Action of Penicillin, Streptomycin and Sulfanilamide Against Heavy Suspensions of Vibrio Fetus Added to Semen Extender. *J. Dairy Sci.*, **37**: .... 1954.

(7) PLASTRIDGE, W. N., AND EASTERBROOKS, H. L. Effect of Antibiotics in Diluted Bull Semen on Vibrio Fetus. *Am. J. Vet. Research*, **13**: 145. 1952.

(8) PLASTRIDGE, W. N., WILLIAMS, L. F., CHERNAK, C., AND EASTERBROOKS, H. L. The Effect of Vibriosis on Breeding Efficiency in Cattle. *J. Am. Vet. Med. Assoc.*, **118**: 367. 1951.

(9) TERPSTRA, J. I., AND EISMA, W. A. Vibrio Fetus Infection in Cattle and Enzootic Sterility. *Tijdschr. Diergeneesk.*, **76**: 433. 1951.

METABOLISM OF BOVINE SEMEN. I. UPTAKE OF GLUCOSE-C^{14} BY BOVINE SPERMATOZOA [1]

R. J. FLIPSE

Dairy Cattle Breeding Research Center
The Pennsylvania State University, State College

McCarthy and associates *(12)* suggested in 1927 that spermatozoa utilized the sugar contained within them as a substrate for glycolysis. Although glycolysis in semen has been investigated by many workers, it was not until 1946 that the sugar present in semen was identified as fructose *(9)*. This discovery came after more than twenty years of investigations by many workers *(2, 4, 6, 7, 8, 10, 11, 13, 14, 15, 16)*, who have sought to determine the nature and importance of the glycolytic process to spermatozoa.

Most of the reported studies have dealt with spermatozoa or with whole semen; however, the importance of seminal plasma also has been recognized *(13, 18)*. Winchester and McKenzie *(18)* demonstrated that even after 5 minutes at 100° C., extracellular respiration by seminal plasma of the boar was not eliminated. Plaut and Lardy *(13)* have reported that small amounts of seminal plasma increase the glycolysis of maltose by spermatozoa.

Studies of the endogenous respiration of spermatozoa have been reviewed by Anderson *(1)*, but prior to the recent report from this laboratory *(5)* no distinct evidence had been presented to indicate whether the substrate involved enters the sperm cell proper or disappears as a result of extra-cellular processes. In order to further investigate this problem, the use of tracer quantities of glucose-C^{14} has been continued to determine the extent to which this compound is taken up by ejaculated bovine spermatozoa.

EXPERIMENTAL

Semen was obtained by means of the artificial vagina from Holstein and Guernsey bulls and gradually cooled until used in the laboratory, usually 1½ to 3 hours after collection. Only the semen samples having an initial motility of at least 50% and a concentration of at least 10^9 spermatozoa per milliliter were used. The semen collected from two to four bulls was pooled and diluted with calcium-free Ringer-phosphate solution (pH 7.4) to a final concentration of 0.5×10^9 spermatozoa per milliliter for each trial.

After the designated incubation period, samples were inactivated by the addition of formalin (except for the first two trials described under *Results*). Spermatozoa were recovered by centrifugation and resuspended in Ringer-phosphate. The washing procedure was repeated until no further reduction in radioactivity occurred in the washed spermatozoa. Usually three washings were

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adequate. After the final centrifugation, spermatozoa were suspended in 1 ml. of Ringer-phosphate, and 0.5 ml. of the suspension was transferred with a micropipette to a 1⅛-in. aluminum planchet. A disk of lens-cleaning tissue was placed on top of the suspension to assure uniform spreading *(3)*, and the sample was dried under an infrared lamp. Radioactivity of the dried preparation was assayed in a windowless flow gas G.M. counter in conjunction with a decade scaling unit. As a general rule, 10^9 spermatozoa were plated for assay; in the few instances where this was not possible the radioactivity was corrected for spermatozoan numbers.

RESULTS

A simple exploratory trial was conducted to determine the feasibility of using labeled compounds to study spermatozoan metabolism. Fresh, pooled semen with an average initial motility of 60% and a concentration of 1.48×10^9 spermatozoa per milliliter was diluted with Ringer-phosphate buffer. The sample was placed in a 50-ml. Erlenmeyer flask and tempered in a water bath at 37° C. Uniformly labeled glucose-C^{14} previously dissolved in Ringer-phosphate was added, 6.16 μc. being added to the 25-ml. sample. After incubation intervals of 10, 60, and 120 minutes, 8-ml. aliquots were removed from the flask and prepared for assay as described above.

The results of this trial are shown in Figure 1. The radioactivity associated with the spermatozoa increased with increase in incubation time, the relationship being approximately linear. Whether this was actual uptake or simply a matter

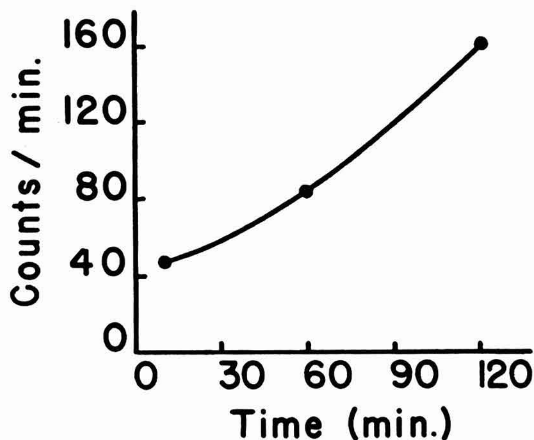

FIG. 1. The effect of incubating for various periods of time on the uptake of glucose-C^{14} by spermatozoa.

of surface adsorption was not determined; no control sample of dead or inactivated spermatozoa was included in the trial.

Inactivation of spermatozoa. From this exploratory trial it was apparent that a quick, efficient method of inactivating spermatozoa was required. Such

a method was needed first to provide metabolically inactive spermatozoa for use as controls and, secondly, to stop the metabolic activity of the spermatozoa at the specified end point of the trial. The latter was particularly important in studies involving brief incubation periods, for considerable time was required for harvesting the spermatozoa and preparing the sample for the assay of radioactivity. Several methods of inactivating spermatozoa were tested, and results of a typical trial are shown in Table 1. Ethanol gave variable results, in some trials stimulating and in other trials inhibiting the uptake of radioactivity. Heating apparently caused some loss of radioactivity from the incubated sample. Distilled water reacted too slowly to be useful in stopping a reaction. Both

TABLE 1

Effect of various methods of inactivation of spermatozoa on the uptake of radioactivity

Treatment	Radioactivity when treated	
	Before incubation	After 30 min. incubation
	(c.p.m.)[a]	(c.p.m.)
Ethanol, 0.2 ml.	125	161
Heat, boiling water bath for 2 min.	32	58
Formalin, 0.2 ml.	25	94
Distilled water, 10 ml.	36	150
5 *N* Sulfuric acid, 0.2 ml.	33	92

[a] Counts per minute per 10^9 spermatozoa.

formalin and 5 *N* sulfuric acid gave satisfactory results; formalin was adopted for subsequent use because it produced lower levels of radioactivity in the controls than did sulfuric acid.

Effect of washing spermatozoa on uptake of C^{14}. The method used in the preparation of the sperm cells obviously is an important factor in working with the requirements of spermatozoa. Although it is desirable to remove all traces of seminal plasma in order to avoid possible interference by this material, too vigorous preparatory treatment must be avoided if damage to the spermatozoa is to be averted.

Trials were conducted in which the uptake of labeled glucose by spermatozoa in diluted semen was compared to the uptake by spermatozoa washed once, twice, or three times with physiological saline. Spermatozoa were resuspended in each washing by gentle pumping action with a glass syringe. A section of polyethylene tubing replaced the usual metal needle on the syringe. Each suspension was finally diluted to 0.5×10^9 spermatozoa per milliliter in Ringer-phosphate. Four ml. of each diluted suspension was tempered in a water bath at 37° C. Two-tenths ml. of Ringer-phosphate containing 0.85 μc. of glucose-C^{14} was added to each sample, and the tubes were incubated for 1 hour at 37° C. Samples were prepared for assay as described above. The mean corrected radioactivity associated with spermatozoa in each treatment is shown in Table 2. Radioactivity increased after both the first and second washings but declined slightly after the third washing.

TABLE 2

Effect of washing spermatozoa prior to incubation with glucose-C^{14} (average of four trials)

Treatment	Radioactivity after incubation for	
	0 min. (control)	60 min.
	(c.p.m.) [a]	*(c.p.m.)*
No washing	30	163
One washing	31	207
Two washings	28	222
Three washings	30	217

[a] Counts per minute per 10^9 spermatozoa.

Effect of length of incubation upon uptake of C^{14}. Spermatozoa were obtained from the pooled ejaculates of two bulls, washed twice with saline and suspended in Ringer-phosphate. Four-ml. aliquots were pipetted to 13 × 125 mm. test tubes, 0.2 ml. formalin was added to each control tube, and all tubes were placed in the 37° C. water bath. After temperature equilibration, 0.2 ml. of Ringer-phosphate containing 0.72 μc. of glucose-C^{14} was added to each tube. After incubation for 60 and 120 minutes, the reaction was stopped by addition of formalin. Spermatozoa were harvested, washed, dried, and assayed for radioactivity as described above. The radioactivity of 60-minute samples averaged 42; that of 120-minute samples averaged 73 more counts per minute than the radioactivity of the comparable control. The 120-minute controls showed no increase in radioactivity over controls incubated for 60 minutes.

Uptake of glucose-C^{14} under anaerobic conditions. Twice-washed spermatozoa were suspended in Ringer-phosphate and flushed with nitrogen for 10 minutes prior to the start of the trial. The results of three trials conducted with spermatozoa from semen above average in quality (mean concentration 1.59 × 10^9 spermatozoa per milliliter, mean initial motility 59%) are presented in Table 3. Although a direct comparison with aerobic conditions was not made in each case, it appears that the maximum uptake occurs earlier under anaerobic than under aerobic conditions. Possibly this is simply a reflection of more rapid glycolysis under nitrogen, with the liberation of C^{14} containing metabolites from the cell.

TABLE 3

Radioactivity of washed spermatozoa after incubation under nitrogen at 37° C., with 2 × 10^9 spermatozoa in 4 ml. of Ringer-phosphate solution

Trial	Length of incubation *(min.)*			
	0 (control)	60	120	240
	(c.p.m.) [a]	*(c.p.m.)*	*(c.p.m.)*	*(c.p.m.)*
1	19	81	95	75
2	24	110	95	99
3	17	90	64	60
Av.	20	94	85	78

[a] Counts per minute per 10^9 spermatozoa.

DISCUSSION

The trials reported herein were of an exploratory nature with the object of establishing whether or not spermatozoa can utilize exogenous sources of energy; no effort was made to recover metabolites or to assay radioactivity possibly associated with them. The data indicate rather conclusively that spermatozoa do utilize exogenous glucose, as considerable radioactivity was found in excess of the low radioactivity of inactivated spermatozoa. The radioactivity of formalin-treated spermatozoa after exposure to glucocse-C^{14} for as long as 4 hours was somewhat variable between trials but apparently did not increase after the first few minutes of exposure.

The cause of the reduced uptake of glucose-C^{14} by spermatozoa in the presence of seminal plasma is subject to speculation. Most of the seminal plasma was removed with the first washing, and this is reflected in the uptake of radioactivity by spermatozoa. The differences in uptake after one, two, and three washings were not great, probably as a result of the counteracting effects of removal of seminal plasma and increased injury to spermatozoa with each succeeding washing. The optimum number of washings is dependent upon the techniques employed, for such factors as the speed and time of centrifuging have obvious effects upon spermatozoan viability. The effect of washing observed in these trials is in agreement with the report of White *(17)*. The effect of seminal plasma could be due to the presence of some substance contained in it which *(a)* is used in preference to the glucose-C^{14}, *(b)* inhibits uptake of glucose-C^{14}, or *(c)* stimulates glycolysis and the release of radioactive metabolites. The report of Vantienhoven *et al.* *(16)* would appear to invalidate the first explanation, inasmuch as fructose is naturally present in seminal plasma, and these workers found that glucose is used in preference to fructose. Work by Plaut and Lardy *(13)* supports the third proposed explanation. They reported that small amounts of seminal plasma increased the ability of spermatozoa to glycolyze maltose. If seminal plasma similarly stimulates the glycolysis of glucose, the liberation of C^{14} through metabolites would account for the apparent reduction in uptake of glucose-C^{14} in the presence of seminal plasma.

SUMMARY

The uptake of glucose by bovine spermatozoa was studied by using uniformly labeled glucose-C^{14} as a tracer. Spermatozoa were inactivated for use as controls or to stop a reaction by addition of formalin to the suspension. The presence of seminal plasma caused an apparent reduction in the uptake of glucose-C^{14} by spermatozoa. Two preexperimental washings prevented this effect; additional washings tended to be detrimental to the spermatozoa. Uptake of radioactivity by washed spermatozoa was five to eight times greater than the uptake (adsorption) by formalin-inactivated spermatozoa when suspensions of spermatozoa were incubated with glucose-C^{14} at 37° C. under air or nitrogen.

REFERENCES

(1) ANDERSON, J. The Semen of Animals and Its Use for Artificial Insemination. Imp. Bur. Animal Breeding and Genetics, *Tech. Communication.* 1945.

(2) ANDERSON, J. Sugar Content of Bull Semen and its Reduction During Storage. *J. Agr. Sci.*, **36**: 260. 1946.

(3) CALVIN, M., HEIDELBERGER, C., REID, J. C., TOLBERT, B. M., AND YANKWICH, P. E. *Isotopic Carbon.* John Wiley & Sons, Inc., New York. 1949.

(4) COMSTOCK, R. E. A Study of the Mammalian Sperm Cell. I. Variations in the Glycolytic Power of Spermatozoa and Their Relation to Motility and Its Duration. *J. Exptl. Zool.*, **81**: 147. 1939.

(5) FLIPSE, R. J. Aerobic Uptake of Glucose–C^{14} by Bovine Spermatozoa. *J. Dairy Sci.* (Abs.), **36**: 579. 1953.

(6) LARDY, H. A., AND PHILLIPS, P. H. The Interrelation of Oxidative and Glycolytic Processes as Sources of Energy for Bull Spermatozoa. *Am. J. Physiol.*, **133**: 602. 1941.

(7) MACLEOD, J. The Metabolism of Human Spermatozoa. *Proc. Soc. Exptl. Biol. Med.*, **42**: 153. 1939.

(8) MACLEOD, J. The Metabolism of Human Spermatozoa. *Am. J. Physiol.*, **132**: 193. 1941.

(9) MANN, T. Studies on the Metabolism of Semen. 3. Fructose as a Normal Constituent of Seminal Plasma. Site of Formation and Function of Fructose in Semen. *Biochem. J.*, **40**: 481. 1946.

(10) MANN, T. Fructose and Fructolysis in Semen in Relation to Fertility. *The Lancet*, **254**: 446. 1948.

(11) MANN, T., AND LUTWAK-MANN, C. The Metabolism of Semen. IV. Aerobic and Anaerobic Utilization of Fructose by Spermatozoa and Seminal Vesicles. *Biochem. J.*, **43**: 266. 1948.

(12) MCCARTHY, J. F., STEPITA, C. F., JOHNSON, M. D., AND KILLIAN, J. A. Glycolysis in Semen. *Proc. Soc. Exptl. Biol. Med.*, **25**: 54. 1927.

(13) PLAUT, G. W. E., AND LARDY, H. A. Metabolism of Ejaculated Bull Spermatozoa with Particular Reference to the Glycolysis of Maltose. *Am. J. Physiol.*, **162**: 598. 1950.

(14) ROSS, V., MILLER, E. G., AND KURZROK, R. Metabolism of Human Sperm. *Endocrinol.*, **28**: 885. 1941.

(15) SALISBURY, G. W., AND VANDEMARK, N. L. Stimulation of Livability and Glycolysis by Additions of Glucose to the Egg Yolk–Citrate Diluent for Ejaculated Bovine Semen. *Am. J. Physiol.*, **143**: 692. 1945.

(16) VANTIENHOVEN, A., SALISBURY, G. W., VANDEMARK, N. L., AND HANSEN, R. G. The Preferential Utilization by Bull Spermatozoa of Glucose as Compared to Fructose. *J. Dairy Sci.*, **35**: 637. 1952.

(17) WHITE, I. G. The Effect of Washing on the Motility and Metabolism of Ram, Bull and Rabbit Spermatozoa. *J. Exptl. Biol.*, **30**: 200. 1953.

(18) WINCHESTER, C. F., AND MCKENZIE, F. F. Relative Metabolic Rates of Semen, Seminal Plasma, and Bacteria in Semen of the Boar. *Proc. Soc. Exptl. Biol. Med.*, **46**: 455. 1941.

BUTYRATED LARD IN THE AD LIBITUM FEEDING OF "FILLED MILK" FOR VEAL PRODUCTION

JOHN H. HOPPER, K. E. GARDNER, AND B. CONNOR JOHNSON
Division of Animal Nutrition
and the Department of Dairy Science
University of Illinois, Urbana

The usual method of feeding veal calves is that of supplementing the milk diet with grain and hay. However, Wiese *et al.* *(9)*, in developing synthetic milk rations for the dairy calf, fed these diets ad libitum with no other supplement and obtained satisfactory gains. Gardner *et al.* *(1)* found that in feeding whole milk ad libitum with no hay or grain offered they could secure excellent daily gains.

Wiese *et al.* observed that when soybean oil was used as a source of fat, the calves grew poorly, scoured, and appeared unthrifty, whereas the animals that received the rations prepared with lard showed good gains in weight, did not scour, and appeared normal and healthy. Krauss *et al.* *(4)* used a milk fat substitute–skimmilk combination along with grain and hay in the feeding of young dairy calves. They reported an average total gain of 44 lb. in body weight when calves were slaughtered at 50 days of age, as compared to 56 lb. gain for whole milk in the same period. Gullickson *et al.* *(2)* have shown that vegetable oils are unsatisfactory as a substitute for butterfat in the feeding of young dairy calves, and Jacobson *et al.* *(3)* showed that these could be greatly improved by hydrogenation. However, although lard seemed to be a satisfactory fat in our synthetic milk studies, the Minnesota experiment indicates that lard, while well used, was not equal to milk fat, and it seemed that the short chain fatty acids of butter might be important in the nutrition of the dairy calf.

Considerable work has been done with antibiotics as growth stimulants in calves. Loosli *et al.* *(5)* found that the rate of daily gain for Holstein calves fed an antibiotic supplement for a 56-day period was 1.16 lb. as compared to 0.95 lb. for the control calves. Murley *et al.* *(7)*, in comparing the effect of aureomycin in different diets fed to calves from 4 to 60 days of age, report that the aureomycin-fed calves in each group were superior in weight gains and in physical appearance to the controls. Rusoff and Davis *(8)* studied the effect of aureomycin on growth of young calves weaned from milk at an early age and fed for 14 weeks. They found that Jersey calves receiving aureomycin feeding supplement and those receiving crystalline aureomycin showed a gain of approximately 25% over the Jersey control calves, whereas the Holstein aureomycin groups showed a gain of approximately 15% over the Holstein control group.

The purposes of this experiment were to compare the gains obtainable with dairy calves on ad libitum feeding of a lard-containing filled milk with those

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obtainable on a butyrated lard-containing filled milk (a lard containing the same butyrated composition as does milk fat), and to test the growth stimulating effect of aureomycin under these very rapid growth conditions.

EXPERIMENTAL PROCEDURE

In this trial, two different fats were used: lard and butyrated lard.[1] These fats were emulsified [2] and mixed with both fluid skimmilk and reconstituted dried nonfat milk solids to provide a 4% fat (liquid basis) filled milk, which was warmed before feeding. No differences were found between fluid and reconstituted skimmilk. Bull calves from the five major dairy breeds were left with their dams for 3 days following birth, after which they were placed in individual stalls bedded with wood shavings. The calves were fed three times daily with a nipple pail, the milk being given in increasing amounts for the first 2 weeks, after which they received all the milk they would consume. Any milk refused was weighed back after each feeding. All calves were continued on experiment until 56 days of age and then slaughtered.

Trace mineralized salt was available at all times, and, in addition, a mineral solution was mixed with the milk. This mineral solution supplied 226.0 mg. of ferric citrate, 11.7 mg. of $MnSO_4$, and 2.34 mg. of $CuSO_4$ per liter of milk. Each calf received 100,000 U.S.P. units of vitamin A weekly by capsule and 50,000 U.S.P. units of vitamin D at the beginning of the trial, and 50,000 units at 4 weeks of age, also by capsule.

Half of the calves on both filled milks received 3 mg. of aureomycin hydrochloride per pound of milk.

The calves were weighed on two succeeding days at birth and at the end of the feeding trial, and weekly throughout the feeding period.

RESULTS AND DISCUSSION

The growth data are reported in Table 1. From this table it can be seen that the calves fed butyrated lard made daily gains similar to those reported by Gardner *et al.* *(1)* for calves which received whole milk ad libitium. As five different dairy breeds were involved in this study, the body gains are also reported as percentages of Morrison's standards *(6)* in order to compensate for breed differences. When expressed in this manner, the differences in effect of type of fat on body gain are even more striking. This would indicate that the butyrated lard can be as efficiently utilized by the calf as milk fat.

In confirmation of the earlier work of Gullickson *et al.* *(2)*, and of Wiese *et al.* *(9)*, lard itself was well used by the dairy calf as a fat source. Even under these conditions of very high intake, scouring, although somewhat more prevalent than on whole milk or the butyrated lard, was not a serious problem.

[1] The butyrated lard was produced by an ester interchange reaction and had approximately the same butyrated content as butter (3%).

[2] The lard and butyrated lard emulsions were prepared by homogenizing in water with an emulsifier (lipogel) to give a 60% fat emulsion.

TABLE 1

Summary of gains made by calves[a] receiving different fats

Ration	No. of calves	Av. initial wt.	Av. final wt.	Av. daily gain	Per cent of Morrison standards[c]
		(lb.)	*(lb.)*	*(lb.)*	
Whole milk[b]	32	86	198	1.98	244
Lard	22	98	204	1.90	201
Butyrated lard	12	97	218	2.16	241

[a] Three calves were removed from the butyrated lard trials, two with respiratory infections and one with navel ill. One calf was removed from the lard emulsion group because of a respiratory infection.

[b] See Gardner *et al.* *(1)*.

[c] Holstein normals were used for Brown Swiss.

There were enough Holstein calves on each of the treatments to enable a statistical analysis of the data for this one breed to be carried out. This analysis is presented in Tables 2 and 3.

TABLE 2

Comparison of gains of Holstein calves receiving butyrated lard and whole milk

Ration	No. of calves	Mean initial wt.	Mean final wt.	Corrected final wt.[b]	Difference	P. value of difference
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	
Whole milk[a]	8	99.9	211.3	211.3	12.7	~0.09
Butyrated lard	8	102.0	226.0	224.0		

[a] See Gardner *et al.* *(1)*.

[b] Differences in mean final weight were adjusted to the same mean initial weight by the covariance method.

When comparisons are made in this manner, the gains made by calves receiving the butyrated lard were at least equal to those made by calves receiving whole milk ($P < 0.1$). When a comparison of butyrated lard and lard is made by the same method, the gains made on the butyrated lard are significantly superior ($P < 0.03$). From these results it appears that this butyrated

TABLE 3

Comparison of gains of Holstein calves receiving lard emulsion and butyrated lard

Ration	No. of calves	Mean initial wt.	Mean final wt.	Corrected final wt.[a]	Difference	P. value of difference
		(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	
Lard emulsion	18	99.7	209.7	210	14	~0.03
Butyrated lard	8	102.0	226.0	224		

[a] See note [b], Table 2.

lard-filled milk can satisfactorily replace whole milk in the feeding of dairy calves. Since this was true in these veal calf experiments, in which all the burden of growth was put on the "milk" ration, it would seem to be equally satisfactory as an early feed for calves when they are converting from a milk diet to dry feed.

From Table 4 it can be seen that there was no appreciable difference observed between the aureomycin and the control calves. This is in contrast to the work cited above. A possible explanation for the lack of a stimulation in this trial could be the health and general condition of the calves, which were strong and vigorous throughout the test. It would appear that under similar conditions and at the rate of gain reported here, little additional stimulation can be expected from the use of an antibiotic.

TABLE 4
Summary of gains of calves receiving aureomycin

Treatment	No. of calves	Av. daily gain	Per cent of Morrison standards
		(lb.)	
Aureomycin supplemented	17	1.99	218
Control	17	1.99	212

SUMMARY

In experiments involving 34 three-day-old male calves from the five major dairy breeds, it was found that ad libitum feeding of an emulsified filled milk containing butyrated lard gave average daily gains of 2.16 lb. over an 8-week experimental period. These gains, which averaged 241% of Morrison standards, were equal to those obtained on whole milk and were significantly better than the gains obtained on a filled milk containing lard. On the basis of this work, it appears that the short chain fatty acids are important for the calf and that such a butyrated lard-filled milk can satisfactorily replace whole milk in the feeding of dairy calves.

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REFERENCES

(1) GARDNER, K. E., NEVENS, W. B., FOLKERTS, T., AND JOHNSON, B. CONNOR. Growth Stimulants and ad Libitum Feeding in the Production of Milk-Fed Veal. *J. Animal Sci.*, 11: 761. 1952.

(2) GULLICKSON, T. W., FOUNTAINE, F. C., AND FITCH, J. B. Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Calves. *J. Dairy Sci.*, 25: 117. 1942.

(3) JACOBSON, N. L., AND CANNON, C. Y. Soybean Filled Milks for Feeding Young Dairy Calves. *J. Dairy Sci.*, 30: 587. 1947.

(4) KRAUSS, W. E., MONROE, C. F., AND HAYDEN, C. C. The Value of a Milk Fat Substitute-Skimmilk Combination for Raising Bull Calves for Veal and Heifer Calves for Replacements. *Ohio Special Circ.* 57. 1939.

(5) LOOSLI, J. K., WASSERMAN, R. H., AND GALL, L. S. Antibiotic Studies with Dairy Calves. *J. Dairy Sci.*, **34**: 500. 1951.

(6) MORRISON, F. B. *Feeds and Feeding.* 21st ed. The Morrison Publ. Company, Ithaca, N. Y. 1948.

(7) MURLEY, W. R., JACOBSON, N. L., WING, J. M., AND STODDARD, G. E. The Response to Aureomycin Supplementation of Young Dairy Calves Fed Various "Practical" and Restricted Diets. *J. Dairy Sci.*, **34**: 500. 1951.

(8) RUSOFF, L. L., AND DAVIS, A. V. Effect of Aureomycin on Growth of Young Calves Weaned from Milk at an Early Age. *J. Dairy Sci.*, **34**: 500. 1951.

(9) WIESE, A. C., JOHNSON, B. CONNOR, MITCHELL, H. H., AND NEVENS, W. B. Synthetic Rations for the Dairy Calf. *J. Dairy Sci.*, **30**: 87. 1947.

THE MICROFLORA OF BLUE CHEESE SLIME [1, 2]

C. B. HARTLEY [3] AND J. J. JEZESKI
Department of Dairy Husbandry
University of Minnesota, St. Paul

Cheese slime may be described as an orange-yellow, mucilaginous accumulation of microorganisms and digested curd which develops over the outer surface of certain types of cheese. The value of the slime in the ripening of Blue cheese has not been completely established. There is no question that the ripening of Blue cheese is due largely to the action of the mold, *Penicillium roqueforti.* Recently, Morris *et al.* *(9)* indicated that under their ripening conditions, normally slimed cheese developed a finer flavor and body than unslimed cheese. Whether this was due to a direct action of the slime or to other factors was not determined.

The slime developing on Blue cheese has been described briefly as consisting of yeasts, micrococci, and rod-shaped bacteria *(3, 7, 8, 14)*. However, no detailed description has been reported of the types of organisms found in the slime of Blue cheese. Numerous reports have been noted regarding the type of slime which develops on Limburger *(4, 7, 16, 18)* and Brick-type cheese *(6, 16)*. Preliminary results indicated that the type of slime developing under our local conditions of curing Blue and Brick cheese did not conform to earlier descriptions.

In order to establish the possible role of the slime during ripening, a detailed knowledge of the organisms making up the slime was considered necessary. Consequently, this investigation was undertaken to describe in some detail various microbiological and chemical characteristics of Blue cheese slime.

METHODS

Six lots of Blue cheese were made from raw milk by the method used at the Dairy Husbandry Department of the University of Minnesota. The cheeses were then ripened in a sandstone cave at temperatures between 46 and 49° F. To establish the effect of the method of manufacture and the effect of different curing conditions on slime development, other cheeses were manufactured in a commercial plant (Plant A), using the Iowa State process, in which homogenized raw milk was utilized. The latter cheeses were then cured in sandstone caves at temperatures of 55 to 58° F.

Slime samples for bacteriological and chemical analysis were obtained at intervals of 7 to 10 days throughout the sliming period. The slime was removed from an entire flat surface of a cheese and from a part of the curved side.

Estimates of the total number of yeasts and bacteria were made using standard

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[2] The data in this paper are taken from a thesis presented by the senior author in partial fulfillment of the requirements for the degree of Master of Science, University of Minnesota.

[3] Present address: Kraft Foods Company, Galena, Illinois.

plate counts. The basal medium (TGE) consisted of 0.5% tryptone, 0.1% glucose, 0.3% beef extract, and 1.5% agar. The pH was adjusted to 7.0 with NaOH prior to sterilization at 15 lb. pressure for 15 minutes. Preliminary platings indicated that supplementing this medium with tomato juice and yeast extract did not significantly increase the counts obtained on cheese slime.

One medium (TGE-5M) contained 5% skimmilk in addition to the above basal medium, and the second (TGE-5M5S) was adjusted so that it contained concentrations of 5% skimmilk and 5% sodium chloride. The skimmilk and concentrated saline (15%) were autoclaved separately and added aseptically to the autoclaved basal medium.

The broth media used for salt and pH tolerance tests were similar to the above basal medium except that 0.5% yeast extract was added and the agar was omitted. For pH tolerance, various values at 0.5 pH unit intervals between pH 2.0 and 10.0 were obtained by adjusting the broth with *N*/1 NaOH and *N*/1 HCl. Final pH values were determined after autoclaving. Salt tolerance tests were made in broths containing NaCl in concentrations adjusted at 2.5% increments from 5 to 20%. Incubations were carried out at 20° C. for 10 days.

Proteolytic counts were made on both the above agar media. Lipolytic counts were determined on nile blue sulfate-butterfat (NBS fat) agar according to the method of Knaysi as described by Stark and Scheib *(13)*. The total yeast count was determined with unacidified malt agar (Difco) at pH 4.8.

The slime was prepared for plating by weighing 1 g. of well-mixed slime into a dilution blank containing 99 ml. of sterile distilled water and one tablespoon of glass beads (1/8 in. dia.). This blank was agitated vigorously at room temperature until the slime was thoroughly broken up and well dispersed. Further dilutions were made in the conventional manner.

Incubation was for a period of 7 days at 20 ± 1° C. unless otherwise stated in the results. This time-temperature combination gave results similar to those obtained in 12-14 days at 10° C.

Slides used for microscopic examination were prepared from the 10^{-2} and 10^{-4} dilutions by the standard Breed technique. These slides were stained by the Hucker modification of the Gram stain *(12)*.

Organisms predominating in the slime at various times were isolated and purified by streaking. Care was always taken to isolate organisms on a medium similar to that on which the colony originally developed. Additional pure culture studies followed, for the most part, methods as described in the *Manual of Methods for Pure Culture Study of Bacteria (12)*. The cultures were identified according to *Bergey's Manual of Determinative Bacteriology (2)*.

In the chemical analyses, the salt concentration was determined by the method of Wilster *et al. (15)*, moisture by the official method *(1)*, and pH with a Beckman, Model H2, pH meter.

RESULTS

Macroscopic examination of the slime. During the first 7 to 10 days in the curing cave at 46 to 49° F., there was very little growth on the surface of the

cheese. At the end of this time, a white slime began to appear, and scattered mold growth was soon observed on the surface. After the first scraping at 39 days of age, the cheese became covered with an orange-yellow, sticky slime. After the second scraping at 78 days, the orange-yellow mucilaginous slime reappeared and persisted until the final scraping. (Taking a sample for analysis is not to be confused with the scraping which is a commercially practiced removal of the heavy accumulation of slime at regular intervals during the ripening period.)

A similar sequence of development occurred on cheese ripened at 55 to 58° F., although the time intervals between scrapings were considerably shortened, the first scraping having been made at 21 days and the second at 49 days. A more profuse mold growth was evident on the surface of the cheese ripened at the higher temperatures.

Microscopic examination. Microscopic examination of slime developing at 46 to 49° F., showed the slime to be composed predominantly of yeasts until about 67 days of curing had elapsed. Large numbers of cocci and some rod forms were present after 46 days. Cocci predominated from this time until rod forms gained ascendancy after about 84 days of ripening.

No detailed microscopic analysis was made of the slime formed at 55 to 58° F.

Bacteriological analysis of the slime. Plate counts obtained from the slime of six lots of Minnesota Blue cheese at intervals during the ripening period

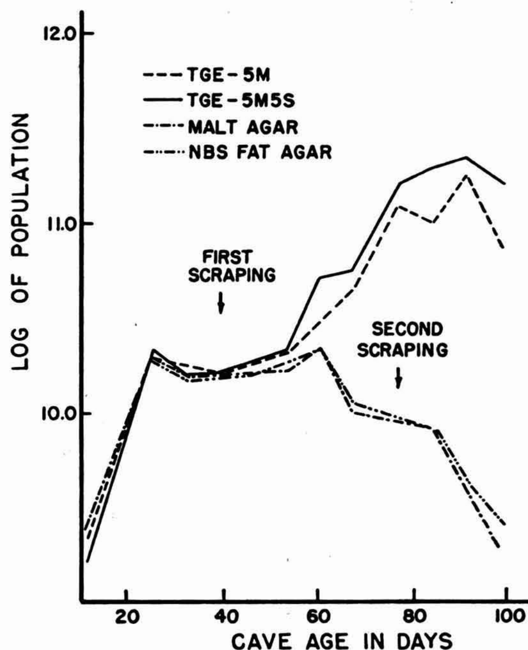

FIG. 1. Numbers of organisms per gram of slime from Minnesota Blue cheese (lot 807) during ripening period in cave.

demonstrated that the slime developed in a similar manner on each lot. A typical example of this development as shown by the plate count is presented in Figure 1.

The flora developing on the Minnesota cheese during the first few weeks was predominantly yeasts. After the first scraping, bacteria began to appear on the plates poured from high dilutions. An orange micrococcus predominated at the end of this period, but before the second scraping a lemon-yellow rod-type organism appeared in relatively large numbers. After the second scraping, this latter organism was the predominant bacterium in the slime. A white micrococcus also was present during these latter two periods in relatively large numbers. During the latter period, slightly pink translucent micrococcus colonies appeared in increasing numbers.

The total count obtained on the TGE-5M5S agar was higher than that on the TGE-5M agar. This, seemingly, was merely a preference for an environment containing salt, since no obligate halophiles were isolated.

The yeast counts during the first part of the sliming period were consistently between 160 × 10^8 and 200 × 10^8 per gram of slime. After 60 days in the cave, however, the numbers of yeasts present in the slime began to decrease and did so throughout the remainder of the curing period. It should be observed that the counts obtained on nile blue sulfate-butterfat agar paralleled the yeast counts.

The total counts on TGE-5M agar increased from a minimum of 230 × 10^8 to a maximum of 280 × 10^9 per gram of slime. On TGE-5M5S agar the total counts increased from 230 × 10^7 to a maximum of 350 × 10^9 per gram of slime. Proteolytic counts obtained on TGE-5M and TGE-5M5S agar were caused by only one type of organism, a lemon-yellow rod-shaped bacterium. This organism made up about 10% of the flora at 60 days; by 77 days it made up 75 to 82% of the bacterial flora in the slime. Accurate proteolytic counts could not be made on the TGE-5M5S agar as the salt apparently exerted an initial inhibitory effect on proteolytic activity; but when proteolysis did appear, zones would diffuse so rapidly over a plate that it was impossible to determine accurately the numbers of proteolytic colonies.

The development of the slime on cheese manufactured at Plant A as measured by the plate count is presented in Figure 2.

The types of organisms developing in the slime at 55 to 58° F. were different from those appearing in the slime of the Minnesota cheese. Initially, the slime consisted predominantly of yeasts which decreased in numbers as ripening proceeded. The bacterial flora consisted of several types of micrococci, as well as rods, the latter seeming to predominate.

The population of the slime reached a maximum rapidly at the higher ripening temperatures, the maximum populations being reached after 35 days in the cave. The total counts obtained on the TGE-5M5S agar were higher than those on TGE-5M agar throughout the cave-ripening period. The count decreased on TGE-5M agar after this point, but that obtained on TGE-5M5S agar remained fairly constant. Here, again, the count seemed to be influenced by the organisms which merely preferred a salt environment, rather than by obligate halophiles. No definitely proteolytic colonies appeared on the plates, although some produced

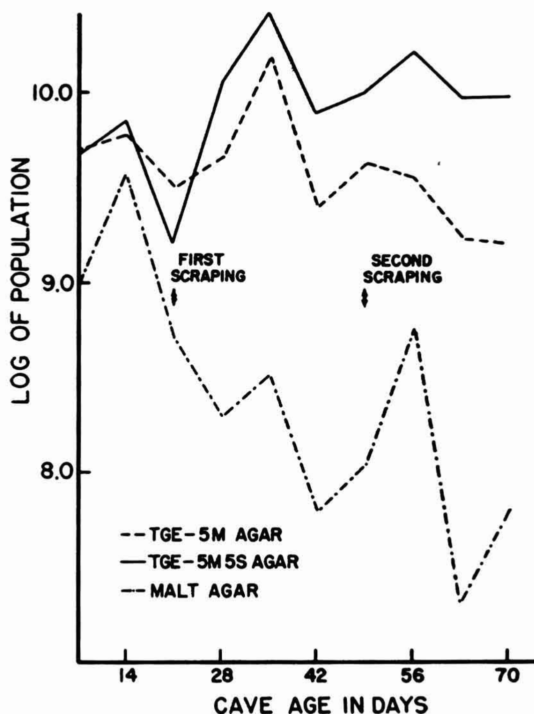

FIG. 2. Numbers of organisms per gram of slime from cheese of Plant A during the ripening period in cave.

questionable zones. These were caused by a rod-type organism, later identified as *Bacterium linens*. Lipolytic counts were not made on this series.

To determine whether the differences observed between slimes developing on the two types of cheese were due to the temperature of the curing cave or a difference in the chemical composition of the cheese or in the type of flora present in each locality, split lots of cheese manufactured by both the Minnesota and the Iowa State processes were uniformly seeded with slime organisms and placed in each of the two caves previously described.

Microbiological analyses of the slimes developing on the two types of cheeses ripening in both caves indicated that two principal factors, namely, temperature of ripening and method of manufacture, had a very marked influence on the type of organisms present in the slime. The lower ripening temperature produced a slime that was slightly higher in total numbers of microorganisms on both types of cheese. In both caves the Minnesota cheese developed a slime with a higher count than the cheese from Plant A. Furthermore, a difference could be observed in the type of slime growing on each type of cheese in the same cave, even though the cheese surfaces had been seeded with similar organisms.

A greater percentage of rods was observed on the cheese made by the Iowa

State process than was observed on the Minnesota cheese when both were ripened at higher temperatures. The Minnesota cheese had a greater variety of organisms in the slime when ripened at the higher temperature than when ripened at low temperature. At low temperature, the cheese from Plant A had more types of organisms present throughout the ripening period than did the Minnesota cheese. Initially, the Minnesota cheese had a variety of types of organisms in the slime, but by the end of the sliming period the pattern was almost normal for the low temperature ripening.

Pure culture study of slime organisms. Three hundred sixty-eight cultures were isolated from the slime of the Minnesota cheese throughout the sliming period. From these, 143 were selected for further study on the basis of their Gram-reaction, colony characteristics, and morphology. An attempt was made to select culture types in proportion to their numerical prominence in the slime at various stages of ripening. This collection of 143 cultures was found to divide itself into five general groups on a basis of morphology-chromogenesis, action in litmus milk, fermentation of dextrose and lactose, liquefaction of gelatin, and utilization of $NH_4H_2PO_4$. Because these results indicated that only a relatively few types of organisms were isolated, only 33 cultures were selected randomly from these five groups for further study.

An additional eight organisms were included in addition to these 33 organisms in the final study. These included five known cultures of *B. linens* [4] and three rod-type cultures resembling *B. linens* isolated from the slime of Plant A cheese.

Eleven of the 41 cultures studied were classified as *Bacterium erythrogenes*. This organism was a lemon-yellow, Gram-positive, nonmotile rod. The biochemical characteristics were as follows: H_2S was produced, nitrites produced from nitrates, and the utilization of ammonium phosphate was variable. Lactose, mannitol, glycerol, sucrose, maltose, and galactose were not fermented; however, after 30 days incubation, acid was produced from glucose and starch was hydrolyzed to dextrins. The action on litmus milk resulted in acid proteolysis, no coagulation, and a final pH of 6.6 to 6.8 after 30 days incubation. Litmus milk was occasionally reduced with the serum being a yellowish white and the sediment in the tubes a yellowish-red color. In the nonreduced litmus milk a deep red-purple color developed with a reddish precipitate. Gelatin liquefaction was stratiform and quite pronounced after 15 to 20 days. Growth response studies at 35, 20, and 10° C., showed maximum growth to be attained at 20° C., followed closely by growth at 10° C. However, at 35° C. no growth occurred.

These organisms exhibited a marked pleomorphism when grown in the presence of salt. In the absence of salt the cells often resembled diplococci or very short rods with slightly enlarged ends. When cultured in a 5% salt broth, long rod forms readily developed.

Three *Micrococcus sp.* were found in the Minnesota cheese slime. Of the 41 cultures, 12 belonged to two species which had similar characteristics, differing only in pigmentation. One species developed an orange pigment and the other

[4] ATCC 9175 and 4 cultures obtained from the Dairy Science Department, University of Illinois, Urbana.

developed a white pigment. The latter closely resembled *Micrococcus candidus*. These two species did not produce H_2S, nitrites from nitrates, utilize ammonium phosphate, or liquefy gelatin. Acid was produced from glucose, lactose, mannitol, glycerol, and sucrose. The litmus milk reaction varied in that initially an alkaline or neutral reaction occurred with a subsequent development of acid. These species possessed no marked proteolytic or lipolytic activity according to the methods used. Maximum growth occurred at 20° C. followed closely by growth at 10° C., and only slight growth occurred at 35° C. The three cultures belonging to the third *Micrococcus sp.* were Gram-positive to Gram-variable noncapsulated, nonmotile organisms. They did not produce H_2S, reduce nitrates to nitrites, utilize ammonium phosphate, liquefy gelatin, or possess any proteolytic or lipolytic activity. Acid was not produced from glucose, lactose, mannitol, glycerol, and sucrose. An alkaline reaction was produced in litmus milk. Maximum growth occurred at 10° C., moderate growth at 20° C., and none at 35° C.

The seven cultures of yeasts studied neither produced H_2S, nitrites from nitrates nor liquefied gelatin but were variable in their utilization of ammonium phosphate. They produced acid from glucose and sucrose but did not ferment lactose, mannitol, glycerol, maltose, or galactose. A slightly alkaline to neutral reaction occurred in litmus milk. The yeasts were not proteolytic but possessed an endocellular lipase. Maximum growth occurred at 20° C., with almost as much growth at 10° C., and no growth occurred at 35° C.

The three cultures resembling *B. linens* were identified as such, their characteristics being identical to those of the known *B. linens* cultures. These cultures produced H_2S, reduced nitrates to nitrites, liquefied gelatin, but did not utilize ammonium phosphate. No fermentation of glucose, lactose, mannitol, glycerol, sucrose, maltose, or galactose occurred. The litmus milk reaction was alkaline with proteolysis. The cultures were slightly caseolytic on milk agar plates but produced no demonstrable lipase. Maximum growth occurred at 20° C., scant growth at 10° C., and none at 35° C.

The salt and pH tolerances of the typical organisms isolated from the slime were determined to seek possible explanations for their appearance at various stages during the ripening period.

All of the organisms studied possessed a high tolerance to salt and grew at concentrations up to 15% salt. Several cultures grew at concentrations of 20% salt.

The bacterial cultures were sensitive to a low pH. *B. erythrogenes* did not grow at pH 5.8 but did at pH 6.4 *B. linens* and two *Micrococcus sp.* grew at pH 5.8, but not at pH 5.4. The pink *Micrococcus sp.* grew over a rather narrow pH range of 6.9 to 8.8. It did not grow at pH 6.4 or 9.1. The unidentified yeasts grew at pH 3.1 but not at pH 2.6. Growth was somewhat limited up to pH 4.2, however. All of the organisms grew at pH 9.4 except the micrococci discussed above.

Chemical determinations on slime and cheese just beneath the slime. The results of pH determinations on the slime from two lots of Minnesota cheese and the slime of Plant A cheese are shown in Figure 3. It may be noted that

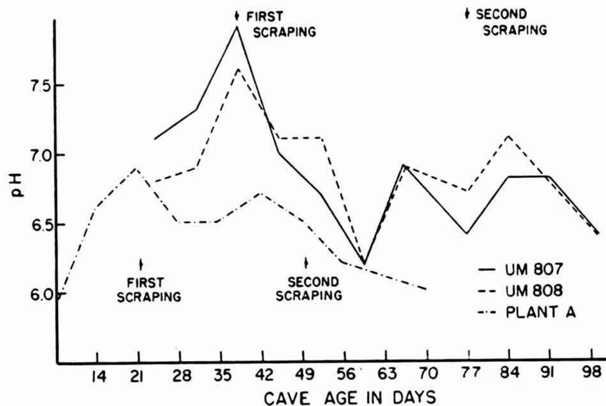

FIG. 3. pH values of the slime of several lots of Blue cheese at intervals in the ripening period.

the same essential pattern exists between the two slimes but beause of a longer sliming period at the lower temperatures, a greater overall effect was noted. Initially the pH rose, probably as a result of the action of yeasts. Later, as the micrococci appeared, the pH decreased and then remained between 6.5 and 7.0, which likewise was the resultant pH of litmus milk containing *B. erythrogenes.*

The pH just beneath the slime on the Minnesota cheese was found to increase from 5.3 to 6.5 during the sliming period. The slime was found to vary between 52 and 60% moisture during curing and the salt content varied from 3.1 to 5.0%. The cheese just beneath the slime had a moisture content of about 42 to 44% and a salt content of 4.0 to 5.2%.

DISCUSSION

B. linens has been associated consistently with slime appearing on various types of cheese *(4, 5, 6, 7, 16, 17)*. It is significant that the data in the above experiments show that *B. linens* is not always the predominant organism in the slime and even may be entirely absent. *B. erythrogenes* was found to predominate in the slime of cheese ripened in the University cave. *B. erythrogenes* resembles the bacterium described as Organism II by Wolff *(16, 17)*. He concluded, however, that this organism was not so important as *B. linens* in the formation of the reddish cheese slime. In this study, however, the only organism found to produce an orange pigment was a micrococcus. The orange-yellow slime apparently results from a blend of colors from the yellow pigmented rod and the orange and the white micrococci.

The important factor determining the type of flora developing in the slime appears to be the cave temperature. *B. erythrogenes* predominates in the slime when cave temperatures are 46 to 49° F. and *B. linens* when the cave temperatures

are near 55 to 58° F. The work of Prouty *(10)* likewise has indicated the absence of *B. linens* on the surface of certain cheeses.

The organisms present in the slime are highly salt-tolerant and enjoy mildly alkaline conditions. Many are inhibited below pH 5.8. These data present a possible explanation of the ecological relationship between these organisms in the slime and also their adjustment to the particular environment. These results are in agreement with other studies on cheese slime *(5, 6)*.

The changes in the pH values of the slime are closely related to the appearance of the different types of organisms. There seems to be little doubt that the yeasts play a major role in reducing the acidity of the cheese surface. This creates an environment suitable for other slime organisms to develop. Possibly the yeasts produce certain growth factors for *B. erythrogenes* and the other slime bacteria as occurs in the case of *B. linens (11)*.

It appears that a specific type of slime developing under a given set of conditions may change over a period of time in a given locality. The organisms studied in the above experiments differed from those isolated by Macy and Erekson *(7, 8)* a number of years earlier from cheese cured in the same cave. Organisms resembling *B. linens* apparently were present in the slime at that time in addition to the types described in this paper. A possible explanation of this phenomenon is that the cave temperature may have been slightly higher because of the large amount of cheese being cured at that time.

SUMMARY

Slime appearing on Blue cheese was shown to develop in a regular pattern depending on local environment, the initial flora being predominantly yeasts and some mold *(P. roqueforti)*. This was followed by an increase in cocci and rod forms. The organism predominating in the slime of cheese ripening at 46 to 49° F. was identified as *B erythrogenes*. *B. linens* was found to be the dominant rod-shaped organism in cheese curing at 55 to 58° F. and was entirely absent from slime developing at 46 to 49° F.

Various factors that can affect the flora of the slime are ripening temperatures, manufacturing techniques, type of seeding, and method of handling during curing.

ACKNOWLEDGMENT

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REFERENCES

(1) Association of Official Agricultural Chemists. *Methods of Analysis*. 6th ed. Assoc. Offic. Agr. Chemists. Washington, D. C. 1945.

(2) Breed, R. S., Murray, E. G. D., and Hitchens, A. P. *Bergey's Manual of Determinative Bacteriology*. 6th ed. Williams and Wilkins Co., Baltimore. 1948.

(3) Evans, Alice C. Bacterial Flora of Roquefort Cheese. *J. Agr. Research*, **13**: 225. 1918.

(4) Kelly, C. D. The Microbiological Flora on the Surface of Limburger Cheese. *J. Dairy Sci.*, **20**: 239. 1937.

(5) KELLY, C. D., AND MARQUARDT, J. C. The Influence of Hydrogen Ion Concentration and Salt on the Surface Flora of Limburger Cheese. *J. Dairy Sci.*, **22**: 309. 1939.

(6) LANGHUS, W. L., PRICE, W. V., SOMMER, H. H., AND FRAZIER, W. C. The "Smear" of Brick Cheese and its Relation to Flavor Development. *J. Dairy Sci.*, **28**: 827. 1945.

(7) MACY, H., AND EREKSON, J. A. Microflora of Cheese Slime. *J. Dairy Sci.*, **20**: 464. 1937.

(8) MACY, H., AND EREKSON, J. A. Unpublished data. University of Minnesota. 1937.

(9) MORRIS, H. A., COMBS, W. B., AND COULTER, S. T. The Relation of Surface Growth to the Ripening of Minnesota Blue Cheese. *J. Dairy Sci.*, **34**: 209. 1951.

(10) PROUTY, C. C. Personal communication. December 22, 1950.

(11) PURKO, M., NELSON, W. O., AND WOOD, W. A. The Associative Action between Certain Yeasts and *Bacterium linens*. *J. Dairy Sci.*, **34**: 699. 1951.

(12) Society of American Bacteriologists. *Manual of Methods for Pure Culture Study of Bacteria.* Biotech. Publication. Geneva, New York. 1948.

(13) STARK, C. N., AND SCHEIB, B. J. A Study of Fat-splitting and Casein-digesting Bacteria Isolated from Butter. *J. Dairy Sci.*, **19**: 191. 1938.

(14) THOM, C., AND MATHESON, K. J. Biology of Roquefort Cheese. Conn. (Storrs) Agr. Expt. Sta., *Bull.* 79. 1913.

(15) WILSTER, G. H., PRICE, W. V., MORRIS, A. J., GOSS, E. F., AND SANDERS, G. P. The Determination of Fat, Moisture, and Salt in Hard Cheese. *J. Dairy Sci.*, **20**: 27. 1937.

(16) WOLFF, A. Welche Mikro-organismen beteiligen sich an der Bildung des rotgelben Farbstoffes auf der Oberfläche der Käse im Reifungskeller? *Milchw. Zentr.* **5**: 145. 1909. (Translation)

(17) WOLFF, A. Nachtrag zu der Frage: Welche Mikro-organismen beteiligen sich an der Bildung des rotgelben Farbstoffes auf der Oberfläche der Käse im Reifungskeller? *Milchw. Zentr.* **5**: 414. 1909. (Translation)

(18) YALE, M. W. The Surface Flora and the Use of Pure Cultures in the Manufacture of Limburger Cheese. New York ((Geneva) Agr. Expt. Sta., *Tech. Bull.* 268. 1943.

THE MECHANISM OF SUNLIGHT FLAVOR FORMATION IN MILK WITH SPECIAL REFERENCE TO METHIONINE AND RIBOFLAVIN [1]

STUART PATTON

Department of Dairy Husbandry
The Pennsylvania Agricultural Experiment Station, State College

The problem of sunlight flavor in milk has received considerable study. A comprehensive review *(12)* of the subject has appeared recently. However, information to date concerning the identity and mode of formation of the flavor substance(s) is inconclusive. A recent report from this laboratory has shown that methionine and riboflavin are of significance in the development of this off-flavor in milk *(8)*. The flavor was found to originate in methionine, and its production was observed to depend in a large measure on the presence of riboflavin. Findings in the present study concern identification of the flavor compound, further observations on its origin, and possible mechanisms of its formation.

EXPERIMENTAL

Materials and methods. In order to avoid confusion which might arise from milk fat oxidation and oxidized flavor, the study has dealt solely with fresh pasteurized skimmilk (State University Creamery) and other fat-free systems. With the exception of DL-methionine (Distillation Products Industries), the L-forms of the amino acids (Nutritional Biochemicals Corp.) were used. Riboflavin (Hoffman–LaRoche Inc.) generally was used at a level (1.5 mg. per quart) comparable to that found in milk. Unless otherwise specified, exposure conditions involved use of conventional square, flint-glass milk bottles as containers, direct sunlight varying in intensity between 300 and 600 Weston units and an exposure time of 1 hour. For each exposed sample a corresponding sample was retained in dark storage. Three taste observers, thoroughly familiar with sunlight flavor, evaluated the quality and intensity of flavors encountered during the investigation.

Identification of the flavor compound. A chance observation in the laboratory revealed that when paper chromatograms of methionine are treated with ninhydrin reagent an odor is given off which bears a striking resemblance to sunlight flavor. It is postulated that one of the products of the reaction between an α-amino acid and ninhydrin is the aldehyde of one less carbon. In the case of methionine this would be β-methylmercaptopropionaldehyde (methional). The possible significance of this aldehyde in sunlight flavor was investigated. The compound was synthesized from acrolein and methyl mercaptan by the method of Pierson *et al.* *(9)*. A 74% yield of product, boiling 71 to 73° C. at

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23 mm., with n_D^{20} 1.484 and d_4^{20} 1.040, was obtained. The ultraviolet spectrum of methional both in water and in 95% ethanol was determined with the aid of a Beckman Model DU spectrophotometer. In both solvents the compound exhibited an inflection at about 285 mμ with a molecular extinction coefficient of 140. The 2,4-dinitrophenylhydrazone of the aldehyde was prepared in conventional manner and was observed to melt at 120.5° C. after two recrystallizations from ethanol. These data for methional are in good agreement with those in the literature *(4, 9)*.

The odor of methional, noted during its preparation, was most intense and disagreeable and perhaps is best described as cooked-cabbage odor. Therefore the flavor qualities of methional at low concentration in skimmilk were investigated. Amounts of methional ranging from 0.05 to 2.0 p.p.m. were employed. It was concluded by the taste observers that methional faithfully reproduces typical sunlight flavor. The flavor was found very strong at concentrations of 0.2 p.p.m. and slight but definitely detectable at 0.05 p.p.m.

As a measure of precaution, several other compounds closely related to methional were tested for their ability to impart sunlight flavor to milk. These were: methyl mercaptan (Distillation Products Industries); β-methylmercaptopropylamine, prepared by the method of Tutiya *(13)*; β-methylmercaptopropyl alcohol, synthesized as by Kirner *(7)*; and β-methylmercaptopropionic acid prepared by the method of Hurd and Gershbein *(4)*.[2] Although the odor characteristics of these compounds appear somewhat similar to those of methional, none was found to reproduce typical sunlight flavor in milk. All of the compounds in question have unpleasant odors in undiluted form similar to the odors of rotten cabbage, garlic, and sauerkraut.

Completely conclusive identification of methional as the sunlight flavor compound would involve demonstration of its presence in exposed milk. Assuming for the moment that it is the flavor agent, preceding data have shown that even large volumes of milk with relatively strong, naturally developed, sunlight flavor could contain only a few milligrams of the flavor compound. Recovery and identification of this quantity of compound from milk would be difficult. Thus, this approach has been rejected, at least for the present. However, methional was demonstrated as a product of the interaction of methionine and riboflavin in an aqueous system exposed to sunlight. A description of a representative experiment follows: Riboflavin, 100 mg., and 3.0 g. of methionine were made to volume in a quart milk bottle. The materials were mixed by inversion of the bottle until complete solution occurred. The bottle and contents then were exposed to strong sunlight for a period of 3 hours, after which the highly odorous solution was saturated with NaCl and extracted twice with 100-ml. volumes of ethyl ether. The ether extracts were combined and treated with a reagent composed of 100 mg. of 2,4-dinitrophenylhydrazine dissolved in 0.75 ml. of concentrated H_2SO_4 and diluted with 7.5 ml. of ethanol. The ether was evaporated on a steam bath. On standing, the extract residue gave rise to

[2] Data concerning these preparations are available on request.

a mass of orange-yellow crystals, which were recovered by filtration, the yield of crude product being approximately 90 mg. This material was recrystallized twice from ethanol. The final product, 30 mg., exhibited a melting range of 117 to 119° C. The infrared spectra of this compound and the authentic 2,4-dinitrophenylhydrazone of methional were determined for the region from 3 to 15μ in a Perkin-Elmer model 21 infrared spectrophotometer. The samples were prepared for analysis by dissolving 20 mg. in 6 ml. of carbon tetrachloride. The

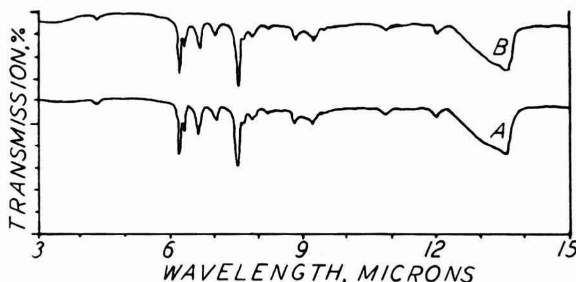

FIG. 1. Infrared absorption spectra of an authentic methional 2,4-dinitrophenylhydrazone (A) and the same derivative prepared from photolytic decomposition product of a methionine-riboflavin system (B). The former spectrum is offset for convenience of comparison.

spectral data clearly established the coidentity of the two compounds (Figure 1). The two spectra could be completely superimposed. Matching absorption maxima were noted at the following wavelengths: 4.35, 6.19, 6.29, 6.63, 7.02, 7.15, 7.35, 8.80, 9.23, 10.85, 11.99, and 13.56μ.

Origin of the flavor. Patton and Josephson *(8)* have shown that methionine may serve as an origin of sunlight flavor but that cystine and cysteine apparently do not. In a further study, a number of additional amino acids and derivatives of methionine were investigated. These included tryptophane, arginine, leucine, alanine, homocysteine, methionine sulfoxide, and methionine sulfone. The compounds were tested by exposure both in skimmilk and in aqueous solutions containing 1.5 mg. of riboflavin and 20 mg. of the amino acid per quart. None of the materials when added to skimmilk gave intensified sunlight flavor upon subsequent exposure of the milk. In the riboflavin solutions, none of them produced flavors or odors resembling sunlight flavor. Thus it appears that methionine is a rather specific source of the flavor compound. Moreover, these results rule out methionine sulfoxide or methionine sulfone as intermediates in the flavor production mechanism.

In earlier studies, the significance of riboflavin in the production of sunlight flavor was not known. For this reason it was considered worth while to reevaluate the various milk proteins as sources of sunlight flavor when exposed to light in the presence or absence of riboflavin. Casein was recovered from pasteurized skimmilk by two methods: *(a)* Skimmilk was supercentrifuged for ½ hour at 30,000 r.p.m., and the protein deposited on the wall of the bowl was

resuspended in pH 6.6 phosphate buffer; *(b)* skimmilk was adjusted to pH 4.7 with HCl. The casein which precipitated was recovered by centrifuging, washed three times with distilled water, and resuspended in phosphate buffer. The wheys resulting from these preparations also were included in the study. Various whey protein fractions were prepared as previously described by Hutton and Patton *(5)*.

The following general observations were made concerning the susceptibility of these systems to sunlight flavor development. In the presence of riboflavin, both casein and whey proteins give rise to sunlight flavor. In the absence of riboflavin, neither of the two systems evidences development of the typical off-flavor. Concerning relative intensity, the whey proteins appear to produce a somewhat greater degree of off-flavor. However, since sols of the whey proteins were more transparent than those of "native" casein (prepared by supercentrifuging skimmilk), light penetration may have been a significant factor in this difference. Of the whey proteins, pseudoglobulin appeared most potent as a source of sunlight flavor. Since casein is present in the greater concentration, contains more methionine, and is a principal factor limiting light absorption, it perhaps is of greater importance as an origin of sunlight flavor than the whey proteins.

DISCUSSION

There are at least three aspects of the sunlight flavor mechanism which may be considered at this time. These are *(a)* the mode of action of dicarbonyl compounds; *(b)* the mode of action of riboflavin; and *(c)* formation of methional from milk constituents.

The degradation of *a*-amino acids in the presence of dicarbonyl compounds to aldehydes of one less carbon has been studied extensively by Schonberg *et al.* *(10, 11)*. The effectiveness of these compounds in degrading amino acids with heat employed as the catalyst is well established. With the exception of sodium pyruvate *(14)*, no dicarbonyl–amino acid reactions involving light as a catalyst appear to have been investigated. Thus, aqueous solutions of methionine, 20 mg. per quart, were exposed to sunlight in the presence of the following dicarbonyl compounds: diacetyl, methyl glyoxal, pyruvic acid, ninhydrin, alloxan, and ascorbic acid. These were used individually at a level of 2 and 3 mg. per quart of methionine solution. With the possible exception of diacetyl, which imparted its own potent flavor, all of the compounds were effective in promoting sunlight flavor. Presumably, it is the dehydro (diketo) form of ascorbic acid which is active. By comparison it was observed that riboflavin gives a much greater degree of flavor than any of the dicarbonyl compounds. However, it is possible that both materials may be involved in production of the flavor in milk.

The evidence indicates that riboflavin is the critical agent in converting methionine to methional in milk. The mechanism of this phenomenon is by no means clear. Attempts to produce sunlight flavor from methionine using the dyes, water soluble chlorophyll or tartrazine, were unsuccessful. However, methylene blue was observed to give results comparable to those obtained with

riboflavin. Brauner *(2)* found that dyes varied in their sensitizing action on the photolysis of indoleacetic acid and that riboflavin was most effective of those studied. The mode of action of such compounds cannot be explained on the basis of a dicarbonyl group since they do not contain this requisite structure *(11)*. Nor do light absorption properties alone seem to afford an adequate explanation. Carter *(3)* has noted that the photodynamic action of dyes appears to depend on the factor of fluorescence, although their effectiveness in photocatalysis is not directly related to this property. It has been shown that in the photo-oxidation of certain amino acids, methylene blue acts also as a hydrogen acceptor *(3, 15)*.

Keeney *(6)* has shown that the diffusate of milk does not develop the typical off-flavor on exposure to sunlight. This diffusate would contain riboflavin and also any free methionine which might be present in milk. The fact that it developed no sunlight flavor suggests that no significant amount of methionine was present. Rather his results, as well as those of others, indicate that milk proteins are the primary origin. Whether methional is liberated directly from the protein by photolytic action or whether proteolysis, resulting in the production of free methionine, is an essential intermediate step is not known. Although the former, in fact, may be the mechanism, it seems reasonably certain that conditions which increase the nonprotein nitrogen content of milk also might increase the susceptibility of the milk to sunlight flavor development.

A recent review *(12)* on activated flavors demonstrates that findings on this subject are not in harmony. At least one reason for such differences is evident. In some investigations no distinction has been made between flavors which result from fat oxidation and those arising from photolysis of serum constituents. The relative amounts of fat and serum in a light-exposed milk product and the comparative susceptibility of these two components to photolysis should have considerable influence on the quality and magnitude of off-flavor produced. It seems probable that in many instances fat oxidation contributes to, or is a dominant factor in, light-induced off-flavors of milk.

Many investigators in attempting to determine the origin of sunlight flavor in milk have relied heavily for their conclusions on results obtained with simplified systems. The fact that commercial rennin contains protease activity places in doubt the significance of observations on rennet whey. The liberation of even minute quantities of methionine by proteolysis could be expected to enhance sunlight flavor development in this medium. In addition, it seems doubtful whether the minor whey protein of Weinstein *et al.* *(16)* is a valid origin of sunlight flavor in milk. This protein is prepared from heated (95° C. for 1 hour) rennet whey, and its existence in market milk is open to question. The exposure to light of simplified systems containing proteins does not necessarily yield results analogous to those obtained with milk. In any event such systems would have compositions and light absorption properties at variance with those of milk. Consideration of the importance of riboflavin in the chemistry of sunlight flavor suggests that the significance of any single milk protein as a sole origin of the off-flavor should be reconsidered.

The fact that methional imparts a detectable flavor to milk at a concentration of one part in 20 million seems worthy of emphasis. Very little milk protein hydrolysis would be required to furnish sufficient methionine for production of this amount of methional. Revealing chemical changes involving materials of this magnitude is very problematical. Perhaps for this reason the importance of methionine decomposition products in the flavor of foods has not been extensively investigated. An exception concerns the work of Akabori and Kaneko *(1)* on the flavor of soy sauce.

SUMMARY

Evidence is presented to show that β-methylmercaptopropionaldehyde (methional) is a compound of importance in the sunlight flavor defect of milk. In the estimation of three experienced taste observers, this compound imparted typical sunlight flavor to milk at a level of 0.1 p.p.m. and was detectable in milk at concentrations as low as 0.05 p.p.m. A number of other compounds closely related to methional, including methyl mercaptan, β-methylmercaptopropylamine, β-methylmercaptopropyl alcohol and β-methylmercaptopropionic acid, were evaluated. None of these appeared to be so specifically an agent of the flavor as methional. Although the presence of methional in milk containing naturally induced sunlight flavor was not shown, the compound was demonstrated, by means of infrared spectral data, as a product of the sunlight catalyzed reaction between methionine and riboflavin.

Additional evidence concerning the origin of sunlight flavor was obtained. It was concluded that the amino acid methionine is a specific source of the flavor. Negative flavor development was noted from a number of other amino acids and several derivatives of methionine. In milk, the proteins are indicated as the primary source of sunlight flavor. Of these, it is suggested that casein is most important as an origin, since it is present in greatest concentration, contains the highest level of methionine, and is the principal component limiting light transmission.

It is shown that riboflavin is of considerable significance in the production of sunlight flavor in milk. The role of riboflavin, as well as certain dyes and dicarbonyl compounds, in the photolysis of methionine is discussed.

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REFERENCES

(1) AKABORI, S., AND KANEKO, T. Aroma of Soy Sauce. *J. Chem. Soc. Japan*, **57**: 832. 1936.

(2) BRAUNER, L. The pH Changes in the Photolysis of Heteroauxin. *Naturwissenschaften*, **39**: 282. 1952.

(3) CARTER, C. W. LXXII. The Photoxidation of Certain Substances in the Presence of Fluorescent Dyes. *Biochem. J.*, **22**: 575. 1928.

(4) HURD, C. D., AND GERSHBEIN, L. L. Reaction of Mercaptans with Acrylic and Methacrylic Derivatives. *J. Am. Chem. Soc.*, **69**: 2328. 1947.

(5) HUTTON, J. T., AND PATTON, S. The Origin of Sulfhydryl Groups in Milk Proteins and Their Contributions to "Cooked" Flavor. *J. Dairy Sci.*, **35**: 699. 1952.

(6) KEENEY, D. G. Some Chemical Reactions Involved in the Production of the Sunlight Flavor in Milk. Master's Thesis, Ohio State University. 1947.

(7) KIRNER, W. R. The Effect of Structure of Organic Halides on Their Rate of Reaction with Inorganic Halides. II. The Effect of the Methylthio Group. *J. Am. Chem. Soc.*, **50**: 2446. 1928.

(8) PATTON, S., AND JOSEPHSON, D. V. Methionine — Origin of Sunlight Flavor in Milk. *Science*, **118**: 211. 1953.

(9) PIERSON, E., GIELLA, M., AND TISHLER, M. Synthesis of DL-Methionine. *J. Am. Chem. Soc.*, **70**: 1450. 1948.

(10) SCHONBERG, A., AND MOUBASHER, R. The Strecker Degradation of α Amino Acids. *Chem. Rev.*, **50**: 261. 1952.

(11) SCHONBERG, A., MOUBASHER, R., AND MOSTAFA, A. Degradation of α Amino Acids to Aldehydes and Ketones by Interaction with Carbonyl Compounds. *J. Chem. Soc.*, **70**: 176. 1948.

(12) STULL, J. W. The Effect of Light on Activated Flavor Development and on the Constituents of Milk and Its Products: A Review. *J. Dairy Sci.*, **36**: 1153. 1953.

(13) TUTIYA, Y. Methionine and Its Derivatives. III. Formation of 3-Methylthiopropyl Alcohol. *J. Agr. Chem. Soc. Japan*, **17**: 619. 1941.

(14) WEIL, L., AND BUCHERT, A. R. Photoöxidation of Crystalline β-lactoglobulin in the Presence of Methylene Blue. *Arch. Biochem. Biophys.*, **34**: 1. 1951.

(15) WEIL, L., GORDON, W. G., AND BUCHERT, A. R. Photoöxidation of Amino Acids in the Presence of Methylene Blue. *Arch. Biochem. Biophys.*, **33**: 90. 1951.

(16) WEINSTEIN, B. R., DUNCAN, C. W., AND TROUT, G. M. The Solar Activated Flavor of Homogenized Milk. IV. Isolation and Characterization of a Whey Constituent Capable of Producing the Solar-Activated Flavor. *J. Dairy Sci.*, **34**: 570. 1951.

THE EFFECT OF FAT-FREE DIETS ON YOUNG DAIRY CALVES WITH OBSERVATIONS ON METABOLIC FECAL FAT AND DIGESTION CO-EFFICIENTS FOR LARD AND HYDROGENATED COCONUT OIL

H. M. CUNNINGHAM AND J. K. LOOSLI

Laboratory of Animal Nutrition, Cornell University, Ithaca, New York

There has been a trend in recent years to replace the whole milk in the ration of dairy calves with cheaper dairy by-products, such as nonfat dry milk solids or whey, and to include various commercial concentrates. However, these diets are low in fat, and calves seldom grow as rapidly as on whole milk. They are often unthrifty and experience a greater incidence of diarrhea. Gullickson and coworkers *(9)* found that young calves did not thrive on a low-fat diet composed of skimmilk, molasses, beet pulp, starch, cerelose and cod-liver oil. The blood fat of these animals fell to very low levels, and normal conditions were obtained only by the addition of fat to the diet. Similarly, Arrington and Reaves *(2)* found that of six Jersey and seven Holstein calves given a diet of skimmilk remade from powder from 3 days of age with grain and vitamin A and D supplements, all the Jerseys and one Holstein died.

Though these and various other experiments indicated that certain low-fat diets may adversely affect the development of young calves, there was no conclusive evidence submitted to demonstrate that calves require a dietary source of fat or of the essential fatty acids. In view of previous experiments *(5, 6)* showing that fat was important in the diet of lambs and kids, it appeared appropriate to study the fat requirements of calves. In addition, values were secured for metabolic fecal fat and the digestibility of lard and hydrogenated coconut oil.

EXPERIMENTAL PROCEDURE

Two experiments were conducted with sixteen 1- to 2-day old colostrum-fed calves representing four dairy breeds. The first study was designed to determine if young calves would survive on a fat-free diet, and the second experiment was undertaken to ascertain if calves would live on a diet containing fat but completely devoid of the essential fatty acids.

The calves were housed in individual wire-floored tie stalls supplied with overhead heat lamps. Upon arrival, each calf received 30,000 I.U. of vitamin A. All calves received 8 g. of sulphathalidine per day for the first 2 weeks, 4 g. the third week, and 8-g. doses thereafter only upon the appearance of severe scours. Aureomycin was included in the diet at the rate of 10 mg. per kilogram of milk during the fourth and succeeding weeks.

The purified diets in Table 1 were designed after those of Clark *(4)* and Wiese *et al. (17)* employing the modifications of Sewell *(15)*. The lard contained 9.37, 0.91, and 0.32% linoleic, linolenic, and arachidonic acids, respectively. It was homogenized in three parts of water with soya lecithin or a combination of

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TABLE 1
Composition of the purified diets

Ingredient	Experiment I: Lard diet	Experiment I: Fat-free diet	Experiment II: Lard diet	Experiment II: Hydrogenated coconut oil diet	Experiment II: Fat-free diet
		(g/kg of artificial milk)			
Cerelose	74.0	96.5	60.1	60.1	80.1
Alcohol extracted casein [a]	40.0	40.0	40.0	40.0	40.0
Fat [b]	10.0	0.0	20.0	20.0	0.0
Minerals [c]	9.9	9.9	9.9	9.9	9.9
H_2O	866.1	853.6	870.0	870.0	870.0
Synthetic vitamin A palmitate	15,000 I.U. per calf per day				
Calciferol (D_2)	400 I.U. per calf per day				
Alpha-tocopherol acetate	50 mg. per calf per day				
B vitamins [d]					

[a] Crude casein was boiled in 95% alcohol for 1 hr. and then extracted in a continuous type extractor for 24 hr. with 95% alcohol.

[b] The lard in Expt. I was homogenized with 6% soya-lecithin and in Expt. II with 0.5% Tween 60 (polyoxyethylene sorbitan monostearate) and 0.5% Tween 80 (polyoxyethylene sorbitan monooleate).

[c] Minerals in g. per kg. of diet: $Ca(OH)_2$, 1.0; KOH, 0.959; NaOH, 0.928; $CaCl_2$, 1.565; $CaCO_3$, 0.140; HCl, 0.101; $FeSO_4.7H_2O$, 0.050; $CuSO_4.5H_2O$, 0.004; $CoCl_2.6H_2O$, 0.0014; $MnSO_4.4H_2O$, 0.0034; $ZnSO_4.7H_2O$, 0.0031; KI, 0.0003; MgO, 0.298; KH_2PO_4, 2.873; citric acid, 1.998.

[d] B vitamins in mg. per kg. of diet: thiamine, 0.65; riboflavin, 0.65; niacin, 2.5; choline chloride, 260.0; folic acid, 0.052; pyridoxine, 0.65; para-aminobenzoic acid, 2.60; calcium pantothenate, 1.30; B_{12}, 0.0074.

Tweens 60 and 80 acting as emulsifying agents. The fat homogenate was stored at 5° C. until used. Fresh milk was mixed every 3 or 4 days, stored at 5° C., and warmed to body temperature just before feeding. The calves were fed twice daily by nipple pail at the rate of 10% of their body weight per day. Upon the onset of severe scours, the milk intake was reduced by 25 to 50% and antibiotics were administered until the scouring subsided. At 1 week of age, the calves in Experiment I received shredded cellophane and a dry mixture of 60% glucose, 15% casein, and 25% potato starch supplied ad libitum.

All calves were weighed weekly and examined daily for falling hair, scaliness, scours, and general thriftiness. Blood samples were drawn from the jugular vein of all animals on the first day of the experiment. Additional samples were secured from the calves in Experiment I before and after supplementation with fat and at weekly intervals from the calves in Experiment II. The plasma lipids were extracted[1] and analyzed spectrophotometrically for linoleic, linolenic, and arachidonic acids, employing modifications of the method of Brice *et al.* (3).

[1] The lipids were extracted from 10 to 20 ml. of plasma by shaking for 15 minutes with an equal volume of absolute alcohol and 50 ml. of petroleum ether (60-70). Aliquots of the petroleum ether layer were evaporated in test tubes under nitrogen and reduced pressure and analyzed spectrophotometrically for linoleic, linolenic, and arachidonic acids. Total lipids were secured by evaporating aliquots of the petroleum ether layer under nitrogen and reduced pressure in 300-mg. aluminum dishes and weighing on a microbalance.

Feces collections were made for a 7-day period every 2 weeks on three calves from each of the lard and coconut oil groups in Experiment II. A preliminary period of 3 days of constant daily feed intake preceded each 7-day collection period. The three calves on the lard diet received a fat-free diet (with the lard replaced by an equal weight of glucose) during the seventh and eighth weeks so that determinations of fecal metabolic fat could be made. In order to determine the effect of roughage on fat digestibility, all calves received shredded cellophane ad libitum beginning 9 days before the 11th week collection period. All intakes of milk and cellophane were accurately weighed. The feces were collected twice daily and stored with chloroform as preservative at approximately 5° C. until the end of the collection period. They were then mixed in a Waring blendor and the slurry was analyzed for dry matter, total lipids, neutral fats, soaps, and free fatty acids according to the method given by Hawk *et al.* *(10)*.

TABLE 2
Average weight gains and plasma lipid values for calves in Experiment I

Calf No.	Weeks on trial	Av. daily wt. gain	Age [a]	Total lipids	Arachidonic acid	Linolenic acid	Linoleic acid
		(g.)	*(days)*		*(mg/100 ml.)*		
			Lard diet				
3	10	218	1	107	2.8	6.9	19.9
			16	147	6.1	0.0	31.8
			58	366	13.9	0.0	90.7
6	8	80	1	103	5.0	1.4	14.7
			42	220	7.1	7.6	57.9
			Fat-free diet				
2	3 [c]	0	2	217	9.6	2.4	27.5
			16	70	3.8	0.0	5.8
			21 [b]	93	4.0	1.6	7.3
4	9	244	2	145	6.4	0.4	17.3
			7 [b]	......	......	....	....
			22	273	10.1	0.0	147.6
			54	210	7.6	0.7	49.7
5	8	107	1	141	5.7	1.6	11.8
			16	56	2.0	1.2	4.2
			37 [b]	72	3.6	0.0	3.5
			45	205	10.1	0.0	44.5
			59	200	7.8	0.2	50.7
7	7	24	2	105	2.8	2.1	6.3
			7	64	1.6	1.9	7.2
			33 [b]	48	1.9	0.0	1.1
			48	226	4.6	1.9	42.9
8	3 [c]	490	1	167	6.2	1.6	16.3
			9 [b]	86	3.2	2.8	9.9
9	7	75	1	154	7.1	1.2	14.4
			9 [b]	86	1.3	9.3	13.7
			47	200	6.7	0.8	46.1

[a] Day blood sample was drawn.
[b] Day calf became too weak to rise. Blood samples were drawn at this time and oral administration of fat was begun within one to two days.
[c] Died.

RESULTS AND DISCUSSION

Experiment I. The practice of limiting the milk intake during the early weeks in an attempt to alleviate scours appeared to be largely responsible for the slow growth rates shown in Table 2. Although the calves receiving 10 g. of lard per kilogram of milk did not display normal weight gains, their diet was apparently adequate for survival. On the other hand, it is doubtful if any of the calves on the fat-deficient diet could have survived without additional fat. Between 1 and 5 weeks of age, these calves displayed symptoms of leg weakness and had great difficulty in rising, similar to that shown by lambs and kids in previous experiments *(5, 6)*. One to 2 days after these first symptoms appeared, the calves were unable to rise and recovered only upon the immediate administration of lard orally. Calves 4, 5, 7, and 9 were given 40 g. of lard per kilogram of milk within 1 day after they were first unable to rise and required 7, 1, 3, and 13 days, respectively, on this diet before they were able to rise. Calf 2, which did not receive the 4% fat diet until 2 days after it was first unable to rise, failed to recover.

In view of the work of Esh *et al.* *(8)* in which colostrum-free calves receiving skimmilk diets failed to survive unless a source of lecithin was provided, Calf 8 was given 10 g. of lecithin per day when it first reached the stage where it was unable to rise. The lecithin failed to induce a favorable response, and the calf succumbed within 9 days.

Esh *et al.* suggested that in their experiments poor vitamin A absorption in the absence of dietary fat was a contributing factor to the death of calves fed skimmilk diets. However, inadequate vitamin A absorption was not considered to be of any significance in the present experiments because the plasma vitamin A of two calves determined at a time when they were too weak to rise was above normal, exceeding 25 γ per 100 ml. of plasma.

Table 2 shows that the total lipids in the plasmas of all calves were fairly low at 1 day of age and in the calves which received fat, rose as the experiment progressed. This is in agreement with the work of Allen *(1)* and Zaletel *et al.* *(18)*, in which the blood fat of dairy calves was found to be low at birth but to rise rapidly during the succeeding few days.

The arachidonic and linoleic acids in the plasma of controls also increased gradually along with the total lipids, but the linolenic acid values were extremely variable. This variability of the trienoic acid content of the plasma lipids of cattle and other species was similarly indicated by O'Connell *(13)*. The calves on the fat-deficient diet showed a decline in total lipids and arachidonic and linoleic acids from birth to the time they were too weak to rise. This was followed upon supplementation with lard by an increase in these lipid components to approach those of controls. Postmortem examination of the calves that died failed to show any abnormalities suggestive of an essential fatty acid deficiency.

Experiment II. All the calves receiving the coconut oil diet survived until the end of the experiment without additional supplementation with lard. Table 3 shows that the calves receiving hydrogenated coconut oil had a slightly lower incidence of scours than those receiving lard. However, the progress of the

TABLE 3

Summary of weight gain, feed consumption, and incidence of scours for calves in Experiment II

Calf No.	Days on trial	Av. daily milk intake	Av. daily cellophane intake [a]	Av. daily wt. gain	Total days of scours
		(kg.)	*(g.)*	*(g.)*	
		Coconut oil diet			
10	98	3.47	159	222	0.0
11	82	3.70	84	145	10.0
12	81	3.80	39	190	3.0
13	77	3.77	59	144	2.5
14	77	2.96	81	164	5.5
Av.		3.74	84	173	4.2
		Lard diet			
15	77	3.91	69	168	8.0
16	77	3.55	61	136	11.5
17	77	4.22	161	191	1.5
Av.		3.89	97	165	7.0

[a] Cellophane fed ad libitum at 61 days.

calves on the lard diet appeared to be retarded during the seventh and eighth weeks when, in order to obtain figures for metabolic fecal fat, the lard was replaced by glucose. They not only failed to gain weight during this period but showed a much greater tendency to scour on the fat-free diet. Neither group grew very rapidly, but it must be remembered that none of the diets were supplemented with hay or concentrates and that in order to maintain constant feed intakes for the digestion trials, the feeding schedule could be adjusted only every second week.

Figure 1 shows that the plasma total lipid values of both groups rose to almost double the initial values during the first 5 weeks of the experiment. Changing calves 15, 16, and 17 from the lard to the fat-free diet produced a sharp drop in the total plasma lipid and linoleic acid levels during the following 2 weeks. Upon the inclusion of lard in the diet at the eighth week, there was a rapid increase in both components, which continued until the end of the experiment. Hydrogenated coconut oil appeared to have no depressing effect on total plasma lipid levels, but there was a decline in plasma linoleic acid during the first 6 weeks and then a gradual leveling off. It is not known whether this leveling off indicated linoleic acid synthesis or whether there was a mobilization of body stores. Plasma linolenic and arachidonic acid levels of both groups of calves were comparatively constant throughout the experiment with little or no variation that could be attributed to changes in diet. Mean plasma arachidonic and linolenic acid values for the lard group were 7.9 ± 2.7 and 0.37 ± 1.10 mg. per 100 ml. and for the hydrogenated coconut oil group were 5.6 ± 1.7 and 0.55 ± 1.11 mg. per 100 ml., respectively. A comparison of the means by a "t" test showed a highly significant ($P < 0.01$) difference between the mean arachidonic acid levels of the two groups but no significant differences between the mean linolenic acid levels.

Recent studies by Lambert *et al.* *(12)* have also demonstrated that calves fed lipid-free diets exhibit a greater incidence of diarrhea and have lower plasma lipid levels than controls fed hydrogenated soybean oil. Though there

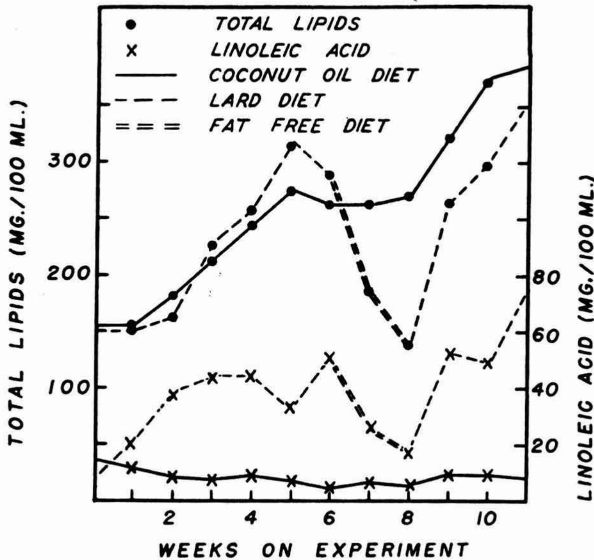


FIG. 1. Average weekly plasma total lipids and linoleic acid levels for calves 11, 12, 13, and 14, which received the hydrogenated coconut oil diet and calves 15, 16, and 17, which received the lard diet.

were no reports of leg weakness, these workers did observe several cases of rough hair coats, dandruff, and partial alopecia, which were alleviated by the inclusion of fat in the diet.

It was interesting to find that when a single dose of 30 g. of lard was given orally to a fat-depleted calf (No. 10) there was an immediate and prolonged elevation of the plasma linoleic acid level. The calf had been on a fat-free diet for 6 weeks when the lard was given, and its plasma contained 5.4 mg. of linoleic acid per 100 ml. Twenty-four hours later the plasma linoleic acid was 23.0; 1 week later, 13.7; and 2 weeks later, 6.4 mg. per 100 ml. The fact that the 30 g. of lard contained only 2.8 g. of linoleic acid would indicate that small stores of this compound may last a calf for long periods of time.

It was also strange to find that by the end of the experiment the calves receiving the hydrogenated coconut oil not only appeared weaker and less thrifty than the lard group, but during the 11th week they all suddenly developed spasms which lasted 15 to 30 minutes. The spasms involved strong muscular twitches over the entire body and violent chewing on the front of their pens, and some of the calves fell down and were unable to rise until the spasms were over.

At the time the spasms occurred, the plasma of all calves was analyzed for calcium, phosphorus, and magnesium by methods given by Hawk *et al.* *(10)* and vitamin E by the method of Quaife and Harris *(14)*. Average values for calcium, phosphorus, magnesium, and vitamin E in the plasma of calves receiving hydrogenated coconut oil were 9.6, 6.2, 1.8, and 0.278 mg. % and in the plasma of calves receiving lard were 9.2, 8.1, 2.0, and 0.159 mg. %, respectively. Since all

values were normal, the levels of these components in the plasma of the calves on the coconut oil diet were not believed to be closely associated with the spasms. Plasma fatty acid levels were no lower than those of the previous week, and postmortem examinations failed to explain the cause of the spasms.

Fat digestibility data. Twenty-eight individual digestion trials, including 13 with hydrogenated coconut oil, 12 with lard and 3 with a fat-free diet, were conducted during the course of this experiment. It may be observed from Table 4 that during the second and fourth weeks the coconut oil had digestion coefficients

TABLE 4
Summary of fat digestibility data for calves in Experiment II

Weeks on trial	No. of trials	Av. milk intake	Av. days of scours	Av. fat dig. coef.	Av. dry matter dig. coef.	Neutral fat	Free fatty acids	Soaps
		(kg/wk)				*(% of the fecal fat)*		
			Calves receiving coconut oil diet					
2	3	21.3	1.5	82.6 [a]	89.4	78.3	7.3	14.4
4	3	22.1	0.0	89.7	94.4	41.9	4.2	53.9
6	3	26.6	0.7	85.5	94.4	33.0	3.8	63.2
8	1	25.2	0.0	88.6	94.9	49.0	4.3	46.7
11	3	28.9 +702 C [b]	0.2	71.9	82.1	32.4	4.0	63.6
			Calves receiving lard diet					
2	3	24.3	0.7	68.0 [a]	83.3	69.6	6.0	24.4
4	3	28.9	0.2	77.0	91.3	39.8	3.4	56.8
6	3	29.9	0.8	92.5	93.4	45.6	5.3	49.1
8	3	26.1	0.3	1.6 [c]	94.3	71.3	3.2	25.5
11	3	32.2 +663 C [b]	1.0	93.7	84.0	60.0	6.9	33.1

[a] One calf in each of the coconut oil and lard groups showed excessive diarrhea during the second week collection period. If the digestion coefficients of these calves are not included in the averages for this period, the fat digestion coefficient for the coconut oil would be 86.4 instead of 82.6 and that for the lard would be 72.6 instead of 68.0.

[b] C refers to the average cellophane consumption (g.) per calf for the 11th week.

[c] Fecal metabolic fat expressed as a per cent of the fat intake on 2% fat diets.

of 86.4 and 89.7 compared to 72.6 and 77.0, respectively, for the lard. However, by the sixth week the lard was 92.5% digested in comparison to 85.5% for the coconut oil, and by the 11th week, when both groups received cellophane, the lard had a digestion coefficient of 93.7, whereas only 71.9% of the coconut oil was digested. These data may be compared with recently published digestion work with calves on filled milks, in which soybean oil, hydrogenated soybean oil, and whole milk fat were 67, 75, and 96% digested, respectively *(7)*.

Severe scours appeared to lower the digestibility of the lard and coconut oil during the first collection period. Consequently, a correction for this factor is supplied in Table 4 for one calf in each group. The consumption of cellophane produced an approximately equal decrease in the dry matter digestibility of both groups, but, since there was no decrease in the digestibility of the lard, the poorer utilization of the coconut oil during the 11th week could only be attributed to a shortcoming of the oil.

It is significant to note that one fat may be better digested during the early

weeks of a calf's life and another fat may be more completely utilized during the preweaning period. This suggests that the age of the calf may be related to the digestibility of dietary fat and that in order to properly evaluate a fat or oil in the diet of young calves, one must secure digestion coefficients for several stages of a calf's early life.

Metabolic fecal fat. During the second week of the fat-free diet (eighth week of Experiment II) calves 15, 16, and 17 excreted 1.5, 0.8, and 1.4 g. of fat per day, respectively. This is equivalent to an average of 1.6% of the total fat that would ordinarily be ingested using a 2% fat milk (Table 4). It also may be expressed as 0.28, 0.19, and 0.26% of the dry matter intake, 4.6, 3.1, and 5.8% of the total fecal dry matter, or 29, 19, and 26 mg. per kilogram of body weight per day, respectively. The latter values compare closely with the endogenous lipid excretion of 42 to 22 mg. per kilogram of body weight per day for dogs *(16)*.

It is of interest to note that with diets in which the fat is well digested the fecal metabolic fat may constitute a large proportion of the fecal lipids. For example, Calf 17 excreted 10.2 g. of fecal lipids while on the fat-free diet and 27.9 g. during the 11th week, when it received 616 g. of lard. The metabolic fecal fat in this case was equivalent to 36.6% of the fecal lipids, one of the highest values recorded in this experiment.

Partition of the fecal lipids. The partition of fecal lipids given in Table 4 shows that during the first collection period the fecal lipids of the calves on both the coconut oil and lard diets were high in neutral fat and low in soaps. The free fatty acids showed no major trends in either group during the course of the experiment, but the soaps and neutral fat displayed considerable variation. Soaps tended to predominate in the feces of all calves during the fourth week and, though they continued high in the feces of the calves receiving the coconut oil, the fecal lipid partition of the calves on the lard diet changed during the later weeks to contain an excess of neutral fat. Howe *(11)*, upon analyzing the feces of 1- to 7-day old calves, found that on the average the fecal lipids constituted 14% of the total fecal dry matter. In comparison, the average total lipids found in the fecal dry matter during the second, fourth, sixth, and eleventh weeks of the present experiment amounted to 25.2, 28.2, 39.8, and 23.7% for the calves receiving coconut oil and 29.5, 40.6, 17.5, and 5.6%, respectively, for the calves receiving lard.

SUMMARY

Studies were carried out with 16 1- to 2-day old dairy calves to determine whether there is a dietary requirement for fat. It was found that calves receiving a fat-free synthetic milk developed leg weakness and muscular twitches within 1 to 5 weeks and died unless a source of fat was supplied. The condition could be cured by feeding an artificial milk containing 4% lard and prevented with one containing 1 to 2% lard. However, the fact that a milk containing 2% of hydrogenated coconut oil also prevented the appearance of these symptoms indicated that the early death of the fat-deficient calves was not the result of an essential fatty acid deficiency. The results suggest that body storage of essential fatty

acids at birth may be adequate to last a calf several months but that dietary fat may be necessary during the first few days.

Plasma total lipid levels were found to vary directly with the quantity of fat in the diet, whereas linoleic and arachidonic acid levels were much lower in calves receiving the hydrogenated coconut oil or fat-free diets. Dietary fat had no apparent effect on plasma linolenic acid values.

Digestibility studies were conducted every second week on calves receiving diets containing 2% lard or coconut oil. The coconut oil was 86.4, 89.7, 85.5, and 71.9% digested, and the lard was 72.6, 77.0, 92.5, and 93.7% digested during the second, fourth, sixth, and eleventh weeks, respectively. Metabolic fecal fat excretion of three calves receiving a fat-free diet during the seventh and eighth weeks amounted to 19 to 29 mg. per kilogram of body weight per calf per day.

REFERENCES

(1) ALLEN, N. N. Blood Fat of Dairy Cattle. II. Factors Seen Influencing the Fat Content of the Blood Plasma. Minn. Agr. Expt. Sta., *Tech. Bull.* 130. 1938.

(2) ARRINGTON, L. R., AND REAVES, P. M. Studies of the Growth and Blood Composition of Dairy Calves Fed Remade Skim Milk after Three Days of Age. *J. Dairy Sci.*, 31: 1. 1948.

(3) BRICE, B. A., SWAIN, M. L., SCHAEFFER, B. B., AND AULT, W. C. Spectrophotometric Determination of Small Proportions of Polyunsaturated Constituents in Fatty Materials. *Oil and Soap*, 22: 219. 1945.

(4) CLARK, W. M. Synthetic Milk as a Basis of Research. *J. Dairy Sci.*, 10: 195. 1927.

(5) CUNNINGHAM, H. M. The Fat Requirements of Ruminants. Ph.D. Thesis, Cornell University, Ithaca, N. Y. 1953.

(6) CUNNINGHAM, H. M., AND LOOSLI, J. K. The Effect of Fat-free Diets on Lambs and Goats. *J. Animal Sci.* (in press).

(7) DEMAN, TH. J. Kalveropfokproef met Gehydeerde Soya-Olie. *Tijdschr. Diergeneesk.* 76: 175. 1951.

(8) ESH, G. C., SUTTON, T. S., HIBBS, J. W., AND KRAUSS, W. E. The Effect of Soya-Phosphatides on the Absorption and Utilization of Vitamin A in Dairy Animals. *J. Dairy Sci.*, 31: 461. 1948.

(9) GULLICKSON, T. W., FOUNTAINE, F. C., AND FITCH, J. B. Various Oils and Fats as Substitutes for Butterfat in the Rations of Young Calves. *J. Dairy Sci.*, 25: 117. 1942.

(10) HAWK, P. B., OSER, B. L., AND SUMMERSON, W. H. *Practical Physiological Chemistry.* 12th ed. The Blakiston Company, Toronto, Canada. 1947.

(11) HOWE, P. E. The Fat Content of the Feces of Young Calves. *Am. J. Diseases Children*, 21: 57. 1921.

(12) LAMBERT, M. R., JACOBSON, N. L., ALLEN, R. S., AND ZALETEL, J. H. Lipid Requirements of the Young Dairy Calf. *J. Dairy Sci.*, 36: 591. 1953.

(13) O'CONNELL, P. W., LIPSCOMB, E., AND DAUBERT, B. F. Studies on the Determination of Polysaturated Fatty Acids in Blood. *Arch. Biochem.*, 36: 304. 1952.

(14) QUAIFE, MARY L., AND HARRIS, P. L. The Chemical Estimation of Tocopherols in Blood Plasma. *J. Biol. Chem.*, 156: 499. 1944.

(15) SEWELL, R. F. The Threonine and Protein Requirements of Suckling Pigs. Ph.D. Thesis, Cornell University, Ithaca, N. Y. 1953.

(16) SPERRY, W. M. Lipid Excretion. III. Further Studies of the Quantitative Relations of Fecal Lipids. *J. Biol. Chem.*, 68: 357. 1926.

(17) WIESE, A. C., JOHNSON, B. C., MITCHELL, H. H., AND NEVENS, W. B. Synthetic Rations for the Dairy Calf. *J. Dairy Sci.*, 30: 87. 1947.

(18) ZALETEL, J. H., ALLEN, R. S., AND JACOBSON, N. L. Lipids in Blood Plasma of Young Dairy Calves. *J. Dairy Sci.*, 35: 1046. 1952.

ASSOCIATION ANNOUNCEMENTS

49TH ANNUAL MEETING

The Pennsylvania State University
State College, Pennsylvania

June 22-24, 1954

Registration, Housing, and Meals

Registration and housing assignment headquarters will be in the main lounge of the West Dormitories dining hall from 10:00 A.M. to 11:00 P.M., June 21, and 8:00 A.M. to 5:00 P.M., June 22-24. Advanced registration applications are being mailed to the membership with the request that they be filled out completely and accurately. This is desired even though the registrant expects to make his own rooming arrangements.

Housing accommodations will be available in campus residence halls, the Nittany Lion Inn (a hotel on the campus), another hotel in the town, and several local motels. All who room in the campus residence halls will be expected to obtain meals in the cafeterias of the residence halls. Meals will be available beginning with dinner on Monday, June 21, and ending with breakfast on Friday, June 25. People staying at the Inn, hotels, or motels may use the residence halls' cafeteria service but must request dining privileges for the entire period of their stay when preregistering. No individual meals may be obtained at the cafeterias but may be had at the Nittany Lion Inn, which is convenient.

The Housing and Meals Committee will make room assignments to the campus residence halls, the Nittany Lion Inn, and the local motels although, except for the residence halls, registrants may make their own arrangements if they so desire. No reservations will be made by the Committee in any off-campus hotel.

All advanced registrations will be confirmed, and incompletely or inaccurately filled-out request forms will be returned for correction. Please make registrations early.

Women and Children

A program of interest to women is being planned, and supervised activities and entertainment for children (over 3 years) will be available in programs for the different age groups. A list of baby-sitters who may be engaged to care for youngsters will be distributed. No cribs are available in the residence halls.

Committee and Special Meetings

Groups desiring rooms for committee or special meetings should write to the Room Assignment Committee, Dairy Department, The Pennsylvania State University, State College, Pa. Please give the date, time, and number participating. Groups desiring special breakfast, luncheon, or dinner reservations should make their needs known to the Special Meals Committee at the above address. Please state date, time, number expected, and name of group. Special meals cannot be arranged after June 5, 1954. Only limited facilities for special meals are available. None are available at the cafeterias of the residence halls.

Facilities for Sessions

The Facilities for Sessions Committee urges all speakers at the various sessions to avoid the use of projection equipment and to bring mimeographed illustrative material with them for distribution at the sessions. All mimeographed and printed material distributed at the sessions should be identified by title, program number (if available), and names of authors.

Information Center

An Information Center will be maintained at registration headquarters during the meetings. Matters pertaining to employment will be publicized on a bulletin board, and information relative to positions wanted and positions available will be on file. All mail for those in attendance will be available at this desk.

PEOPLE and EVENTS in the Dairy Science World

Pioneers in the Dairy Industry

Failing to get an appointment to West Point in 1880, a disappointed young man by the name of SAM H. GREENE, following Greeley's advice, decided to leave his boyhood home in Saco, Maine, where he was born in 1870, and set out for the West to seek his fortune. At Evanston, Wyoming, he started working for the Union Pacific railroad. In a few years the urge to travel again overtook him, so he wrote himself a pass and traveled on to San Jose, California, where he arrived in 1887.

S. H. Greene

After various business ventures, Greene became half owner of the Western Creamery Co., manufacturing and selling butter and cheese. During this time he was active in the organization of the San Francisco Wholesale Dairy Produce Exchange. Selling his business interest to his partner, he joined the United States Food Administration as Chief of the Dairy Section of the California organization.

Greene served as manager of the California Dairy Council from its inception in 1919 until his retirement in January, 1947. He still serves on the board of directors. At present he and his wife live in Berkeley beside the campus of the Univ. of California, close by the Faculty Club, of which he is an associate member.

Through the years, the California Dairy Council initiated many movements for the betterment of the dairy industry. First, a study was made, in cooperation with the State Board of Education, of the diets of 130,000 children, and this dramatized the importance of milk products. The Pacific Slope Dairy Show was established and between 1920 and 1930 brought the industry prominently to public attention. The first milk bar was established in the livestock show in Los Angeles. "Scrub bull trials" were held to emphasize the need of herd improvements through proven sires. DR. WILLIAM R. P. EMERSON, famous nutritionist, and DR. E. V. McCOLLUM, renowned discoverer of the vitamins, were brought from the East to lecture to large audiences under the auspices of Dairy Council.

A series of "Dairy Products for Health" campaigns was conducted. With the cooperation of parent-teacher associations and milk dealers, milk was introduced into schools. Leadership was taken in the fight against foot-and-mouth disease, which invaded California in 1924. The Council pioneered in radio programs in behalf of milk and milk products. It introduced the "Land O' Health" program into California schools, creating the famous character "Dario." The lecture "How a Quart of Milk Provides Half the Food Needs of an Adult for One Day" was given by the Council nutrition workers to hundreds of thousands of persons. The Dairyland exhibit at the Golden Gate International Exposition was created and operated by the Council. Finally, the Council conducted the campaign that established the California Dairy Industry Advisory Board. Greene also advised in the formation of the Oregon and Washington Dairy Councils. On his twenty-fifth anniversary in 1944 as manager of the Dairy Council, Mr. Greene was honored by the Univ. of California as a most valuable citizen. This is one of three such awards given by the University.

As one time chairman of the Section on Agriculture of the Commonwealth Club of California, Greene directed a two year survey of water problems of the state as related to agriculture. The section's report has been widely used. He is still active in this club and has membership in the State Chamber of Commerce, California Creamery Operators Assoc., California Dairy Industries Assoc., and the Shrine Club. He was a member of the American Dairy Assoc. for many years, until after his retirement. Although technically retired, he is still an advisor to the dairy industry. He attended the Dairy Industry Conference at the Univ. of California at Davis, February 8-10, a function that he rarely misses.

Sam H. Greene has had a fruitful and colorful career, and his contributions will be long remembered. His keen intellect, his ability to organize, and his fine personality have enabled him to contribute much to the welfare of dairying on the west coast. His record, beyond a doubt, qualifies him as one of the great pioneers of the dairy industry.

A.D.A. Holds Annual Meeting

The annual meeting of the American Dairy Association was held March 23-24 in Chicago. A number of distinguished service awards were presented for leadership in promoting increased

use of dairy foods and the advancement of a better standard of living for the entire nation. The following winners were announced at the banquet, attended by 482 delegates and guests from 45 states.

Magazines:
- Better Homes and Gardens
- Good Housekeeping
- McCall's
- Look
- Woman's Day

Related Food Groups:
- Super Market Merchandising
- Cling Peach Advisory Board
- Knox Gelatine
- National Biscuit Co.
- Ralston Purina Co.
- Wrigley's Stores

Television Station:
- KNOE-TV — Monroe, Louisiana

Newspapers:
- Chicago Daily News
- Chicago Tribune
- Dallas Times Herald
- Denver Post
- Milwaukee Journal
- Dayton News
- Greenwood Commonwealth
- Mobile Press
- Seattle Post-Intelligencer

Dairy Industry Advancement:
- Professor H. F. DeGraff
- Paraffined Carton Research Council

Dr. DeGraff, who spoke at the meeting, stated that milk is one of the greatest food bargains in America, providing 30% of the necessary food nutrients for only 15% of the food dollar. He brought out in his discussion the fact that while children drink plenty of milk, the per capita consumption of adults falls far short of the nutritional requirements.

Utah's Twentieth Annual Dairy Manufacturing Short Course

Utah's 20th annual Dairy Manufacturing Short Course was held March 1-5 on the Utah State Agricultural College campus. The meeting was attended by more than 150 dairy leaders from all parts of Utah and nearby states. At the banquet, quality awards were made for milk, buttermilk, cottage cheese, ice cream, sherbet, Cheddar cheese, and butter.

Ira H. Gould, Ohio State Univ., W. I. Winder, Univ. of Wis., and M. N. Warnich, president of A.D.A., were guest speakers.

Exhibition of Dairy Products at Ghent

In the framework of the 9th International Fair of Ghent, September 11-26, 1954, an exhibition of dairy products and connected industries will be organized in Belgium for the first time. This exhibition, placed under the patronage of the Minister of Agriculture, will have a scientific, technical and an industrial character.

Missouri Dairy Festival Days Increase Butter Sales

It has been announced by A.D.A. that the dairy farmers and businessmen of Southwestern Missouri have increased the sale of butter in the food stores of that area 40% and the dairy farmers have upped their own purchases of butter 50%. This was not for 1 week or for 1 month, but for a whole year, according to W. T. Crighton, manager of the Producers Creamery Co., Springfield.

The territory covers 34 counties in Southwestern Missouri, with a total urban population of more than 250,000. These dairy promotion programs, at first called Butter Days, were held in 17 communities in the territory for the first time in the summer of 1953.

Mansfield, Mo., was where the Butter Day idea was born. The vocational agriculture teacher, Tom Freeman, suggested it to the Business Club of Mansfield. The club heartily agreed on the plan, thereby starting a chain of events which has snowballed the sales of butter throughout the area.

1954 Contest to Be Held at Atlantic City

The 1954 Collegiate Students' International Contest in the judging of dairy products will be held in Atlantic City, N. J., October 25. This contest will be held in conjunction with the Dairy Industries Exposition and the meetings of the Milk Industry Foundation and the International Assoc. of Ice Cream Manufacturers.

Cherry-Burrell Announces New Midwest District Appointments

A number of personnel appointments for the recently formed Midwest District of Cherry-Burrell, which covers the St. Paul, Chicago, and Cedar Rapids branches, were recently announced by J. W. Farley, manager of the company's field sales division. George Foote, midwest district manager, will have the following staff: A. H. Barber, Jr., Chicago branch area sales manager; Charles (Chuck) Sundberg, St. Paul branch area sales manager; Ralph Baker, Cedar Rapids branch area sales manager; Frank Neill, district credit manager; Jack Colfer, district inventory control manager; Carl Nelson, district office mana-

ger; BOB NORDSTROM, district service manager; and SHELLY THOMPSON, district engineering manager.

Georgia Offers Major in Plant Management

In keeping with the growing need for graduates trained with a major in commerce and a minor in dairy manufactures, the Univ. of Georgia College of Commerce has recently adopted a 4-year program leading to the degree of Bachelor of Business Administration, which combines a technical knowledge of the dairy manufacturing industry with training in business administration. Similar arrangements are now in effect at the Oregon Agricultural College and the Univ. of Illinois.

In the new Georgia program, the subject matter courses will be divided as follows:

Freshman — English composition, college algebra, general chemistry, principles of economics, history of western civilization, and orientation of business.

Sophomore — European literature, elements of organic chemistry, general microbiology, American government, principles of accounting, business correspondence, physical science, American economic history.

Junior — Elementary economic statistics, money and banking, principles of marketing, labor economics, principles of organization and management, business law, dairy chemistry, dairy microbiology, market milk.

Senior — Personal adjustment to business, principles and problems of retailing, sales administration, purchasing, dairy plant management, butter and cheese manufacture, ice cream making, electives (income tax accounting, advertising, advanced business law, personal finance, public speaking).

The department will continue to offer a major in dairy manufactures in the College of Agriculture.

Campbell to Spend Year in Egypt

M. H. CAMPBELL, dean of the College of Agriculture at the Univ. of Rhode Island and director of the Agricultural Experiment Station, has accepted an invitation to become chief agriculturist in Egypt for the Foreign Agricultural Service of the U.S.D.A. He has been granted a year's leave of absence starting March 1, 1954, for this assignment.

As chief agriculturist, Dr. Campbell will direct the Point IV technical aid in agriculture program in Egypt, correlating this work with the agricultural programs of the Egyptian government. He will be located in Cairo.

During his absence, E. P. CHRISTOPHER, vice-dean of the College, will supervise the instructional program, and W. H. WILEY, associate director of the Experiment Station, will direct the research program.

New Book on Butter

G. H. McDOWALL, of the Dairy Research Institute, New Zealand, has published a Buttermaker's Manual (2 volumes, 1,589 pages). In addition to the usual chapters dealing with the butter industry, there is one on dry butterfat and ghee and one on the manufacture of margarine.

Milking Parlor and Farm Tank Acceptance in Montana

It is estimated that approximately half of all the grade A milk produced in Montana is produced on farms that have milking parlors. Almost all of these milk producers also use the pen-stabling system of housing the milking herd. One milk pasteurizing plant has 110 producers, 106 of which have milking parlors.

Three Montana milk pasteurizing plants, located in different areas of the state, are changing from can to farm tank–tank truck transportation of milk from the farm to the plant. Two of these plants are now getting most of their milk from farm tanks. These plants are on a project to change over completely from can delivery of milk to bulk tank delivery to the plant in the next few years. The trend in Montana is definitely toward pen-stabling of the milking herd, milking parlors, and farm tanks.

Marketing Butter in Paper Crocks

A 2-lb. wax cardboard container, simulating the pottery crocks popular in the '30's, has recently been introduced by Wrigley's Stores, Inc., a Detroit chain, to encourage butter sales.

According to ANDY DE KONINCK, general sales manager for Wrigley's, only 93 score, AA grade butter is packed in this way, and the price is comparable to that of leading national brands. The new pack has been well accepted by the public, and sales for "Wrigley's Old-Fashioned Country Crock Butter" have mounted in volume until now, less than a year after its introduction, 50% as much is sold as of the regular brands.

The crock, which was advertised in newspapers and on television, may have opened a new market, since sales of other butter have not been affected, according to the chain. Bulk butter may have particular appeal to large families and housewives who believe that it stretches farther than print.

Minnesota Students Sponsor Milk Hour

For several years it has been customary for various student organizations on the St. Paul campus of the Univ. of Minnesota to sponsor Thursday afternoon coffee hours at their student union. This year the Dairy Science Club decided to depart from the traditional pattern and recently sponsored a milk hour. In place of coffee, plain milk, chocolate milk, and buttermilk were offered to some 335 students and faculty members in attendance. Cheddar cheese, Nuworld cheese, crackers, and cookies rounded out the menu. The venture proved so successful that it is planned to repeat the milk hour, which promises to become a regular club project. This is the report of E. L. THOMAS, who is one of the faculty advisers to the Dairy Science Club.

Trend in Number of Dairy Students of Concern to Industry

A Guest Editorial

Of concern to the dairy industry is the decided decline in the number of students in our colleges and universities who are studying dairying. The Milk Industry Foundation and the Dairy Industry Committee both have begun with vigor to obtain all the facts underlying this trend.

Those engaged in the industry are perplexed, and a study of its own house is being made to see whether industry itself has contributed to the trend. The colleges and universities should also evaluate their programs to be sure the teaching schedule produces the proper end result. Should our colleges turn out men fitted for research, or should a middle course be drawn in which the student is prepared for other things?

F. B. Baldwin, Jr.

This problem does not lend itself to a hasty solution. The industry is composed of men who have had college training and many who have not. Somewhere these two groups can contribute much to a final understanding of the problem and a way to the solution of it. This industry is big, and the men in it are big. The help of these men in serious planning with those in our colleges who are responsible for training the future key people in the dairy industry is a must. The downward trend of enrollment in dairy courses must be halted. The dairy industry must prove it needs the graduates and encourage the enrollment of the high school graduates.

F. BRUCE BALDWIN, JR.
Abbotts Dairies, Inc.
Philadelphia

LETTERS TO THE EDITOR

Australian Meeting

The Annual Conference of the Australian Society of Dairy Technology will be held in Brisbane, Queensland, early in October, 1954. It has been our custom to endeavour to arrange for a guest speaker to address the Conference on some matter of technical or general interest to the Dairying Industry.

I am taking the liberty of writing to you to ascertain if you know of any members of your association or any persons connected with the dairy industry who are likely to be visiting Australia about October next. With such a wide-spread association as yours I thought it possible that some information along the lines indicated may be available. May I suggest that you circulate this letter amongst the members of your council to make the enquiry as wide as possible. I would be very grateful if you would agree to assist our society in this manner.

May I take this opportunity of sending greetings to you and to the members of your association from the Australian Society of Dairy Technology.

C. J. MACDERMOTT, *President*
Australian Society of Dairy Technology

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

USE OF NUTRITION INFORMATION IN PROMOTING THE USE OF MILK AND MILK PRODUCTS

ZOE E. ANDERSON
Director, Department of Research and Nutrition Service
National Dairy Council

Milk and the products made from milk are *foods for which there is no adequate substitute.* Ample scientific evidence is available to support the contention that these foods will benefit the consumer's health, appearance, and pocketbook. The National Dairy Council has promoted milk and its products on their nutritive value since 1915. However, these activities need to be supplemented and emphasized by the advertising and merchandising of the entire dairy industry. How can the dairy industry coordinate its promotional efforts so that the same nutrition story is told by all industry companies and organizations? What nutrition facts should the dairy industry highlight in today's promotion of milk and its products?

Principles for Proper Use of Nutrition Information in Dairy Promotion

The dairy industry should follow sound principles in the use of nutrition information in the sales promotion of milk and its products. Each statement made should give the consumer information about the food products he buys. Nutrition terms should not be used in meaningless phrases. This is a common fault of current promotion of some products. *For example:* A nondairy product is currently promoted on the basis that it is "calorie-controlled." Such a description is without meaning. Telling the calorie content of a specified quantity of the product would be informative. Saying merely that it is "calorie-controlled" is noninformative and is misleading. This product is further promoted on the idea that "it is possible to drink all you want of the product without being too full." This could not be true unless one wanted only that amount which his system could comfortbly handle.

There is so much that can be said about the food values of milk and its products that it is possible to tell a persuasive story without exaggeration. Nature has provided the industry with a superior product to sell, and technical know-how has made it possible to market a number of highly palatable and wholesome food products. All can be sold on their merits alone.

Statements Should Be Accurate in Their Implications

Possibly the most damaging characteristic of poor advertising is false implication. The promotion referred to above implies that the product cannot make a person fat, even if consumed to excess on top of a food intake which has already satisfied the individual's needs — because it is "calorie-controlled" and will not cause one to feel "too full." The responsibility of the dairy industry to the health of the American public is too great to risk making false implication. This important industry should be a leader in supplying the consumer with accurately interpreted nutrition information.

Each Statement Should Be Directed Toward a Specific Audience

The nutritional needs of the individual vary with age, stage of growth, sex, health, and physiological stress. Therefore, nutrition information promoting dairy foods should be directed to the interests and needs of a specific audience. To capture the market of expectant mothers, tell them what their needs are and how these needs may be met with the right level of food. To promote dairy foods for the use of small children, address their parents with the story of what the child needs and how dairy foods meet that need. When appealing to the adolescent, tell them what they need from food, what it will do for them, and how they can combine foods to achieve the desired results. *The same approach would be effective with other age groups.*

Show How Dairy Foods Combine with Other Foods to Provide the Individual's Nutrient Needs

A complete and adequate diet is best achieved by combining desirable amounts of animal and plant food products of wide variety. The present food supply in the United States is adequate to meet the nutrient needs of our population, provided food is wisely selected and equitably distributed. Milk is the most versatile of all of these foods and combines with other foods to make highly palatable meals. Cooperation with other industries promoting the products included in the seven basic food groups is the most effective use of nutrition information. The different segments of the dairy industry and the other food industries should

work with each other, not against each other. They have a common competitor — those who exploit nutrition information to sell nostrums and drugs on the theory that our foods are robbed of their nutrient values by modern processing methods.

Safeguarding Dairy Foods Promotion Against Pitfalls

The dairy industry should safeguard itself against the pitfalls of misinformation or misimplication in nutrition information used in promotion of dairy foods. The company or organization which intends to use nutrition information for the promotion of dairy foods should employ the services of a nutrition expert to advise them on promotion copy and layouts. Such a person could work either with the company or organization that is having copy developed or with the agency which is developing the advertising and merchandising materials.

The people who are responsible for the development of copy should be provided with the basic educational materials, such as that provided by the National Dairy Council. Such material has the advantage of: (a) authorship by an authority in the field being discussed; (b) review by the Council on Foods and Nutrition of the American Medical Association; or (c) review by the Council on Dental Health of the American Dental Association, and/or (d) review by the authors of scientific reports cited in the material. Use of the information contained in these pieces of material, unless distorted in meaning by being taken out of context, will give the advertiser assurance of accuracy.

Coordination of Dairy Foods Promotion by the Industry

The dairy industry should coordinate its promotional efforts so that the same nutrition story is told by all industry companies and organizations.

The National Dairy Council has sponsored the organization of the Dairywide Coordinating Committee on Nutrition Research. This committee has a five-point program as follows:

1. To assemble and evaluate from available sources the consumer and professional attitudes toward dairy foods.
2. To review scientific data and provide the factual background for public information about dairy foods.
3. To suggest additional needed nutrition research which will encompass all phases of human nutrition as affected by dairy foods.
4. To present to the dairy industry authentic information about dairy foods and to formulate recommendations for its use in public relations, advertising, and other educational work.
5. To make available to dairy groups an annual summary of dairy industry sponsored nutrition research projects in progress, including the name of the director, the location of project, and the objectives.

The Dairywide Coordinating Committee on Nutrition Research embraces all segments of the industry, including the American Dairy Science Association. The value of this program to the industry remains to be demonstrated since to this date its activities have been limited to the tedious job of organization and delineation of responsibilities and functions.

Nutrition Facts for Today's Promotion of Milk and Its Products

Certain nutrition facts should be highlighted in today's promotion of milk and its products.

Promote all dairy foods for weight reduction and tell the public how to intelligently use dairy foods to fit this situation. The dairy industry has been taking advantage of the intense interest evidenced at the present time in weight reduction. This is good. The public is weight-conscious and will be interested to know that milk does more for the reducer than any other single food. Too many publicly-prescribed diets do not recognize this fact. Even too many physician-prescribed diets do not recognize it. Actually, the lower the calorie intake, the greater the need for choosing foods of high nutritional value in relation to their calorie content — and dairy foods have a high ratio of nutrients to calories. The dairy industry has a good and sound story to tell about dairy foods in weight reduction.

The National Dairy Council, in cooperation with Dr. Margaret A. Ohlson and her staff at Michigan State College, developed a documentary film and educational materials based on research studies which clearly show the role of milk and other dairy foods in weight reduction. The results of the study emphasize that weight reduction can be comfortably achieved with a diet which contains approximately equal amounts by weight of protein, fat, and carbohydrate. Such a diet can include some of all dairy foods.

However, instead of taking advantage of this investment and correlating public information with the information that is going to professional people in order to tell a good story on weight reduction for all dairy foods — the industry has been concentrating on the promotion of skimmilk, a minor product in terms of total volume. Unfortunately, some of the promotion done on skimmilk products for weight reduction has left the implication with the public that milk fat is something that should be avoided. Milk fat represents close to 40% of the total milk solids sold by the industry. Surely, the industry cannot afford to give con-

sumers the false impression that milk fat is not good for them. It is a highly important product of the dairy industry, whether we think of it from the standpoint of nutrition, taste satisfaction, or economics. Do not under-promote milk fat by inference nor over-promote it by omission or distortion of facts.

TABLE 1

Summary of selected data on U.S.A. nutrient needs and supplies

Nutrients	Units	Recommended Daily dietary allowances Range for all groups beyond age one	Amounts available per person per day, 1950				
			From all foods	From all animal food products		From all dairy foods	
				Amount	% of total	Amount	% of total
Food energy	Cal.	1200-4500	3270	1200	(36.7)	543	(16.6)
Protein	g.	40-100	94	61	(64.6)	24	(25.0)
Fat	g.	33-135	146	92	(63.0)	37	(25.3)
Carbohydrate	g.	—	399	31	(7.7)	31	(7.7)
Calcium	g.	1.0-2.0	1.06	0.87	(81.2)	0.8	(75.5)
Iron	mg.	7-15	16.3	6.1	(37.6)	0.6	(3.6)
Vitamin A	I.U.	2000-8000	8500	2754	(32.4)	1547	(18.2)
Thiamine	mg.	0.6-1.8	1.90	0.77	(40.6)	0.21	(10.9)
Riboflavin	mg.	0.9-3.0	2.35	1.66	(70.6)	1.13	(48.1)
Niacin	mg.	6-18	19.0	9.3	(49.2)	0.7	(3.6)
Ascorbic acid	mg.	35-150	117	8.8	(7.5)	6.8	(5.8)

Dairy Foods as Protein Foods

It would be advisable to help the American consumer to realize that the various forms of milk and cheese — and even of ice cream — are excellent protein foods. Dairy foods provide one-fourth of the available protein in the food supply of the United States (See Table 1). That is not the whole story. Milk protein, by virtue of its particularly favorable balance of amino acids has a higher biological value than has any other food protein except egg. Our bodies can do more with less of a food protein that has a high biological value. From the standpoint of cost, dairy foods are the cheapest source of high quality protein.

A study of food consumption of urban families living in areas representative of all sections of the United States showed that 60% of the protein was supplied by animal food products. Dairy foods supplied 24% at only 16% of the food cost; meat, poultry, and fish supplied 29% at 27% of the food cost; and eggs supplied 7% at 4% of the food cost. Grain products made the only other significant contribution of protein in these diets, 24% at 11% of the food cost. The quality of grain protein is low compared with milk protein — although foods made from grain and dairy foods supplement each other with respect to essential amino acids when consumed together.

Promotion of Dairy Foods as a Source of Calcium

It can be seen in Table 1 that dairy foods provide three-fourths of the calcium in our national food supply. It can also be seen that there is not the same margin of extra calcium in our food supply which we have for the other nutrients.

Although, as previously indicated, most people get enough protein, calcium is the nutrient in diets most often falling below the recommended level. In the aforementioned dietary survey dairy foods contributed 66% of the calcium at only 16% of the food cost. Grain products made the only other significant contribution of calcium, 14% at 11% of the food cost. Without dairy foods, the average mixed diet consumed by people in the United States supplies only about one-fourth or less of the recommended calcium allowance.

Promotion of Dairy Foods as a Source of Riboflavin

Table 1 indicates that dairy foods contribute about one-half of the riboflavin in our food supply. Again in the dietary survey animal food products made the major contribution of this nutrient, supplying 66% of that present. Dairy foods furnished 42% at 16% of the food cost; meat, poultry, and fish furnished 17% at 27% of the food cost; and eggs furnished 7% at 4% of the food cost. The only other significant economical contribution of riboflavin to these diets was from grain products, which supplied 18% at 11% of the cost. The report did not make clear to what extent the addition of milk solids to bread and baked goods furnished calcium, protein, and riboflavin to the grain products, such data not being available.

A Better Job Should Be Done with the School Lunch Program

Chart I shows clearly the importance of milk in the school lunch program and the economy resulting from the inclusion of milk as a source

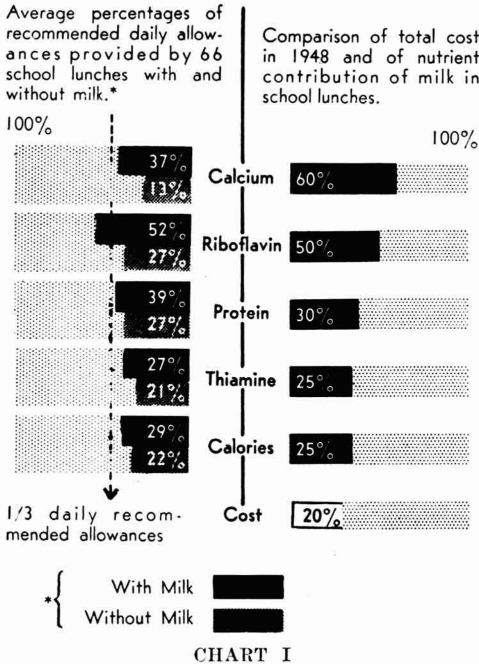


CHART I

Emphasizing the proteins in dairy foods may help to overcome the current belief that dairy foods are fatty foods. Actually, two soft boiled eggs provide 11 g. of fat; one cup of whole milk provides 9.5 g. of fat; 1 oz. of Cheddar cheese provides about 9 g. of fat; and 3 oz. of beef provide about 20 g. of fat. When such a comparison is made of the fat content of comparable servings of these protein foods, one readily sees how unjustified it is to label whole milk and cheese as fatty foods. It is interesting to compare the ratio of fat to protein with respect to these foods. When this is done the following values are obtained: eggs, 0.90; whole milk, 1.12; Cheddar cheese, 1.28; beef chuck, 1.30.

Promoting Milk on the Basis of Economy

Milk and most of the products made from it are economical foods. The economy of milk with respect to protein and some other nutrients has already been discussed. It is often stated in the press that "milk and dairy foods cost too much." In relation to other foods and in relation to other commodities, this is not true. The economy of milk can be demonstrated on a nutritional basis. (See charts 2, 3, 4)

TABLE 2

Total amount of food served by National School Lunch Program in 1952

Milk, beverage	359,000,000 qt.	Fruits and vegetables	570,000,000 lb.
Other dairy products	248,000,000 lb.	Cereals and bread	142,000,000 lb.
Meats, poultry, fish	117,000,000 lb.	Fats and oils	34,000,000 lb.
Eggs	25,000,000 doz.	Other foods	43,000,000 lb.

of specific nutrients. The school lunch program is vital to the dairy industry. The quantity of dairy foods used in this program is clearly seen in Table 2. The importance of this program to the dairy industry as a means of teaching good food habits to present and future consumers is not fully realized.

The school lunch program is an effective means not only of teaching good food habits (which includes dairy products) but also of providing nutritious meals for the children.

Do a Better Job of Promoting Milk for Small Children and Infants

One quart of milk can provide 85% of the recommended daily protein allowance of the child 1 to 3 years of age and 65% of the recommended protein allowance of the child 4 to 6 years of age. The late Dr. P. C. Jeans recommends, in the American Medical Association Handbook of Nutrition and in the National Dairy Council Baby Care Digest, that a minimum of 1 qt. of milk each day for the child throughout growth is a desirable level of intake toward which to strive.

Emphasize Foods as a Source of Nutrients

The American food supply is more than adequate to provide the nutritional needs of our population with respect to those nutrients for which there is a demonstrated need. The American public does not fully realize that the nation's food supply is adequate for good nutrition. It is a joint responsibility of all of the

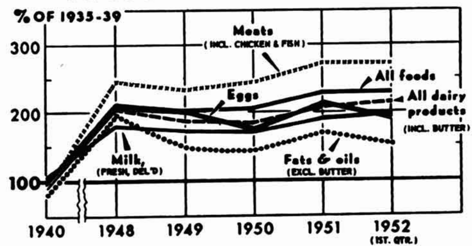


CHART II

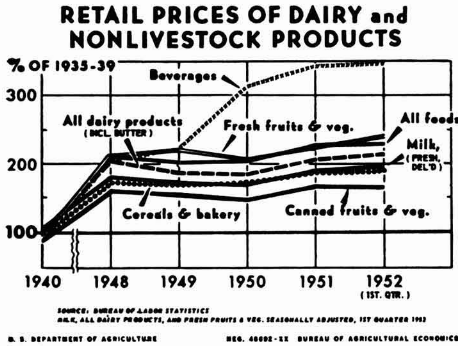
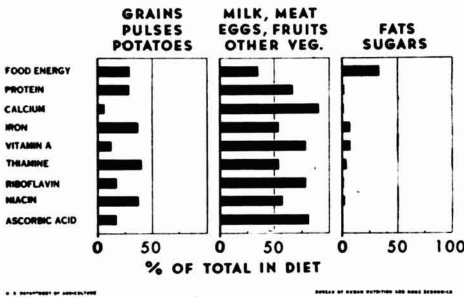
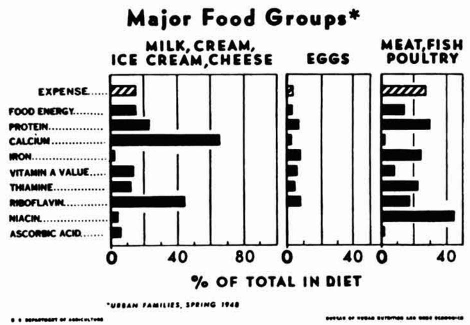
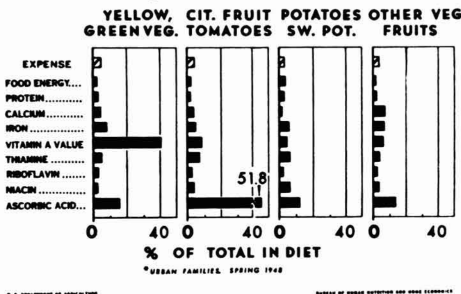


CHART III

Respect the prerogatives of the medical profession.
Be sure of all facts.
Be sure of the implications of all statements.
Address all promotion to a specific audience.
Appeal to the interests of the audience.
Provide the specific nutrition information that applies to the audience.
Tell only a single idea in a single message.
Keep all statements simple and to the point.
Place the promotion efforts on major products.
Avoid contrasting one dairy food with another.







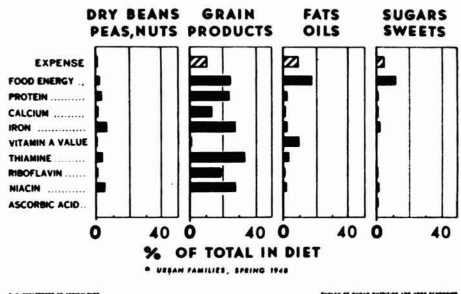


CHART IV

food industries to make this clear and to show the housewife how she can have an adequate diet for herself and her family by a proper choice of food. The information in Table 1 will help do this.

A Guide for Those Who Prepare Dairy Advertising

In conclusion, here are a few do's for dairy foods promotion that the nutritionist would like to recommend to sales promotion experts.

Take advantage of the wealth of source material about nutrition which is so readily available.
Take advantage of the organizations that are equipped to help with nutrition information and interpretation.
Pull together as a team to sell dairy foods by coordinating your promotion efforts within the dairy industry and with other food industries.
Remember that a healthy consumer is an asset to the dairy industry.

THE ROLE OF THE DAIRY INDUSTRY IN UTILIZING OUR INEXHAUSTIBLE RESOURCES

J. P. LaMaster
*The Clemson Agricultural College
Clemson, S. C.*

The cycle of life involves soils, atmosphere, plants, and animals, using the same materials over and over again. Photosynthesis is the process by which living leaves of green plants use sunlight to combine the water taken from the soil by plant roots with the carbon dioxide of the air to form sugar. This reaction requires six molecules of carbon dioxide and twelve molecules of water, plus the presence of sunlight, to produce one molecule of glucose, six molecules of water, and six atoms of oxygen. This is the most vital chemical reaction in the world. It is the source of our food supply, maintains the proper quantity of oxygen in the air we breathe, and provides a major part of the raw materials and the energy for industry.

It is estimated that 1¼ tons of glucose are synthesized by plants each year for each acre of the earth's surface. The ocean has a 300 ft. layer of algae, in which the photosynthesis should be at least twice as great per acre as that of the land surface. There are 37 billion acres of land surface and 90 billion acres of ocean surface to provide an estimated total world production of 270 billion tons of glucose per year. The value of this world-wide energy production amounts to the equivalent of 1,654 billion horsepower per hour.

Energy Reserves Are Plentiful

Improved methods for locating fuel reserves, more efficient refining procedures, and new designs for boilers promise to make the coal and oil supplies last hundreds of years longer than predicted a few years ago. At the present time the known oil reserves are the largest in history. The atomic scientists give hope of tapping other important energy sources. Einstein calculates that petroleum has latent energy equal to 11.3 billion kw.-hr. per pound, yet when burned it yields only 6 kw.-hr. per pound. It is reasonable to believe that technology will provide a way to release much of this latent energy for our use.

The atmosphere below a height of 12½ miles has a rather constant composition other than for water vapor. The volume percentages of the important elements required for plant and animal life in the atmosphere are nitrogen, 78.03; oxygen, 20.99; carbon dioxide, 0.03; and hydrogen, 0.01. There are about 5.8 metric tons of nitrogen over each square yard of the earth's surface. Continual interchange takes place between atmospheric nitrogen and the nitrogen combined in the bodies of plants and animals. Some nitrogen is returned to the soil as nitric acid in rain, and large amounts are fixed by bacteria on the roots of leguminous plants. The decay of the bodies of plants and animals releases nitrogen to the soil for productive purposes. In recent years methods of fixing nitrogen from the air in forms usable as fertilizers have become commercially established.

Animals Are Benefactors to Man

Animals must obtain their protein and energy from plants or from the tissues of other animals. All animals and all plants without green color, such as yeast cells and bacteria, become the agencies for revolving the elements of the universe. As a result of these life principles animals become benefactors to mankind by converting plant materials unavailable for human use into food and products for commerce.

Every dairy cow is equipped with a large stomach, which works 24 hours each day as a fermentation vat capable of digesting pasture crops, hay, and silage. Dr. C. F. Huffman, of the Michigan State Agricultural College, has glamorized rumen digestion under the title of "The Romance of the Gut," in which he describes how the enzyme urease breaks down simple nitrogenous compounds to ammonia, which is then built up by the rumen bacteria and protozoa into the complete amino acid balance needed to produce the complex proteins of milk and body tissues.

This relationship between the rumen microbes and the stomach contents implies that this process is really rumen nutrition. It is similar to applying fertilizers to crops. The bacteria and protozoa produce quality proteins from fertilizer-like chemicals and synthesize many vitamins, including thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, vitamin B_{12}, biotin, and vitamin K. The carotene contained in the leafy cattle feeds becomes the precursor of vitamin A. Cattle also have the ability to produce vitamin C in their bodies. This process is unknown, but the production of vitamin C is thought to be associated with the presence of vitamin A. The rumen microbes consume fibrous feeds to obtain energy supplies that become available to the cow when the microbes are digested.

Veal Protein Economically Produced

All the productive energy of animals may be classified as work or growth. Milk, eggs, and veal have a composition resembling growth. Dr. Samuel Brody shows in *Bioenergetics and Growth* that the percentage of gross efficiency for converting the energy of plants into animal tissues and products ranks in the following order: prenatal growth, 60; postnatal growth, 35; milk production, 33; hog carcass, 29; egg production, 16; and beef production, 7. The dry matter contained in the new-born calf is almost identical in composition to the dry matter

of milk. The prenatal development of the calf occurs at a uniform temperature of 101° F. There is no activity factor. Ninety per cent of the development takes place in the last 6 weeks of gestation to greatly reduce the maintenance requirements. After birth, the efficiency drops from 60 to 35%. These situations indicate that veal is one of the most economical food proteins to produce.

Cow Converts Roughages to Foods Suitable for Humans

Seventy-five per cent of the dairy cow ration consists of pasture, hay, silage, and other roughages, all of which are low in energy and not suitable for human food. The major portions of the concentrate feeds used by dairy cattle are by-products of food processing and industry, such as citrus pulp, wheat bran, oatmeal factory by-products, cottonseed meal, urea, and ammoniated molasses, which are not used for human food or feeds for poultry or hogs.

Grassland farming is an important development resulting from the symbiotic relation of the cow and the microbes of her rumen. Crop farming has depleted and caused erosion of the soils in sections of America to such an extent that large applications of fertilizers are required to produce food, feed, and fiber crops. The use of sod crops for cattle feed with the return of manure to the soils prevents erosion and improves soil fertility with limited applications of limestone and phosphate materials.

Quality of Beef from Dairy Cows Can Be Improved

From 40 to 50% of the beef used in the United States comes from dairy cattle. This includes all the veal and some good grades of beef obtained from dairy steers and barren heifers. There is a real opportunity to increase the acceptability of beef from dairy cows to improve the food supply and to augment the dairy farm income. The excessive feeding often practiced with beef cattle creates a wastage of feed supplies in too great production of body fat. Most of this tallow is not edible. Effective research on ways for making the lean meat of dairy cattle more tasty will make a major contribution to the dairy industry.

Better Breeding Necessary

The artificial insemination of dairy cattle, started on an organized basis in May, 1938, has developed into a successful program for the mass improvement of dairy cattle. The introduction of the technique for freezing semen promises to speed up the trends for increasing the milk production per cow. The frozen-semen program makes it possible to use proven bulls for obtaining dairy cattle improvement comparable to the advances gained with hybrid corn. The only system of breeding dairy cattle to prove successful over a long period has been the use of unrelated proved bulls, generation after generation. This system has been followed by the Bureau of Dairy Industry, U.S.D.A., since 1920 and is the basic work supporting the proved bull idea. The widespread use of artificial insemination has created a great demand for proved bulls. The turnover of old bulls is so rapid that it is becoming difficult to locate suitable replacements. Shopping around over many states or throughout the United States for proved bulls to meet this demand could result in a mass selection program from which further improvement would be limited.

It is now important for each bull stud to establish ways to insure the proving of young bulls from superior families with progeny-tested pedigrees. It is preferable that these young bulls be proved in artificial service so that they may be sampled under many herd conditions.

It is unreasonable to believe that someone will have bred the bull needed and that this bull will have had the opportunity to be adequately proved at the right time to meet every need. The Chinese have a saying that "the way to have a good orchard is to plant trees a long time ago." The tools for building breeding systems are inbreeding, crossbreeding, and selection. Improvement in the inherited ability of dairy cattle to produce more milk per cow awaits the application of the methods which have been applied so successfully to corn breeding. In the absence of proved facts, it is legitimate to develop a philosophy for applying the principles of genetics to the art of breeding dairy cattle. It is not possible or desirable to apply to dairy cattle the intensity of inbreeding used with corn.

The nearness of the lines to each other in Figure 1 represents the closeness of relationship between the mates indicated. The cases to the left of "random breeding within the breed" represent inbreeding and those to the right, crossbreeding. The closest inbreeding with cattle is obtained from matings of full brother to full sister and parent to offspring. Inbreeding is used to designate the mating of related individuals, and crossbreeding refers to the mating of unrelated or remotely related individuals within a breed or between individuals of different breed origin.

Although Table 1 shows that matings of brother to full sister, sire to dam, and sire to daughter have the same percentage of inbreeding and the same percentage of relationship, the gene combinations produced by these matings are very different. The sire to daughter mating increases the inheritance of the sire in the offspring. The son to dam mating increases the inheritance from the dam in the offspring and the full brother to full sister mating maintains in their offspring gene combinations similar to those possessed by the full brother and

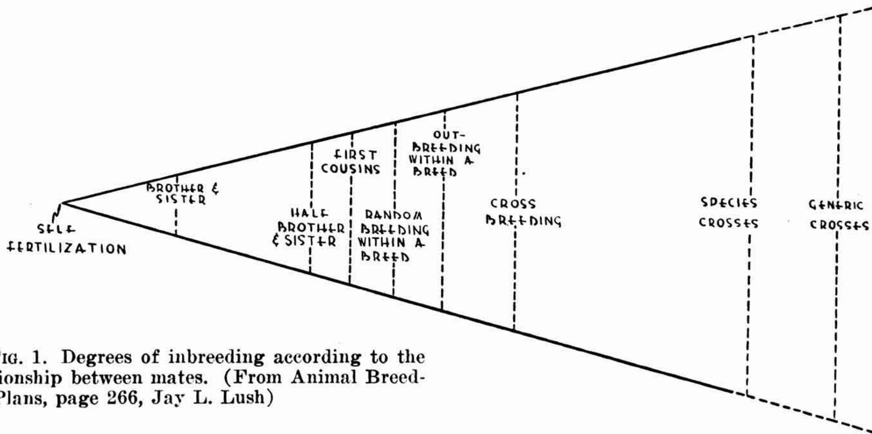


FIG. 1. Degrees of inbreeding according to the relationship between mates. (From Animal Breeding Plans, page 266, Jay L. Lush)

the full sister. The mating of a superior proved sire to his best daughters to produce sons provides the greatest opportunity for selection and permits the possibility for preserving the best inheritance for milk production in the highest immediately obtainable concentration. This plan makes it unnecessary to carry a large number of inbred females to produce inbred bulls.

TABLE 1

Percentage inbreeding and percentage relationship obtained from different systems of mating

Kind of mating	*Coefficient of inbreeding*[a]	*Coefficient of relationship*[b]
	(%)	(%)
Brother × full sister	25	50
Sire × dam	25	50
Sire × daughter	25	50
Brother × half sister	12.5	25
Sire × granddaughter	12.5	25
Double cousins	12.5	25
Single cousins	6.25	12.5
Nephew × aunt (half)	6.25	12.5

[a] The coefficient of inbreeding measures the probable similarity of the germ cells which united to produce the offspring.

[b] The coefficient of relationship measures the probable similarity of the genetic constitutions of the two individuals that were mated.

Through the selection of the best sons produced by this plan a system of crossbreeding can be employed with daughters of other superior sires within the same breed or with daughters of superior sires from other breeds, depending upon whether a purebred or grade herd is being used. The crossbred cows will possess the physical strength required for heavy milk production.

No one has ever been able to create a gene. All that can be done is to locate and utilize the best genes that have been produced by nature. Systems of breeding move genes around. Inbreeding makes the inbreds different from other lines. The crossing of inbred bulls with daughters of other inbred bulls from different family lines unites different genes to produce hybrid vigor and to bring together different quantitative genes that are additive in their effects. The next step is to locate two or three lines or families with favorable combining effects. This system of breeding is being used experimentally in the Clemson College dairy herd.

The fact that no two animals produced by sexual reproduction are alike, except identical twins, insures unlimited opportunity for improving the inherited capacity of dairy cattle for milk production.

Good Milk the Ideal Food

Pasteurized, vitamin D, homogenized, 4% milk of table quality deserves to become the American standard — pasteurized to make it safe, vitamin D-fortified to insure this protective vitamin, homogenized to give the same good taste to the last drop and to aid assimilation, 4% butterfat for uniform full food value, table quality to avoid objectionable flavors.

The 4% butterfat standard is based on the fact that the solids-not-fat in milk increase with increasing butterfat but at a slower rate. The two lines cross at 4%, thus making this point the standard at which dairy farmers can provide the most solids-not-fat in proportion to the butterfat at the least cost. Fluid milk is the protective food of the greatest importance to people of all ages. Milk and milk products are all 100% edible and practically 100% digestible.

Can Man Produce Enough Food for His Needs?

In 1798, Thomas Robert Malthus developed the idea that populations increase by geometric progression and the food supply by arithmetic progression. He concluded that the race of plants and the race of animals shrink under

this restrictive law and that the race of man cannot by any efforts of reason escape from it. Among plants and animals the effects are waste of seed, sickness, and premature death; among mankind, misery and vice. The prediction that man would increase faster than the food supply did not materialize at that time because of the industrial revolution that swept over Europe and the colonization of the Americas and Africa, which produced conditions for lower birth rates in the manufacturing centers than in the farming sections.

There is no food shortage in the United States. Our concern with world food shortages is due to political policies and revolutionary situations in other parts of the world. The Food and Agricultural Organization of the United Nations reports that "Two-thirds of the people of the world are now undernourished all the time, and marginal millions do not get enough to eat. Production in most of the undernourished areas is failing to keep pace with population growth. This means more undernourishment, less resistance to disease, more funeral processions of those who die young. Hunger is steadily haunting our civilization."

The countries of the world with the greatest hunger conditions have the highest birth rates. Dr. Samuel Brody in his *Facts, Fables and Fallacies on Feeding the World Population* concludes that increasing food production is meaningful only when expressed in relation to increasing population. "The human population has the capacity and will for indefinite exponential growth, whereas the population-supporting capacity of the earth is limited. Hence, the freedom-from-want ideal cannot be made a world-wide reality unless the decline in birth rate is adjusted to approximate the decline in death rate."

Josue de Castro in *The Geography of Hunger* takes the position that hunger itself is the cause of over population. His thesis is that all well-fed nations have low birth rates and those with low intake of proteins have the high birth rates. He quotes a popular saying in Latin America that "The table of the poor is meager, but fertile is the bed of misery."

TABLE 2

Estimated world population, population increase, and the percentage relation of food production to population

	Estimated population[a]				Population increase				Percentage distribution[b]—1948-1950		
Region	1936	1948	1949	1950	1949	1950	1949	1950	Population	Production	Ratio prod. to pop.
		(millions)			*(millions/yr)*		*(%)*	*(%)*			
Far East	1,072	1,175	1,187	1,198	12	11	1.0	1.0	54.5	32.0	0.5:1
Europe	372	390	394	397	4	3	1.0	0.8	18.0	23.5	1:1
Near East	108	124	125	127	1	2	0.8	1.6	5.5	4.4	0.8:1
Africa (ex. Near East)	127	147	150	153	3	3	2.0	2.0	7.0	4.7	0.7:1
Latin America	125	156	159	162	3	3	1.9	1.9	7.0	10.1	1:1
U.S. & Canada	140	160	163	166	3	3	1.9	1.8	7.5	22.6	3:1
Oceania	10.7	12.2	12.4	12.6	0.2	0.2	1.6	1.6	0.5	2.8	5:1
Total	1,955	2,164	2,190	2,216	26	26	1.2	1.2	100	100	

From the Report of Director General, Food and Agricultural Organization of U.N. Rome 1951; also Item III Provisional Agenda, Conference of F.A.O. Nov. 19-Dec. 7, 1951. Arranged by Samuel Brody.

[a] Figures refer to end of each year

[b] Percentage based on totals, excluding USSR.

There may be opportunity for argument as to whether high birth rate is the cause or the result of inadequate food supplies. It does seem that in either case it is now time for the political and monetary limitations on exports to be revised to give the American farmer an opportunity to make friends and influence people.

The Challenge

The production of the dairy industry in the United States in 1952 was valued at $6,304,-322,000 by the Milk Industry Foundation. Thanks to the application of scientific methods, the producing capacity and the efficiency of dairy farms and milk plants and factories are being increased, yet there is more to be done. Men of energy and ability must develop our inexhaustible resources in order to meet the increasing needs of the peoples of America and the world. This is the challenge our scientists must and can meet.

THE MANUFACTURE OF LOW HEAT NONFAT MILK SOLIDS[1]

S. T. COULTER, ROBERT JENNESS, and HERBERT HARLAND

Departments of Dairy Husbandry and Agricultural Biochemistry
University of Minnesota, St. Paul

The rapid increase in the use of nonfat milk solids in the home and in cottage cheese manufacture has placed emphasis on low heat powder. Actually, low heat nonfat milk solids never has been adequately defined with the result that the products sold under this designation have varied considerably in heat treatment. In the absence of information on the extent of heat treatment which may be tolerated for different uses and on objective criteria of heat treatment, a definition or specification is impossible. This paper was prepared to present a summary of present information with respect to its manufacture.

Effects of Heat on Milk and Methods for Evaluating Them

Methods for controlling the manufacture of low heat nonfat solids presumably might be based upon any of the changes that occur on heating milk. The ones most studied in this connection depend upon serum protein denaturation and curd tension.

1. *Effects on the serum protein solubility.* The noncasein or serum proteins of milk constitute a mixture of protein entities which to date has been only partially resolved *(21)*. The principal homogeneous components that have been isolated are β-lactoglobulin (50%), α-lactalbumin (12%) *(8)*, an albumin identical to that of bovine blood (5%), and two immune globulins (each 5-10%). At least some of these proteins are denatured by heat treatment above 145° F., and interaction very likely occurs with the result that a large part of the mixture is rendered precipitable by acidification to pH 4.6 or by saturation with salt *(11)*. Heat treatment does not precipitate the serum proteins or cause them to aggregate to very large particles or to combine with caseinate particles since the latter can be removed centrifugally from heated milk leaving the serum proteins in dispersion *(23)*. A maximum of about 80% of the total serum protein is rendered acid precipitable by heat treatment but somewhat more (up to 95%) is rendered precipitable by sodium chloride. Figure 1 depicts the time-temperature relations for given extents of denaturation as measured by sodium chloride precipitability *(14)*. Naturally, the increase in precipitability has been widely adopted as an index for the extent of heat treatment. Of further importance is the fact that the denatured serum protein, coprecipitated with casein in acid curd, may directly hinder the syneresis of the latter in cottage cheese curd *(27)*.

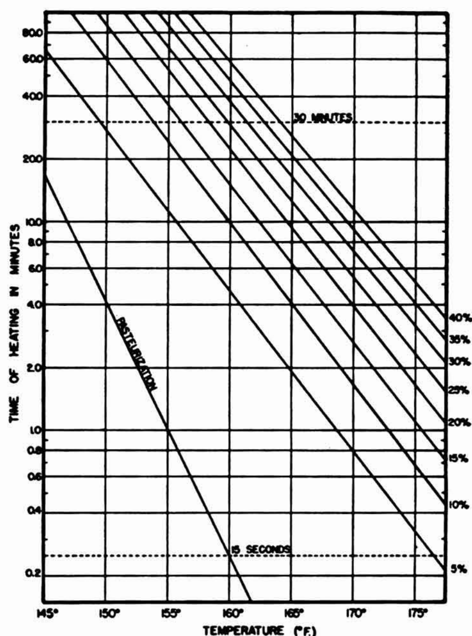


FIG. 1. The time-temperature relationships for heat denaturation of serum proteins in fresh skimmilk. (Data of Harland, Coulter and Jenness, 14.)

Denaturation may be measured as follows:

(a) Kjeldahl procedure of Rowland (25, 26). The casein and heat denatured serum proteins are removed from the sample by means of acetate buffer at pH 4.6 and the nitrogen content of the filtrate is determined by the Kjeldahl method. According to the Rowland procedure, the undenatured serum protein N is that fraction not precipitable by the acetate buffer but precipitable by 12% trichloracetic acid. The following variation of this technique also yields satisfactory results. The noncasein nitrogen is determined by the Kjeldahl method on the fluid sample before and after a 20-minute heat treatment in a boiling water bath. The difference between the values obtained represents the undenatured serum protein nitrogen. Although the accuracy of the Kjeldahl method is unquestioned, any method based upon it has the disadvantage of requiring considerable time and equipment.

(b) Harland-Ashworth method (12). This procedure depends upon the removal of casein and heat denatured serum proteins from milk by means of saturation with sodium chloride and subsequent measurement of the turbidity produced by acidification of the diluted filtrate.

[1] Paper No. 849, Miscellaneous Journal Series, Minnesota Agricultural Experiment Station.

This method was proposed originally as an objective test for the baking quality of nonfat dry milk solids. In the case of the low heat product, greater stability and more appropriate density of the turbidity developed by the undenatured serum proteins is secured by greater dilution of the sodium chloride filtrate.

Experience to date with this method indicates satisfactory agreement between laboratories in the rating of samples of nonfat solids of variable heat treatment history but poor agreement on absolute levels of undenatured serum protein reported *(3)*. Furthermore, there is considerable variability among milks in both the amount and the heat lability of the serum proteins.

In a recent survey *(15)* of 81 samples of raw skimmilk obtained at various seasons of the year from widely-separated milk sheds, the average serum protein nitrogen content was found to be 0.756 mg. per milliliter, with a standard deviation of 0.062. The amount of serum protein denatured by a given heat treatment in these samples increased with increase in their concentration in the raw milk. In spite of these limitations, measurement of serum protein solubility is a satisfactory index of the extent of heat treatment in processing a given lot of milk. Difficulties in cottage cheese manufacture may be encountered if serum protein denaturation exceeds 6-10% *(22)*.

2. *Effects on –SH groups of serum proteins.* Another manifestation of the heat denaturation of milk serum proteins is the increase in activity of sulfhydryl groups. These are confined largely, if not entirely, to the β-lactoglobulin fraction. The uncoiling and unfolding of the protein which occurs in denaturation increases the activity of these groups so that they react more readily with various oxidizing, alkylating, and mercaptide-forming reagents.

Raw milk does not contain active sulfhydryls, but heating to 145° F. or higher "liberates" or "activates" them. However, the absolute level of sulfhydryl groups is not a good index of heat treatment because these groups are readily oxidized by atmospheric oxygen during and following heating. As a matter of fact, at temperatures below 160° F., activated –SH groups do not accumulate because they are oxidized as fast as formed. The active –SH groups can be measured by either thiamine disulfide or nitroprusside *(10 13, 16)*. It should be recognized that a low value may result either from low heating or from high heating followed by oxidation. Furthermore, the nitroprusside method is not especially quantitative and the thiamine disulfide procedure is too laborious for routine work.

The total sulfhydryl content of the milk serum proteins can be estimated by an iodimetric method *(18, 19, 20)*. This is at a maximum in raw milk but decreases on heat treatment due to activation and subsequent oxidation of the sulfhydryl groups. This decrease tends to parallel the loss of solubility of the serum proteins. Although capable of yielding very precise results for the unoxidized –SH content of milk or milk proteins, the iodimetric method has several disadvantages for routine work. The ascorbic acid content of the system must be determined independently and the appropriate correction applied to the titer. Furthermore, the product under study must be exposed to oxygen for a considerable period of time to insure complete oxidation of "heat sensitized" –SH groups. Finally, as would be expected, –SH measurements are complicated by natural variations in the serum proteins.

3. *Effects on enzymes and bacteria.* The various enzymes and bacteria present in milk are each inactivated or killed by specific heat treatments. This is illustrated by the data on the time-temperature relationships necessary for inactivation of phosphatase, lipase, and peroxidase, and for the destruction of *M. tuberculosis* and *E. coli* which have been compiled in Figure 2. Phosphatase inactivation, of course, has become widely accepted as a criterion of adequate pasteurization from the standpoint of destruction of pathogens. A milk enzyme that is inactivated in the proper range of heat treatment would provide the basis of a satisfactory test for low heat powder. Such an enzyme should be inactivated by treatments slightly more drastic than necessary to inactivate phosphatase. Satisfactory low heat powders would still contain appreciable amounts of the enzyme. On the basis of present knowledge, β-amylase seems to offer some promise for this purpose *(9, 24)*.

4. *Effects on the caseinate.* Casein, consisting of at least three separate protein entities (α, β, and γ), exists in milk as complex colloidal particles containing calcium, magnesium, phosphate, and citrate. These particles are in equilibrium with the ions and salt complexes in solution in milk which consist of sodium, potassium, calcium, magnesium, phosphate, citrate, chloride, and bicarbonate.

Among the milk proteins, the caseins are often said to be "undenaturable," meaning that they themselves are not altered in solubility and other properties in the range of heat treatments (140-200° F. for times up to 5 hours) that denature other proteins. Apparently, the isolated caseins exist in more or less uncoiled, random structures rather than in a tightly coiled specific structure and, consequently, are not susceptible to heat denaturation of the type exhibited by the serum proteins. Drastic heat treatments do split out the esterified phosphate which is a hallmark of the caseins and also cause degradation as manifested by the appearance of nonprotein nitrogen. Such heat treatments also cause heat coagulation of casein in milk, a phenomenon which has been much studied but little understood. Figure 2 shows the range of heat treatments necessary for coagulation of unconcentrated and concentrated milk.

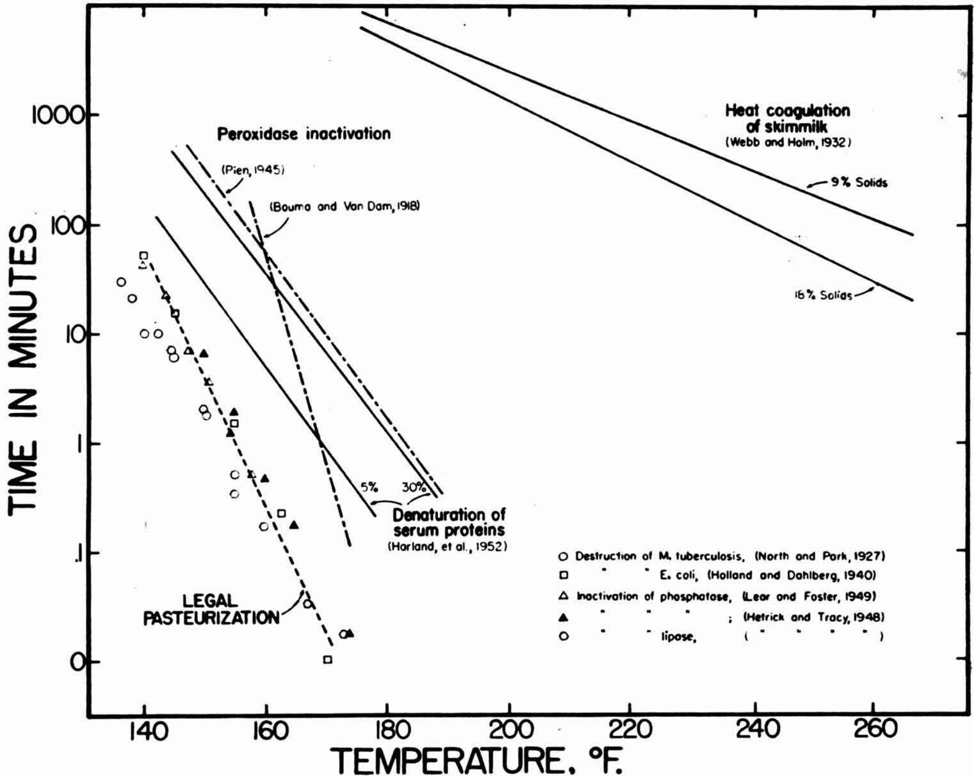


FIG. 2. Time-temperature relationships for inactivation of enzymes, destruction of bacteria and denaturation of proteins in milk.

Although mild heat treatments do not "denature" the isolated caseins, the complex caseinate particles existing in milk are altered in their clottability by rennin or pepsin. The immediate effects of treatments in the range of 140-200° F. are slower clotting and lower curd tension. On holding after heating, the clotting properties are still further impaired. This latter phenomenon seems to be related to transfer of dissolved calcium and phosphate to the colloidal state during heating and their release following the treatment.

It is natural that curd tension should be considered as an index of heat treatment for nonfat dry milk solids. The method most commonly used *(1)* consists of the preparation of a coagulum by treatment of milk with pepsin and hydrochloric acid and subsequent measurement of the resistance of the curd to cutting by a specially constructed knife.[2]

There are abundant data in the literature *(7)* showing the variability in the curd tension of normal fluid milk. Nonfat milk solids may be reconstituted to a constant level, thus eliminating one major source of variation. There are no published data adequately evaluating the effect of heat treatment on the curd tension of nonfat milk solids, but a few data on fluid milk *(2, 7)* indicate that the depression of curd tension by heating is irregular and, hence, that curd tension would not be an especially precise index of heat treatment. In our laboratory, curd tension measurements have been much less satisfactory than serum protein determinations in evaluating heat treatment in the preparation of low heat nonfat solids.

5. *Effects on lactose and lactose-protein interaction.* The well-known "browning reaction" may occur when milk is heated. The first step in this reaction appears to be condensation of amino groups of the protein with aldehyde of the sugar. The chemistry of further stages is complicated but, among other things, results in an intensely brown color, insolubilization of the protein, and production of substances that reduce ferricyanide at pH 5.0-6.6. The rate increases with increase in concentration to about 90% solids *(13)*.

Heat treatment of the liquid milk more drastic than is normally used in the manufacture of low heat nonfat solids must be used to appre-

[2] The semi-automatic device formerly manufactured by the Submarine Signal Company for making the measurement is no longer available.

ciably increase the ferricyanide reducing capacity when measured in the entire product *(4, 6)*. A modification proposed by Choi *(5)* measures the reducing capacity of the proteins alone and appears to be more sensitive in detecting heat treatment at low levels.

The sugar protein interaction occurs in stored powders at rates determined by the temperatures and moisture content. It has been shown that this reaction may occur to an appreciable degree in stored powders without adversely affecting its curd making properties *(27)*. These facts, together with the lack of specificity limits the use of the method for evaluating low heat powder.

Sources of Heat During Processing

Pasteurization of the fluid products prior to spray drying is almost mandatory because the fluids may not reach a sufficiently high temperature during drying to insure destruction of pathogens. Since the fluid skimmilk must be heated before flowing to the evaporator, pasteurization at this stage is most convenient. Precautions similar, if not identical, to those used in the market milk industry should be taken to insure the adequacy of heat treatments. Temperature and time controls which are sufficiently precise are not generally used in the dry milk industry. Thus, to insure adequate bacterial destruction, temperature and time exposures considerably in excess of normal pasteurization sometimes are used, although any heat exposure in excess of that required for pasteurization may be considered undesirable, particularly in view of the additional heat treatments during condensing and drying. Some idea of the effect of various heat treatments may be had by reference to Figure 1. Within the temperature limits observed, the temperature required to produce a given amount of serum protein denaturation varies inversely with the log of time. For example, 170° F. for 0.8 seconds and 145° F. for 65 minutes both denature 5% of the serum proteins.

Heat treatment during condensing will vary with the equipment used and processing procedures. In a single effect pan operated under normal conditions (i.e. vapor temperature below 130° F.), heat treatment is not likely to exceed that sufficient to denature more than 5% of the serum proteins. In multiple effect evaporators, however, heating in terms of serum protein denaturation may be much greater. Production drops sharply in most double effect evaporators when the vapor temperature in the first effect is held at 160° F. or below. The vapor temperatures in the first effect of triple effect evaporators may readily exceed 185° F. The actual temperature of the milk is higher than the vapor temperature by the elevation in boiling point attributable to dissolved and suspended solids, and by an amount equivalent to the effect of the weight of the liquid itself in increasing the pressure on any given portion of the liquid over that of the vapor space. Thus, the temperature of the milk at the bottom of the tube nest may be many degrees above that of the vapor in the vapor space. The total heat exposure (temperature and time) of the milk in any given evaporator may be computed with a fair degree of accuracy.

As the concentration of the milk is increased by water removal, the rate of serum protein denaturation does not change significantly. This may be noted by reference to Figure 3.

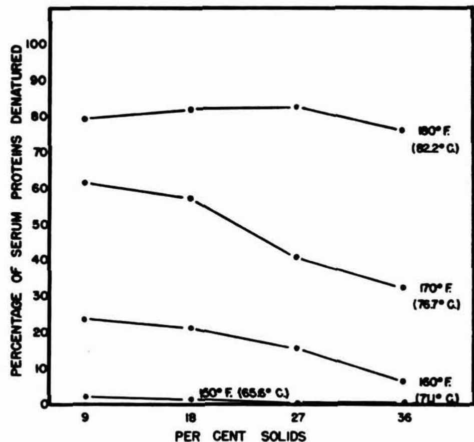


Fig. 3. The influence of the solids content on the percentage of the serum proteins denatured during 30 minutes heating at various temperatures. (Data of Harland, Coulter and Jenness, 14.)

Thus, heating of the condensed skimmilk has about the same effect on serum protein denaturation as heating the fluid skimmilk. The condensed skimmilk, after pumping from the evaporator, may be further heated before spraying.

The effect of heating milk in the liquid state on serum protein denaturation is additive. Figure 1 may be useful in computing the amount of serum protein denaturation to be expected if the processing treatments are known. All processing treatment must be converted to the equivalent time at a given temperature. This can readily be done by running lines from the temperature and time observed parallel to the serum protein denaturation lines and reading the equivalent time at the base temperature.

The heat exposure of milk during spray drying may vary considerably depending upon the design of the drier, the operating conditions, and the length of time the powder is held before cooling. Kitzes *(17)* has shown that under conditions of parallel air-milk flow, the particle immediately after spraying is at a temperature only slightly above the wet-bulb

temperature of the air and, as it dries, eventually reaches the temperature of the outgoing air. Although no driers provide strictly counter current air-milk flow, this condition is approached in some. Since in these, the dry particles may come into contact with the hot inlet air, they may be heated to considerably higher temperatures than in concurrent driers. The powder itself may remain at elevated temperatures unless continuous removal from the drier and powder cooling are provided.

The solubility index, commonly used as a measure of the extent of heat treatment during drying, is largely a reflection of casein destabilization, the rate of which is a function of the concentration up to about 90% solids *(28, 29)*. Thus, during the latter stages of drying, the casein is particularly susceptible to destabilization. On the other hand, serum protein denaturation does not increase with increase in concentration. Even under conditions of abnormally high drier temperatures, we *(15)* have observed no serum protein denaturation unless the powder was held hot for several hours.

Summary

Presumably, the permissible heat treatment during the manufacture of low heat nonfat milk solids might vary with the use of the product. For use in cottage cheese manufacture, the ideal product would be one subjected to no more heat than is necessary to insure pasteurization. However, equipment limitations may make it difficult for manufacturers to avoid excessive heat treatment. Evidence has been presented that the cumulative heat treatment at all stages of handling the liquid systems should not exceed 160° F. for 10 minutes or the equivalent of this at other times and temperatures.

Present information indicates that serum protein denaturation is the best objective index of heat treatment but has the definite limitation that the serum protein content of the raw milk must be known.

REFERENCES

(1) A.D.S.A. Final Report of Committee on Methods of Determining the Curd Tension of Milk. *J. Dairy Sci.*, **24**: 825. 1941.

(2) Berry, M. H. Soft-Curd Milk Studies. Md. Agr. Expt. Sta., *Bull.* 388. 1935.

(3) Bradley, W. B., Frey, C. N., Geddes, W. F., and Jenness, R. A Collaborative Study of Methods for Evaluating the Quality of Nonfat Milk Solids for Use in Breadbaking. *Trans. Am. Assoc. Cereal Chemists*, **11**: 217. 1953.

(4) Chapman, R. A., and McFarlane, W. D. A Colorimetric Method for the Estimation of Reducing Groups in Milk Powders. *Can. J. Research*, **23B**: 91. 1945.

(5) Choi, R. P., Koncus, A. F., Cherrey, G., and Remaley, R. J. Determination of Protein Reducing Value of Milk as an Indication of the Presence of Nonfat Dry Milk Solids. *J. Milk Food Technol.*, **16** (5): 241. 1953.

(6) Crowe, L. K., Jenness, R., and Coulter, S. T. The Reducing Capacity of Milk and Milk Products as Measured by a Modified Ferricyanide Method. *J. Dairy Sci.*, **31**: 595. 1948.

(7) Doan, F. J. Soft Curd Milk. *J. Dairy Sci.*, **21**: 739. 1938.

(8) Gordon, W. C., and Semmett, W. F. Isolation of Crystalline β-Lactalbumin from Milk. *J. Am. Chem. Soc.*, **75**: 328. 1953.

(9) Guy, E. J., and Jenness, R. (Unpublished Data, 1953.)

(10) Harland, H. A., and Ashworth, U. S. The Use of Thiamine Disulfide for the Estimation of Reducing Substances in Processed Milk. *J. Dairy Sci.*, **28**: 15. 1945.

(11) Harland, H. A., and Ashworth, U. S. The Preparation and Effect of Heat Treatment on the Whey Proteins of Milk. *J. Dairy Sci.*, **28**: 879. 1945.

(12) Harland, H. A., and Ashworth, U. S. A Rapid Method for Estimation of Whey Proteins as an Indication of Baking Quality of Nonfat Dry Milk Solids. *Food Research*, **12**: 247. 1947.

(13) Harland, H. A., Coulter, S. T., and Jenness, R. Some Factors Influencing the Reducing Systems in Dry Whole Milk. *J. Dairy Sci.*, **32**: 334. 1949.

(14) Harland, H. A., Coulter, S. T., and Jenness, R. The Effect of the Various Steps in the Manufacture on the Extent of Serum Protein Denaturation in Nonfat Dry Milk Solids. *J. Dairy Sci.*, **35**: 363. 1952.

(15) Harland, H. A., Coulter, S. T., and Jenness, R. Limitations of the Use of Serum Protein Determinations in Evaluating Heat Treatment of Milk. *J. Dairy Sci.*, **35**: 487. 1952.

(16) Josephson, D. V., and Doan, F. J. Observations on Cooked Flavor in Milk — Its Source and Significance. *Milk Dealer*, **29** (2): 35. 1939.

(17) Kitzes, H. S. Factors Influencing the Design and Operation of a Spray Drier. Ph.D. thesis, Univ. of Minnesota. 1948.

(18) Larson, B. L., and Jenness, R. The Determination of Protein Sulfhydryl Groups with Iodine and o-Iodosobenzoate by an Amperometric Titration. *J. Dairy Sci.*, **33**: 890. 1950.

(19) Larson, B. L., and Jenness, R. The Reducing Capacity of Milk as Measured by an Iodimetric Titration. *J. Dairy Sci.*, **33**: 896. 1950.

(20) Larson, B. L., Jenness, R., Geddes, W. F., and Coulter, S. T. An Evaluation of the Methods Used for Determining the Baking Quality of Nonfat Dry Milk Solids. *Cereal Chem.*, **28**: 351. 1951.

(21) McMeekin, T. L., and Polis, B. D. Milk Proteins. *Advances in Protein Chemistry*, **5**: 201. 1949.

(22) Morris, H. A., Coulter, S. T., Combs, W. B., and Heinzel, L. R. Estimation of the Serum Protein Content as a Method of Evaluating Nonfat Dry Milk Solids for Use in Cottage Cheese Manufacture. *J. Dairy Sci.*, **34**: 487. 1952.

(23) Ramsdell, G. A., and Whittier, E. O. The Effect of Heat on the Albumin and Globulin in Milk. *J. Dairy Sci.*, **36**: 437. 1953.

(24) Richardson, G. A., and Hankinson, C. L. Amylase in Cows' Milk. *J. Dairy Sci.*, **12**: 761. 1936.

(25) Rowland, S. J. The Determination of the Nitrogen Distribution in Milk. *J. Dairy Research*, **9**: 42. 1938.

(26) Rowland, S. J. The Heat Denaturation of Albumin and Globulin in Milk. *J. Dairy Research*, **5**: 46. 1933.

(27) Rudnick, A. W., Jr. Effect of Heat During Processing on Nonfat Dry Milk Solids for Cottage Cheese Making. Ph.D. thesis, Univ. of Minnesota. 1954.

(28) Webb, B. H., and Holm, G. E. The Heat Coagulation of Milk. II. The Influence of Various Added Salts Upon the Heat Stabilities of Milks of Different Concentrations. *J. Dairy Sci.*, **15**: 345. 1932.

(29) Wright, N. C. Factors Affecting the Solubility of Milk Powders. I. The Effect of Heat on the Solubility of Milk Proteins. *J. Dairy Research*, **4**: 122. 1932.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

447. A study on the relationship of vitamin A to the development of hyperkeratosis (X-disease) in calves. W. G. HOEKSTRA, R. E. HALL, and P. H. PHILLIPS, Univ. of Wis., Madison. Am. J. Vet. Research, **15**, 54: 41. 1954.

Blood composition studies of field cases of X-disease compared to control healthy calves showed only two significant changes. Plasma vitamin C in the affected calves averaged 0.54 ± .2 mg. % compared to 0.42 ± 0.2 mg. % in the controls. 70% of the affected calves had less than 10μg % of plasma vitamin A compared to 6% of the control calves. Some X-disease calves which were supplemented with 600,000 to 750,000 units of vitamin A in 10 days obtained normal blood values, while others did not change. After 7 days the responsive calves had returned to their subnormal level. Toxic calf starter pellets fed to 6 calves along with limited milk resulted in death at 15 to 80 days. Plasma vitamin A in all calves was very low and death was due to terminal pneumonia with only limited symptoms of X-disease.

A second experiment in which more milk was fed allowed the calves to survive through the milk feeding period. Toxic pellets caused rapid decline in plasma vitamin A in these calves even though weight gains were normal. Vitamin A supplementation prevented the drop in blood vitamin A in 2 of 3 calves treated while receiving the toxic pellets. After weaning the starter consumption increased from about 0.2 lb. daily to about 1 lb. All calves receiving the toxic pellets declined further in blood vitamin A and showed typical X-disease symptoms. All of the supplemented calves died before 80 days and one of the vitamin A supplemented ones died at 92 days, after the toxic pellets had been discontinued at 60 days. The other two supplemented calves maintained nearly normal blood vitamin A after the toxic pellets were removed and then gained weight at a normal rate. Thus daily supplementation of 25,000 units of vitamin A, although not preventing X-disease, did prevent death in some cases; and, after removal of the toxic feed, enabled the calves to return to normal. The principle from these feeds which was toxic to calves had no effect on the vitamin A metabolism in mice, rats or chicks, and it was not active against vitamin A *in vitro*.

E. W. Swanson

448. Production of hyperkeratosis in calves with a topically applied oil-based insecticide carrier. W. G. HOEKSTRA, R. J. DICKE, and P. H. PHILLIPS, Univ. Wis., Madison. Am. J. Vet. Research, **15**, 54: 47. 1954.

Lots of two calves, 2 to 6 mo., were each treated with mineral seal oil alone and mineral seal oil with 0.05% lindane, 0.5% methoxychlor, or 3.2% lethane plus 2.3% thanite. Calves also were observed untreated and orally dosed with highly chlorinated naphthalenes. Blood vitamin A was determined at weekly intervals to 8 wk. after treatment. After slaughter liver vitamin A was determined. Treatments amounted to a daily equivalent of 17 ml. per calf, or ¼ oz. per 100 lb. body weight; this was twice the safe level recommended by the manufacturers. All treated calves developed typical hyperkeratosis. This was accompanied by immediate declines in vitamin A of the blood. Liver depletion did not occur in the oil treated calves, but did in those fed chlorinated naphthalenes. Since the oil alone produced the condition, the effect of the insecticides *per se* was considered insignificant.

E. W. Swanson

449. The survival and transmission of Trichomonas foetus in diluted bovine semen. P. R. FITZGERALD, D. M. HAMMOND, and M. L. MINER, Utah Agr. Expt. Sta., Logan. Am. J. Vet. Research, **15**, 54: 36. 1954.

The survival of *T. foetus* was compared in culture medium at room temperature and at 39° F., in saline solution, yolk-citrate semen diluent, and in semen diluent plus antibiotics, all at 39° F. The average survival in 14 trials was, respectively, 10.9, 11.1, 7.2, 5.0 and 5.9 days when measured by direct examination. When *T. foetus* was added to diluted bull semen the sperm survived 7 days but the *T. foetus* more than 8 days at 39° F. A strain of *T. foetus* isolated from vaginal fluid survived longer (over 9 days) in semen diluent than in vaginal fluid (4 days) or saline (1.6 days). The routine use of semen from an infected bull diluted 1:15 in yolk-citrate diluent containing penicillin, streptomycin and sulfanilamide resulted in no infection in 32 cows inseminated. The actual conception rate was 62.5%, which was similar to that from non-infected bulls used at the same time. The concentration of *T. foetus* secured from the preputial material of this bull was low,

and it was considered that dilution of the semen likely reduced the organisms to an ineffective level. E. W. Swanson

450. Effects of abnormal machine milking on the histopathology of udders of first-calf heifers free of mastitis. C. F. Helmboldt, R. D. Mochrie, W. N. Plastridge, H. D. Eaton, H. L. Easterbrooks, and H. H. Hale, Storrs Agr. Expt. Sta., Storrs, Conn. Am. J. Vet. Research, **15**, 54: 15. 1954.

Eleven first lactation heifers which were shown by tests during the first month of lactation to be free of udder infection were divided into groups milked at 10, 13, and 17 in. of Hg. Each heifer was milked from 2 quarters for a normal period, and from the other 2 for twice normal time. At the end of the 44th wk. of lactation the cows were slaughtered and representative areas of each teat and gland were examined for histological evidences of mastitis. The teats usually were normal, but some cases of thelitis, hyperplasia, and metaplasia were noted. These were not correlated with the excessive milking time. One cow developed clinical mastitis on 5 different occasions, 3 on the normal side and 2 on the twice-normal side. Post mortem examination of her udder revealed no histopathological evidence of mastitis. One other cow had 2 mastitis attacks on the normal side and showed sites of focal acute mastitis in alveoli examined. The other 9 cows had no clinical or bacteriological evidence of mastitis throughout the lactation, but 5 of them had histological signs of focal acute lobular mastitis. These findings were not related to either the vacuum level or the duration of machine milking. The acute foci of mastitis seemed to result in involution rather than fibrosis. Normal involution due to advanced lactation gave a histological picture similar to that often given as being due to mastitis, i.e. collapse of the alveoli and marked lymphocytic infiltration of the interalveolar tissue. E. W. Swanson

451. A method of reproducing teat topography (structure) for evaluation of teat erosion. R. P. Pirozak, R. D. Mochrie, and C. F. Helmboldt, Storrs Agr. Expt. Sta., Storrs, Conn. Am. J. Vet. Research, **15**, 54: 140. 1954.

In order to provide permanent objectively obtained specimens to evaluate teat erosion, a method was developed to make plaster casts which faithfully reproduce the teat surfaces. Teat impressions are taken in plaster. Casts are prepared by use of plaster and a vibrator. Illustrations and complete directions are given.

E. W. Swanson

BOOK REVIEWS

452. Mammalian Germ Cells. A Ciba Foundation Symposium, edited by G. E. W. Wolstenholme, Margaret P. Cameron, and Jessie S. Freeman. Little, Brown, and Company, Boston 6, Mass. 302 pp. $6.75. 1953.

This volume contains the proceedings of a symposium, held originally under the title of the "Physiology of Mammalian Germ Cells" and attended by a variety of investigators engaged in many aspects of experimental research and in both human and veterinary practice.

The first half of the book deals with spermatozoa and consists of 13 presentations, as follows: (1) Biochemical Aspects of Semen, by T. Mann; (2) The Seminal Amino-Acid and Carbohydrate Pattern of Bulls with Normal and Abnormal Testes Function, by F. X. Gassner and M. L. Hopwood; (3) Excretion of Neutral Steroids in the Urine of Bulls with Warmth-Induced Aspermia. The Effect of Transport on Spermatogenesis and Excretion of Neutral Steroids in the Urine of Bulls, by P. Meschaks; (4) Aerobic Metabolism and Semen Quality, by C. Terner; (5) Factors Controlling Rates of Metabolism in Mammalian Spermatozoa, by H. A. Lardy; (6) Proteolytic Enzymes in Human Semen, by F. Lundquist; (7) Some Factors Influencing the Longevity of Bull Sperm Cells in Vitro, by J. C. N. Kok; (8) The Effect of Streptomycin on Bull Semen, by D. R. Melrose; (9) Semen Characteristics and Fertility in the Bull, by M. W. H. Bishop and J. L. Hancock; (10) The Preservation of Spermatozoa at Low Temperatures, by C. Polge; (11) The Movements of Spermatozoa, by Lord Rothschild; (12) Human Spermatozoan Production in Health and Disease, by J. MacLeod; and (13) Physiological Processes Involved in Spermatozoan Transport in the Cow, by N. L. VanDemark.

The second half of the book is concerned with ova and includes the following discussions: (1) The Effect of Controlled Ovulation upon the Fertility of the Mammalian Egg, by S. A. Asdell; (2) Hormonal Mechanism of the First Polar Body Formation in the Follicle, by R. Moricard and S. Gothie; (3) Research on the Formation of the Second Polar Body in the Tube After Entrance of the Sperm into the Oocyte, by R. Moricard; (4) Some Aspects of Ovulation, Recovery and Transplantation of Ova in the Immature Rabbit, by C. E. Adams; (5) In Vitro Experiments with Rabbit Eggs, by A. U. Smith; (6) Fertilizability of Rabbit Germ-Cells, by M. C. Chang; (7) Experiments on Fertilization of Rabbit Ova In Vitro With Subsequent Transfer to Alien Does, by O. Venge; (8) Early Death of the Mammalian Ovum With Special Reference to the Aplacental Opossum, by C. G. Hartman; (9) Some Factors Affecting Fertilization and Embryonic Death, by L. E. Casida; (10) Post-Coital Tests, by P. M. F. Bishop; and (11) Results of Post-Coital Tests Where Pregnancy Ensued, by I. Donald.

As previously stated, this book is the published proceedings of a symposium and makes

no attempt to introduce beginners to the study of mammalian germ cells. The presentations are highly technical and presume a knowledge of biochemistry, embryology, endocrinology, anatomy, and reproductive physiology. However, the book will be of great interest to those who are concerned with research in the field of reproductive physiology. The discussions which follow each presentation are especially valuable and frequently contain ideas for further research. References are cited by nearly all of the writers and constitute a vast source of further data. This book is a welcome addition to the literature on physiology of mammalian germ cells. D. Olds

CHEESE

453. Harmlessness of sorbic acid as a dietary component. H. J. Deuel, Jr., and R. Alfin-Slater, Dept. of Biochem. and Nutrition, Univ. So. Calif., Los Angeles, and C. S. Weil and H. F. Smythe, Jr., Mellon Inst. Pittsburgh, and Carbide and Carbon Chemicals Co., New York City. Food Research, 19, 1: 1. 1954.

Comprehensive tests in two independent laboratories using dogs and rats showed sorbic acid to be harmless when fed in diets up to levels of 5% by dry weight. The authors conclude that sorbic acid is considerably less toxic than the commonly used sodium benzoate.
F. J. Doan

454. Metabolism of α, β-unsaturated fatty acids with emphasis on sorbic acid. H. J. Deuel, Jr., C. E. Calbert, L. Anisfeld, H. McKeeban, and H. D. Blenden, Dept. of Biochem. and Nutrition, Univ. of So. Calif., Los Angeles. Food Research, 19, 1: 13. 1954.

The intermediary metabolism of sorbic acid in rats was found to be identical with that of normally occurring fatty acids. It was concluded that complete oxidation to CO_2 and H_2O is obtained and sorbic acid yields its equivalent of calories to the animal. F. J. Doan

455. Spectrophotometric determination of sorbic acid in cheese and in cheese wrappers. D. Melnick and F. H. Luckmann, Research Labs., The Best Foods, Inc., Bayonne, N. J. Food Research, 19, 1: 20. 1954.

A spectrophotometric method for determining sorbic acid in cheese employs distillation of the substance from the sample in the presence of $MgSO_4$ and calculation of the concentration from the absorbancy reading at the maximum, which varies with the pH. The method is claimed to be precise and specific.
F. J. Doan

456. Migration of sorbic acid from wrapper into cheese. D. Melnick and F. H. Luckmann, Research Labs., The Best Foods, Inc., Bayonne, N. J. Food Research, 19, 1: 28. 1954.

Sorbic acid migrates rapidly from the wrapper into all varieties of cheese. In treated packages held for 6 weeks at 45° F. an apparent loss occurs varying in percentage with variety of cheese and amount added to the wrapper surface. F. J. Doan

457. Resistance of sorbic acid in cheese to oxidative deterioration. D. Melnick, F. H. Luckmann, and C. M. Gooding, Research Labs., The Best Foods, Inc., Bayonne, N. J. Food Research, 19, 1: 33. 1954.

Sorbic acid is oxidizable by the same agents and at the same rates as noted for polyunsaturated fatty acids found in vegetable oils and butterfat and the degradation products are similar. In cheese, however, oxidation was not found to occur. Sorbic acid cannot sublime through the wrapper. The loss noted in held cheese, therefore, is due neither to oxidation by air nor to sublimation. F. J. Doan

458. Metabolic degradation of sorbic acid in cheese by molds and mechanism of mold inhibition. D. Melnick, F. H. Luckmann, and C. M. Gooding, Research Labs., The Best Foods, Inc., Bayonne, N. J. Food Research, 19, 1: 44. 1954.

The disappearance of sorbic acid from cheese was found to be caused by metabolic oxidation by molds. It is concluded that since α, β-unsaturated fatty acids are normal, but transitory metabolites in the oxidation of saturated fatty acids by molds, the high initial concentration of such substances as sorbic acid exercises an inhibitory action on the dehydrogenase enzyme systems of molds. This causes fungistatic and under certain conditions even funcidical action.
F. J. Doan

459. Effectiveness of sorbic acid in protecting cheese. D. P. Smith and N. J. Rollin, Product Development Lab., Milprint, Inc., Milwaukee, Wis. Food Research, 19, 1: 59. 1954.

Thermoplastic-coated cellophane wrappers dusted with 2.5 to 5.0 g. of sorbic acid per 1000 sq. in. of surface were found to prevent mold development in wrapped cheese, effectively. Sorbic acid mixed into process cheese at a concentration of 0.05% also proved inhibitive. In the amounts suggested, sorbic acid does not affect the taste, odor, color or emulsion stability of the cheese. Tentative approval for the use of this fungistatic agent has been given by F.D.A. F. J. Doan

460. Greater yields of cottage cheese. R. W. Mykleby and B. M. Zakariasen, Land-O-Lakes Creameries, Inc., Minneapolis, Minn. Milk Dealer, 43, 5: 45. 1954.

Low-heat nonfat dry milk solids have been accepted by the dairy industry for the manufacture of cottage cheese. Fortification of fluid skimmilk with this product resulted in ave. yields of 2.29 to 2.38 lb. of cottage cheese curd

per lb. of nonfat dry milk solids used, as compared to ave. yields of 1.42 to 1.45 lb. of curd per lb. of nonfat solids when only fluid skimmilk was used. At a 10.42% solids level, the yield of cheese curd per lb. of nonfat dry solids dropped to 2.10 lb. It was, however, apparent from the time that the curd was cut throughout the entire cooking process that the curd was easier to handle, could stand more abuse, and was more uniform in size, with increased fortification. There was some evidence that the beneficial effects of fortification were greater during certain seasons, or in certain areas where the solids content of the fluid skimmilk was low or when the curd forming quantities of the milk were poor. Slightly faster acid development occurred in most vats that were fortified with nonfat dry milk solids thereby shortening the manufacturing procedure. The cooking period was generally shorter and curd shrinkage faster in the fortified vats of cottage cheese. The curd particles in the finished cottage cheese were slightly larger with increased fortification and an over-all improvement in curd quality was generally apparent as a result of fortification. C. J. Babcock

461. Step type modernizing cuts cheese costs. V. N. Smith, Langlois Cheese Makers, Langlois, Ore. Food Eng., **26**, 2: 97. 1954.

While blue cheese making operations were continued at the Langlois, Ore. plant, modernizing was performed in a stepwise program utilizing up-to-date receiving and processing equipment and special innovations. All-metal palletized racks replaced the wooden shelves. Three types of movable shelves are used in the racks for the early curing stages. A special rack is designed for mechanically turning cheeses at the rate of 14,520 cheeses/hr. Salting operations are facilitated. Perforating of the cheese is accomplished with a semi-automatic air-operated unit. With the rack system, cheese is handled manually only 10 or 12 times during curing instead of the previous 75. Better mold growth, less breakage and improved quality are obtained. After 30 days curing, the cheese is washed and waxed. When further cured, the wax is removed and the cheese dried and wrapped in foil and parchment. The modernization has resulted in a reduction in losses, and in labor and processing costs.

T. J. Claydon

CONDENSED AND DRIED MILKS; BY-PRODUCTS

462. Milk customers: R.F.D. Anon. Milk Dealer, **43**, 5: 42. 1954.

For over 2 yr., farm folk in the vicinity of Ames, Iowa have been customers for fresh concentrated milk. This rural delivery answers the need for Grade A pasteurized milk on farms. This program, undertaken by Iowa State College, has resulted in increased use of milk per capita among the farm consumers. In 30 mo., over 300 farm families purchased 250,000 lb. of the product, equivalent to 750,000 lb. of whole milk. The product is prepared by pasteurizing Grade A milk at 180° F. for 15 sec., homogenizing it, concentrating it in a vacuum pan at a temperature of 125° F. to 1/3 of its original volume, then repeating the pasteurization and homogenization. Vitamin D concentrate then is added. When reconstituted by mixing with 2 parts of water, it is equivalent to whole milk containing 3.5% butterfat and 9% solids-not-fat. This project has developed a new market for milk, and farm families have a nutritious high quality milk available to them at all times. C. J. Babcock

DAIRY PLANT MANAGEMENT AND ECONOMICS

463. The challenge to the dairy farmer. L. L. Getten, Land O' Lakes Creameries, Inc., Minneapolis, Minn. Milk Prod. J., **45**, 3: 46. 1954.

The three important policies developing in the dairy industry are: additional advertising, self-help program, and greater production to reduce costs. A combination of these three policies should be a big factor in bringing about a stability in the dairy industry.

J. J. Janzen

464. Saving on maintenance repair with electric arc welding of piping. Anon. Milk Prod. J., **45**, 3: 30. 1954.

The use of electric arc welding in equipment repairs and installations has resulted in savings of 50% on maintenance labor and materials costs. This system has been found quite satisfactory for new piping installations and various phases of maintenance repair work.

An E-6010 class electrode is commonly used for the majority of the jobs with welding at 120 amp. J. J. Janzen

465. Why plant inspection? F. L. Hart, Food and Drug Adm., Boston, Mass. Ice Cream Field, **63**, 2: 78. 1954.

Public law 217, passed by the 83rd Congress and approved by the President, August 7, 1953, provides for factory inspection authority that was previously assumed to exist under provision of Sec. 704 of the Federal Food, Drug & Cosmetic Act.

It is stated that more than 80% of the work of the inspection service grows out of what is found by factory inspection. It is also claimed that factory inspection materially improves the effectiveness of the inspection service, thereby, aiding in the protection of the public health and welfare. W. C. Cole

466. Automatic dairies build new business. Anon. Milk Dealer, **43**, 5: 48. 1954.

Milk-O-Mat Distributors, Inc. recently installed 20 outdoor automatic dairies in Trenton

and Greater Trenton. The biggest sales through these automatic dairies are on Fridays, Saturdays, Sundays and holidays when as many as 200 qt. of milk per machine have been moved in a 24-hr. period. These sales are additional business and to a great extent are made when other outlets for milk are closed. In order to be profitable, sales per machine should average 100 qt. per day. Single qt. paper containers are vended at a price of 24½¢. Capacity of each automatic dairy is 144 qt. on the conveyor belt and 1,000 qt. in storage. Some 80% of the 20 machines are located at high-traffic gas stations and the remainder are near fruit stands or new housing developments. The requirement for a suitable location is a composite of visibility and accessibility. C. J. Babcock

FEEDS AND FEEDING

467. Feed supplements. Antibiotics in the nutrition of ruminants. J. T. Reid, R. G. Warner, and J. K. Loosli, Cornell Univ., Ithaca, N. Y. J. Agr. Food Chem., **2**: 186. 1954.

A review and analysis of the effects of antibiotics upon the nutrition of ruminants are presented. It is well established that certain antibiotics repress various infectious diseases in young ruminants, stimulate appetite, and result in rapid growth rates. The influence of various factors upon the response to antibiotic supplementation and the significance of these responses to applied feeding and husbandry are examined. S. Patton

468. Vitamin stabilization. Effect of added stabilized animal fats on stability of vitamin A in feeds. A. J. Siedler and B. S. Schweigert, Div. of Biochem. and Nutrition, Am. Meat Inst. Foundation, and Dept. of Biochem., Univ. of Chicago, Chicago, Ill. J. Agr. Food Chem., **2**: 193. 1954.

This work was initiated to determine the stability of vitamin A (as fish liver oil) in mixed feeds with either no added fat or 6% stabilized animal fat added, when the feeds were stored at room temperature. The vitamin A stability of the ration was increased when 6% stabilized choice white grease (pork fat) was added to the ration. This increase was noted primarily in the later phases of the storage periods (after 4 to 12 mo. storage), and was observed in two series of experiments conducted in two different years. In addition to increased feed efficiencey shown with dogs, chicks, and other animals, decreased dustiness of the feed, ease and speed of pelleting of the rations, and improved appearance associated with the addition of stabilized animal fats to feeds, increased vitamin A stability also was observed. S. Patton

GENETICS AND BREEDING

469. Streaked hairlessness in Holstein-Friesian cattle. F. E. Eldridge and F. W. Atkeson, Kan. Agr. Expt. Sta., Manhattan. J. of Heredity, **44**, 6: 265. 1953.

A narrow dorso-ventral pattern hairlessness (hypotrichosis) in Holstein-Friesian females affects the region of the thurls, body and legs with considerable bilateral variation in expression but without regard to color of hair in a general area. A large amount of variation between animals also was noted. Close clipping of the hair revealed the presence of the trait in cases that otherwise would be missed. The affected cattle were reported to be more sensitive to "extreme cold" as manifested by shivering in a humped position. Extra sensitivity to sun and scrubbing was also noted.

All 17 observed and reported cattle descended from one cow, four of whose daughters were observed to be affected. Except for one unknown case, all affected cattle came from affected dams.

Although the trait could be identified at an "early age" in females, no cases were observed in males of any age. The sex ratio of calves from affected dams approximated a 2:1 ratio justifying the conclusion that the trait behaves as a sex-linked lethal. Thus, for a specifically manifested trait, the authors present the best evidence yet available for the presence of a sex-linked trait in cattle.

The trait appears to be caused by a gene behaving as a sex-linked lethal manifesting itself in the hemizygous condition. L. O. Gilmore

ICE CREAM

470. Making a dry product. C. D. Dahle and R. M. Hamilton, Pa. Sta. Coll., State College. Ice Cream Field, **62**, 4: 78. 1953.

The addition of 0.1% calcium sulfate to an ice cream mix resulted in a drier appearing ice cream as it came from the freezer, but the addition of 0.2% calcium sulfate coagulated the product. "Tween 60," 0.05%, had a greater effect upon dryness than did 0.1% calcium sulfate. The addition of 2% sodium caseinate and 2% calcium caseinate had little and no effect, respectively, on dryness.

The use of 2% buttermilk powder produced a dry appearing ice cream. Substituting concentrated sweet cream buttermilk for condensed skimmilk likewise increased dryness of ice cream.

The use of 0.2% sodium citrate or 0.2% disodium phosphate failed to increase dryness whereas 0.2% "Calgon" resulted in a slightly drier product. W. C. Cole

471. Special products research. W. S. Arbuckle and L. F. M. Cremers, Univ. of Md., College Park. Ice Cream Field, **62**, 4: 86. 1953.

The effects of sodium caseinate, delactosed milk solids, special modified milk solids, and calcium sulfate on the properties of the mix and the finished ice cream are reported. These

special products were used according to the manufacturers recommendations.

Sodium caseinate had a marked influence on whipping properties of the mix and had a desirable effect on texture of the ice cream. It decreased mix viscosity, produced a slight ingredient flavor and increased the rate of melting of the ice cream.

The delactosed product increased mix acidity and viscosity, but had little influence upon whipping properties or melting of the ice cream. Ice cream texture and storage properties of the ice cream were improved by the delactosed product.

Special processed non-fat-milk-solids reduced mix acidity, but had little effect on whipping properties of mix or melting properties of ice cream and keeping qualities of the ice cream.

Calcium sulfate increased the acidity of the mix, produced a dry stiff ice cream as it came from the freezer and reduced the rate of melting, but otherwise had little effect.

W. C. Cole

472. Retail sanitation study. J. J. SHEURING, Univ. of Ga., Athens. Ice Cream Field, **62**, 4: 72. 1953.

Regular inspection of over 75 soft-serve retail stores during the year served as a basis of the author's report. The score card used in rating the stores is given and includes six major divisions.

In most cases, the store surroundings were considered excellent; improper drainage was given as the most common criticism in this category. Interior of stores were generally not criticized. Securing dependable help was one of the biggest problems in some of the stores. A decided improvement was noticed during the year in methods of cleaning and sanitizing equipment. Improvement was also noticed regarding giving proper sized servings, the use of good flavorings, and other factors concerned with profitable operation.

Insect control improved during the period of study. The use of milk dispensers and other equipment which aid in improving store efficiency was increased.

In general, the bacterial counts of products were excellent. W. C. Cole

473. Orange flavor formula now uses corn syrup. W. A. KRIENKE, Fla. Agr. Expt. Sta., Gainesville. Ice Cream Field, **62**, 5: 56. 1954.

An orange injection material formula for use in variegated ice cream was developed. The formula recommended contained: sucrose, 40 lb.; corn syrup, 49 lb.; pectin (150 grade), 2.1 lb.; water, 17.6 lb.; frozen concentrated orange juice, 40 qt.; and color 20 ml. Directions for combining the ingredients are given.

On the Univ. of Fla. campus variegated orange ice cream has equaled 10% of the total ice cream sales since Oct., 1952. W. C. Cole

474. Condensing your mix. G. H. WILSTER, Ore. State Coll., Corvallis. Ice Cream Field, **63**, 2: 74. 1954.

Calculations for standardizing mixes made by condensing are illustrated, using several combinations of ingredients for various types of mixes. W. C. Cole

475. Ice Cream Field's annual study of industry trends. ANON. Ice Cream Field, **63**, 2: 25. 1954.

Ice cream merchandising is dominated by food stores, 48.42% being sold through grocery stores. The trend towards half-gallon packages also continues, with over 20% of the volume sold in this manner. Packaged ice cream accounted for 43.7% of ice cream sold. Last year, in states where mellorine is sold, the survey shows a breakdown of 71% ice cream, 4.5% ice milk and 24.5% mellorine. Expectations are that the mellorine figure will rise to 30% in 1954. W. C. Cole

476. Vanilla-like synthetic cuts ice cream flavor cost. W. S. ARBUCKLE, L. M. F. CREMERS, and D. H. SEELY, Dairy Dept., Univ. of Md., College Park. Food Eng., **26**, 2: 84. 1954.

Propenyl guaethol, a vanilla-like flavoring substance under the trade name of Vanitrope, was used in ice cream flavor studies. Mixes were prepared under commercial ice cream plant conditions. It was found that Vanitrope could be introduced into ice cream in a flavoring formula at a 1:7 or 1:20 ratio to vanillin or pure vanilla to extend its flavor. Butter oil made a satisfactory solvent and had several advantages over other solvents tried. A stock 4% solution was made by warming 6 lb. butter oil to about 140-160° F. and stirring in 4 oz. of Vanitrope until dissolved. Observations indicated that Vanitrope alone or blended with vanillin or natural vanilla extract gave satisfactory results in vanilla ice cream. Results were also suitable with chocolate, nut and butter type flavors but were somewhat less satisfactory with fruit flavors. The flavor strength of the new product was about 25 times greater than vanillin in these applications. At the concentration used, it is estimated that Vanitrope costs about 1/3 as much as pure vanilla flavor.

T. J. Claydon

477. Fruit concentrates. Flavor-fortified high-density frozen citrus concentrates. R. G. RICE, G. J. KELLER, R. J. MCCOLLOCH, and E. A. BEAVENS. Fruit and Vegetable Chem. Lab., U. S. D. A., Pasadena, Calif. J. Agr. Food Chem., **2**: 196. 1954.

Full-flavored, high-density frozen orange juice and grapefruit juice concentrates were prepared successfully by adding appropriate amounts of cold-pressed peel oil to juice concentrates of the desired level of concentration. Packs of different concentrations from fourfold

to sevenfold were prepared and their storage behavior was studied at –18° C. (0° F.), –7° C. (20° F.), and 5° C. (40° F.). Flavor and cloud stability at –7° C. (20° F.) and 5° C. (40° F.) increased with increasing concentration of the juice above fourfold. The degree of cloud stability obtained at the sixfold level of concentration was equal to that obtained by stabilization of fourfold concentrates by partial heat treatment. In addition to savings in refrigeration costs from reduced bulk and lower freezing point, large size containers of sixfold concentrates were more easily reconstituted than such packs of the standard fourfold product, because they were more fluid at the usual frozen storage temperatures. S. Patton

478. Schedule your sales plan for greater gallonage. D. Merlin, Editorial Staff, Ice Cream Review. Ice Cream Rev., **37**, 7: 42. 1954.

The sales promotional plan followed by Hutchinson Ice Cream Division of the Borden Company at Cedar Rapids, Iowa, includes monthly sales bulletins which are made available to the sales manager, salesmen, driver salesmen, production supervisors, switchboard girls, and foremen on the loading platform. The bulletins indicate what flavor or specialty item is to be featured, what display material is available, and anticipates possible questions which may come up in connection with special flavor being featured. These bulletins are valuable aids in keeping everyone concerned with the operation informed as to future sales plans of the company. W. J. Caulfield

479. Opportunity for individuality in ice cream stencils. Anon. Ice Cream Rev., **37**, 7: 46. 1954.

The stenciled ice cream slice provides a low cost specialty item which can be turned out by small as well as large plants. The stenciling may be done either with whipped cream containing confectioners sugar and color or with an artists air brush in which case vegetable or other food coloring is added.

In using stencils the surface of the ice cream must be kept as hard as possible. This may be accomplished by doing the work on a slab of dry ice covered with waxed cardboard. Bakelite stencils are very satisfactory and may be sterilized in boiling water. It is important that the stencil be wiped dry before use.

Popularity of the stenciled slice is attested to by the fact that the Franklin Ice Cream Co., of Toledo, Ohio, sold 10,364 doz. last year. The slice produced by this company is not only stenciled but is decorated much like a miniature cake. These decorated slices sell for $2.00 per doz. W. J. Caulfield

480. What the consumer likes in ice cream. R. T. Smith, R. T. Smith Laboratories, Scranton, Pa. Ice Cream Rev., **37**, 7: 44. 1954.

Results of taste test panels, conducted over a period of years, have provided a basis for determining what the consumer likes about ice cream in terms of its color, texture, and flavor.

Color plays an important part in the acceptance and enjoyment of ice cream. The consumer groups tested indicated a marked preference for vanilla ice cream colored to resemble cream. Chocolate ice cream with a reddish type color was preferred to samples which were too light or too dark in color. Peach ice cream with a pink shade was preferred to pale or highly colored samples. A pink natural appearing color, slightly on the pale side was preferred in the case of strawberry ice cream. White ice cream with whole or broken cherries showing was the most acceptable for cherry-vanilla ice cream. Nut ice cream should have a light brown color.

Texture is of first importance in that ice cream which is coarse, icy, sandy or sticky will be rejected immediately by most consumers. Consumers like smooth ice cream with a certain chewiness, but it should not be too smooth, livery, or salvy. The importance of proper storage conditions to avoid texture changes in ice cream in the retail cabinets and in home freezers is stressed.

The ice cream must taste like the flavor it is supposed to represent for the best consumer acceptance. In vanilla ice cream it was found that the natural vanilla flavor must be evident to the taste. Heavy or strong vanilla flavored samples were rejected as well as very mild flavored samples. Chocolate ice cream with a rich natural chocolate flavor was preferred to samples that look like chocolate but which have no true chocolate flavor. Heavily flavored chocolate ice cream samples with a bitter character were also rejected. Fruit and nut flavored ice creams with a true natural fruit or nut flavor were preferred over samples in which artificial flavoring was used. W. J. Caulfield

481. Use of sucaryl in frozen desserts. K. M. Beck, Abbott Laboratories, North Chicago, Ill. Ice Cream Rev., **37**, 7: 47. 1954.

Sucaryl (cyclamate) is the registered trade name of a new sweetening agent introduced in 1950 for use in a wide variety of products where sugar must be eliminated or greatly reduced. It is 30 times as sweet as sugar, is stable toward baking, cooking and other food processing, and exhibits no bitter after taste when used in proper concentration.

Sucaryl is used in beverages, fruits and fruit juices, flavoring extracts, vegetables, gelatine, jellies, jams, cookies, candy, salad dressings, dentifrices, pharmaceuticals, and frozen desserts produced for diabetics or obese people.

Suggestions are offered for the formulation of diabetic and dietetic ice creams and ice milk mixes. Lowering of the freezing point of the mix can be achieved through the use of sorbitol.

mannitol, glycerine or a combination of glycerine and dextrose. Sorbitol and mannitol have special merit for diabetic patients because they require less insulin than sugar when utilized by the body. They will, however, have the same caloric value per gram as sugar.

There exists in the United States a potential market of six million diabetics and sixty million overweight people for a low calorie, low sugar frozen dessert. W. J. Caulfield

482. Quality key to success in soft freezer dessert business. P. E. Piper, Piper's Dairyland, Paris, Ill. Ice Cream Rev., **37**, 7: 49. 1954.

A quality product served by neat-appearing, courteous employees and from a clean, orderly, well lighted store is the key to success in the soft served frozen dessert business.

Every operator should view his place of business through the eyes of his customers. Some of the things a customer may see which will lose business are listed.

W. J. Caulfield

MILK AND CREAM

483. Homogenized and nonhomogenized milk in the preparation of selected food products. E. S. Wegner, R. Jordan, and H. A. Hollender, Purdue Univ. Agr. Expt. Sta., Lafayette, Ind. J. of Home Econ., **45**, 8: 589. 1953.

Homogenized milk was compared with nonhomogenized milk in preparing chocolate beverages, rennet desserts, and puddings made from commercial mixes. Chocolate beverages made from homogenized milk were heavier in body and showed less sedimentation. Rennet desserts made with homogenized milk scored higher in flavor and consistency, but lower in texture. Puddings made with homogenized milk were somewhat creamier in texture and were less translucent. In general, the flavor of products made with homogenized milk was less pronounced than that of products made from nonhomogenized milk. J. Hoover

484. How to limit oxidization of frozen cream. A. J. Gelpi, Jr., La. State Univ., Baton Rouge. Ice Cream Field, **62**, 4: 90. 1953.

Summer and winter cream of good quality containing 40% fat, pasteurized at 150° F. for 30 min. and cooled to 40° F. was used. In each case, one portion contained 0.5 ppm of added copper, whereas the other portion did not. Samples frozen and stored at –10° F. were examined at intervals of 30 d. for the first 6 mo. and after 12 mo. of storage. The following antioxidants were used: Sustane (butyl hydroxyanisole), ethyl caffeate, Tenox II (butyl hydroxyanisole + citric + propyl gallate in propylene glycol), and Tenox BHA (similar to Tenox II, but without the added synergists. With summer samples containing no added copper, ethyl caffeate in concentrations of 0.022% and 0.04% prevented oxidized flavor development in samples for a 12 mo. period. When present at the level of 0.004% a slight oxidized flavor was present after 12 mo. In samples containing 0.5 ppm added copper 0.04% ethyl caffeate prevented oxidized flavor development for 12 mo., 0.022% allowed slight oxidized flavor at 5 mo. and 0.004% allowed slight oxidized flavor at 3 mo.

Sustane at 0.04% and 0.022% levels without added copper inhibited oxidized flavor for 6 mo., slight oxidized flavor was present after 12 mo. At the 0.004% level, a slight oxidized flavor developed at 4 mo. All samples containing copper in this trial showed some oxidized flavor after one mo.

With winter cream, all antioxidants used were effective in preventing oxidized flavor development during 6 mo. storage. All samples with added copper (0.5 ppm) developed oxidized flavors within a month.

It was concluded that the four antioxidants were equally effective in preventing oxidized flavors. W. C. Cole

PHYSIOLOGY AND ENDOCRINOLOGY

485. Retained placenta — experimental production and prevention. L. E. McDonald, S. H. McNutt, and R. E. Nichols, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **15**, 54: 22. 1954.

Following removal of the corpus luteum at about the 60th day of gestation, injections of 75 to 100 mg. progesterone daily were continued to the 162nd to 237th day of gestation in eight cows. Parturition occurred at 254 to 282 days and in 7 of the cows the placenta was retained. Histological study showed a firm attachment of the cotyledon and caruncle epithelium. Corpus luteum removal from 5 other cows at 78 to 230 days of gestation was followed by progesterone injections of 100 mg. daily from the 248th to the 278th day of gestation. Parturition developed from the 278th to the 283rd day and in all cows the placenta was expelled within 24 hrs. after delivery. A histological specimen of the cotyledon-caruncle junction before expulsion of the placenta showed separation of the epithelium. These observations suggest the possible relationship of naturally low progesterone to early calving or abortion followed by retained placenta. E. W. Swanson

SANITATION AND CLEANSING

486. ABC's of using acid cleaners. T. Lewandowski. Milk Plant Monthly, **43**, 2: 21. 1954.

Acid cleaners used in dairy clean-up operations are composed principally of mild inorganic acids such as phosphoric or sulfamic and organic acids such as gluconic, glycolic and hydroxyacetic, levulinic, etc. On the basis of corrosion for stainless steel there is little to choose between acid cleaners as all are practi-

cally noncorrosive. For use on tinned steel, however, it is believed that phosphoric acid cleaners are superior to organic acid cleaners from the standpoint of comparatively small weight loss, no pitting and little or no staining or tarnishing of the metal surface. As most dairy farms and plants use both tinned and stainless steel equipment, phosphoric acid cleaners seem to fit the bill for general all-around use with less possibility of corrosion occurring than with organic acid cleaners.

C. J. Babcock

487. Portable equipment for whole-can testing of cream for extraneous matter. K. L. HARRIS and L. L. WARDEN, Food and Drug Admin., Washington, D. C. Milk Prod. J., **45**, 3: 32. 1954.

Two devices for whole-can testing of cream for extraneous matter have been tested. One utilizes a positive pressure sanitary pump that lifts the cream from the can by means of a 1 in. hose, passes it through the pump, and then through a nylon tulle strainer held between the two halves of a filtering chamber held together by bayonet-type fittings.

The other uses a self-priming centrifugal-type pump with neoprene impeller to suck the cream from the can and through the filter before it reaches the pump; the nylon filter is held between the two hemispheres of an 8 in. water filter.

Experiments by representatives of the F.D.A. using both devices indicated that the self-priming centrifugal pump with a modified bayonet-type filter placed on the pressure side will handle more viscous cream than would the positive action sanitary type under the same conditions, and that it met the other necessary performance requirements. Illustrations are presented showing the basic principles involved.

Preliminary tests indicated that all of the houseflies and approx. 75% of the vinegar flies present in sample of thick churning cream will be recovered using the described apparatus.

J. J. Janzen

488. Effect of type of installation and cleansing procedure on the sanitation of milk farm milk pipelines. J. E. HUNTER, E. H. MARTH, and W. C. FRAZIER, Dept. of Bacteriol., Univ. of Wis., Madison. J. Milk and Food Technol., **17**, 43: 46. 1954.

The bacteriological efficiency was studied on a cow to cooler pipeline system and a similar outfit in which the pipeline could be dismantled for cleansing. The swab contact and the brush and rinse methods were used to determine the degree of sanitation of each.

High quality milk was allowed to flow through both pipeline systems. The bacteriological results were comparable, with a low microbial count in each system.

The counts increased as the frequency of brushing decreased, but the milk exposed in the pipeline did not show any appreciable increase in bacterial population. The swab and rinse tests had little direct relationship to each other.

H. H. Weiser

489. The adherence of organisms and soil to surfaces of eating utensils. G. J. HUCKER, N. Y. Agr. Expt. Sta., Geneva. Appl. Microbiol., **17**, 2: 48. 1954.

This study has shown that microorganisms and experimental soil adhered more securely to melamine-formaldehyde plastic surfaces than to china table service. Therefore, the swab technique for counting organisms on eating surfaces is not efficient, because soil is more difficult to remove from plastic surfaces than from china surfaces by the swab procedure. Swabs from cultures on plastic dishes made after a prolonged period showed a marked reduction in bacterial counts.

H. H. Weiser

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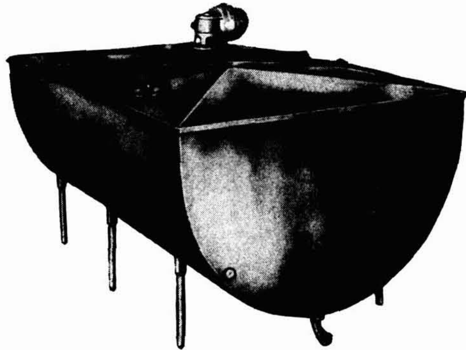

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